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Angiotensin-Converting Enzyme I/D Polymorphism and Preeclampsia Risk: Evidence of Small-Study Bias

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Abbreviations: ACE, angiotensin-converting enzyme; *ACE-I/D*, angiotensin- converting enzyme gene; CI, confidence interval; OR, odds ratio

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ABSTRACT

Background

Inappropriate activation of the renin–angiotensin system may play a part in the development of preeclampsia. An insertion/deletion polymorphism within the angiotensin-I converting enzyme gene *(ACE-I/D)* has shown to be reliably associated with differences in angiotensin-converting enzyme (ACE) activity. However, previous studies of the *ACE-I/D* variant and preeclampsia have been individually underpowered to detect plausible genotypic risks.

Methods and Findings

A prospective case-control study was conducted in 1,711 unrelated young pregnant women (665 preeclamptic and 1,046 healthy pregnant controls) recruited from five Colombian cities. Maternal blood was obtained to genotype for the *ACE-I/D* polymorphism. Crude and adjusted odds ratio (OR) and 95% confidence interval (CI) using logistic regression models were obtained to evaluate the strength of the association between *ACE-I/D* variant and preeclampsia risk. A meta-analysis was then undertaken of all published studies to February 2006 evaluating the *ACE-I/D* variant in preeclampsia. An additive model (per-*D*-allele) revealed a null association between the *ACE-I/D* variant and preeclampsia risk (crude OR = 0.95 [95% CI, 0.81–1.10]) in the new case-control study. Similar results were obtained after adjusting for confounders (adjusted per-allele OR = 0.90 [95% CI, 0.77–1.06]) and using other genetic models of inheritance. A meta-analysis (2,596 cases and 3,828 controls from 22 studies) showed a per-allele OR of 1.26 (95% CI, 1.07–1.49). An analysis stratified by study size showed an attenuated OR toward the null as study size increased.

Conclusions

It is highly likely that the observed small nominal increase in risk of preeclampsia associated with the *ACE D*-allele is due to small-study bias, similar to that observed in cardiovascular disease. Reliable assessment of the origins of preeclampsia using a genetic approach may require the establishment of a collaborating consortium to generate a dataset of adequate size.

The Editors' Summary of this article follows the references.

Introduction

Preeclampsia is a maternal disease of pregnancy associated with increased blood pressure and proteinuria after 20 weeks of gestation. It is a major cause of maternal and neonatal mortality and morbidity worldwide and has particularly high incidence in Latin American and Caribbean countries, in which hypertensive disorders during pregnancy account for 25.7% of maternal deaths [1,2]. Preeclampsia is thought to be the result of the interplay between important genetic components and environmental influences; however, the factors and the mechanisms that lead to preeclampsia remain elusive [3]. As a result, there is a lack of effective preventive interventions [4].

With the exception of smoking, established risk factors for cardiovascular disease, including high blood pressure, diabetes, and obesity, are also risk factors for preeclampsia [5]. In addition, women who suffer from preeclampsia have an increased risk of later cardiovascular disease, which clearly suggests a shared aetiology [6].

Inappropriate activation of the renin–angiotensin system may play a part in the development of many cardiovascular disorders, including preeclampsia [7,8]. A common insertion/deletion polymorphism within the angiotensin-I converting enzyme gene *(ACE-I/D)* has been reliably associated with substantial differences in the plasma and tissue angiotensin-converting enzyme (ACE) activity in a codominant (additive) fashion not only in persons of European descent, but also in other populations such as Hispanics [9–11]. Individuals carrying the *D* allele have higher ACE activity, which has been proposed as an intermediate phenotype of potential relevance for the development of high blood pressure and subclinical atheroma (i.e., higher intima-media thickness of the carotid artery) [10,12]. Despite the biological plausibility and the consistency of the effect of the *ACE-I/D* polymorphism on ACE activity, associations of the *ACE-I/D* polymorphism and coronary heart disease, coronary artery restenosis, stroke, and renal disease have been inconsistent [13–16]. Moreover, systematic reviews and meta-analyses have indicated the presence of small-study bias in the published literature [13–16].

Several studies, also usually small in size, have reported that women carrying the *D* allele of the *ACE-I/D* polymorphism have higher ACE activity and higher measures of uterine artery resistance, which is a marker for development of intrauterine growth retardation and preeclampsia [8,17]. These observations led to the proposal that the *ACE-I/D* polymorphism may be a good candidate in the search for a cause of preeclampsia. However, to date, studies evaluating the role of *ACE-I/D* polymorphism in preeclampsia have been individually underpowered to detect plausible genetic effect sizes, being much smaller than more recent studies in cardiovascular disease. We hypothesized that the published literature on the *ACE-I/D* polymorphism in preeclampsia might be similarly affected by small-study bias. To test this hypothesis, we conducted a new large genetic association study on the *ACE-I/D* polymorphism and preeclampsia in a geographical region with a high incidence of preeclampsia. We then set this study within the context of a systematic review and a meta-analysis of all studies conducted to date.

Methods

Case-Control Study Participants

A prospective case-control study was conducted in 1,711 unrelated young pregnant women (665 preeclamptic participants and 1,046 healthy pregnant controls) recruited from five Colombian cities at the time of delivery between January 2000 and December 2005. A verbal interview with a structured questionnaire was conducted by trained personnel at the time of the delivery to ascertain maternal age, gestational age, parity, smoking status during pregnancy, family history of preeclampsia, ethnic background, and socioeconomic position. On two separate occasions, the mean of two readings of blood pressure was obtained (four measurements in total) at the time of delivery. Blood pressure was measured in the right arm after a five-minute period of rest in a seated position using mercury sphygmomanometers or electronic devices calibrated against a mercury standard.

A case was defined as a primigravid woman younger than 26 years old with blood pressure 140/90 mm Hg or above, and proteinuria 0.3 grams or above in 24 hours, or a reading 2+ or above on a dipstick in a random urine determination with no evidence of urinary tract infection after 20 weeks of gestation [18]. At least one control was recruited after each case within a window of 24 hours from the same hospital that provided the cases. A control was defined as a primigravid woman younger than 26 years old without preeclampsia and in labour after 37 weeks of pregnancy. To improve the homogeneity of the phenotype under evaluation, women with a prior history of autoimmune, metabolic (including diabetes or gestational diabetes), renal, or cardiac (including chronic hypertension) diseases were excluded from the study. All participants signed the informed consent document approved by the Ethics Committee from the Universidad Autónoma de Bucaramanga, Colombia.

DNA extraction and genotyping. Blood was drawn from the antecubital vein into EDTA and samples stored at −50 °C for DNA extraction, using the QIAamp DNA blood mini-kit (QIAGEN, Hilden, Germany). The *ACE-I/D* polymorphism in intron 16 was detected according to the method described by Rigat, et al. [9]. PCR is known to have a tendency to preferentially amplify the short deletion *(D)* allele in contrast to the larger insertion *(I)* allele in a competitive amplification reaction when both alleles are present, as occurs in individuals with the *ID* genotype. This leads to mistyping of *ID* individuals as *DD* in approximately 4%–5% of the samples. To avoid any mistyping of *ID* as *DD*, a second PCR amplification using insertion-specific primers was conducted for all participants who were homozygous for the *D* allele [19]. For a detailed description of the genotyping methods, see Protocol S1. All available DNA samples were genotyped and included in the present report, with no exclusions. For quality control, a random sample ($n = 156$) was subjected to a second PCR and genotyping to minimize any possible misclassification. The Cohen's kappa value among the samples regenotyped was equal to 0.95 (95% CI [confidence interval], 0.94–0.96), and the error rate was 2.56% (95% CI, 0.70–6.43). Genotyping was conducted blinded to the clinical status of the participants.

Statistical analysis. To evaluate the presence of differences between groups, unpaired Student's t-, χ^2, or Mann-Whitney tests were used as appropriate. A test for departure from

Table 1. Maternal and Neonatal Characteristics of the Sample Studied

Category	Variable	Cases ($n = 665$)	Controls ($n = 1,046$)	*p*-Value
Ethnic background	European descent	63 (9.5%)	141 (13.5%)	
	African American	117 (17.6%)	176 (16.3%)	
	Native American	9 (1.4%)	11 (1.1%)	
	Mixture	476 (71.6%)	718 (68.6%)	0.092
Socioeconomic	Low socioeconomic status	585/642 (91.1%)	922/1,011 (91.2%)	0.958
Maternal	Maternal age (y)	19.0 ± 2.9	18.7 ± 2.6	0.013
	Smoking during pregnancy	11/660 (1.7%)	37/1,045 (3.5%)	0.023
	Systolic blood pressure (mm Hg)	146.1 ± 12.1	110.9 ± 9.2	< 0.001
	Diastolic blood pressure (mm Hg)	96.5 ± 8.1	68.1 ± 7.1	< 0.001
	Multiple pregnancy	19/663 (2.9%)	4/1,038 (0.4%)	< 0.001
	Gestational age at delivery (wk)	37.0 ± 3.6	39.2 ± 1.2	< 0.001
Maternal *ACE-I/D* genotype	*DD*	140 (21.1%)	230 (22.0%)	
	ID	382 (57.4%)	607 (58.0%)	0.72
	II	143 (21.5%)	209 (20.0%)	
Allele frequency	*D*	662 (49.8%)	1,067 (51.0%)	
	I	668 (50.2%)	1,025 (49.0%)	0.483
Newborn characteristics	Newborn weight (g)	2,626 ± 730	3,126 ± 434	< 0.001
	Newborn height (cm)	46.9 ± 4.6	49.6 ± 2.4	< 0.001
	Low (< 7) Apgar score at first min	83/671 (12.4%)	67/1,025 (6.5%)	< 0.001
	Low (< 7) Apgar score at fifth min	34/671 (5.1%)	5/1,024 (0.5%)	< 0.001

Data are presented as *n* (%) or mean ± SD.
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Hardy-Weinberg equilibrium was performed by χ^2 analysis. The principal a priori hypothesis was that the association between the *ACE-I/D* polymorphism and preeclampsia follows an additive model according to the number of *D* alleles. The additive "per-allele" model (in a log-scale) was based on the effect of the *ACE-I/D* variant on its intermediate phenotype (ACE activity), established in large studies and overviews as being a reliable association [10]. However, recessive and dominant models and multiple pairwise comparisons (*ID* versus *II* and *DD* versus *II*) were also evaluated for completeness as secondary outcomes.

Multivariate analysis using logistic regression methods was also conducted to control for potential confounders (maternal age, ethnic background, place of birth of the women, recruitment centre, socioeconomic position, urinary or vaginal infections during pregnancy, and smoking status during pregnancy).

To explore the prior hypothesis that the genotypic odds ratio (OR) is greater in women with an enriched phenotype, an analysis using an additive model was repeated for women stratified according to the presence of family history of preeclampsia (positive versus negative family history in mother or sisters), and disease severity with severe preeclampsia being defined as blood pressure above 160/110 mm Hg or proteinuria of 5 grams or more in 24 hours, eclampsia, or the HELLP syndrome. For these analyses 99% CIs were used to make some allowance for their exploratory nature. All statistical analyses were conducted using Stata, Version 9 (Stata Corporation, College Station, Texas, United States).

Meta-Analysis

Search strategy and selection criteria. Electronic databases (MEDLINE, EMBASE, LILACS, KoreaMed, and Google Scholar) were searched up to February 2006 for all genetic association studies evaluating the *ACE-I/D* polymorphism and preeclampsia in humans in all languages. The search strategy contained both medical subject heading terms and text words as follows: "angiotensin-converting enzyme" or "ACE" or "peptidyl-dipeptidase A," in combination with "pre-eclampsia" or "preeclampsia" or "pregnant hypertensive disorders" or "pregnancy hypertension," and combined with "genetic" or "polymorphism(s)" or "mutation" or "genotype" or "gene(s)." No limits were used in the search strategy. We searched for any additional studies in the references of all identified publications, including previous relevant meta-analyses, and used the MEDLINE option "related articles" for all the relevant papers.

For inclusion, studies had to involve unrelated women and examine the association between the *ACE-I/D* polymorphism and preeclampsia. Studies published as full-length articles or letters in peer-reviewed journals in any language were included, as well as abstracts taken from reference lists of identified publications. Authors were contacted (on at least three occasions) to obtain information on the genotype frequency by case-control status and by disease severity (severe and nonsevere preeclampsia), the use of blinding of genotyping staff to clinical status, the definition of outcomes, and, in a few cases, to clarify possible overlapping of study results. A positive reply was obtained in 15 out of 21 study authors contacted.

Data extraction. The following information was extracted (entered into databases by two of the authors, JPC and MCP) from each study and disagreements resolved by consensus: year of publication, total cases, total controls, number of individuals by each genotype, study design, source of controls, matching variable, thresholds used to define preeclampsia, country of origin, ethnicity, χ^2 goodness of fit for Hardy-Weinberg equilibrium and its *p*-value, use of blinding, mean age of participants, frequency of nulliparous women, and main exclusion criteria. In the few instances in which genotype frequencies provided by the investigators in tabular data differed slightly from published figures, the tabular data

Table 2. Estimate of the Effect of the *ACE-I/D* Polymorphism on Preeclampsia Risk Modeled with Logistic Regression

Association	Unadjusted OR (95% CI)	Model-1 OR (95% CI)	Model-2 OR (95% CI)
Dominant model (*D*-carriers[a] versus *II*)	0.91 (0.71–1.17)	0.86 (0.67–1.11)	0.84 (0.65–1.09)
Recessive model (*DD*[a] versus *I*-carriers)	0.95 (0.75–1.21)	0.93 (0.72–1.18)	0.92 (0.71–1.18)
Additive model (per- in increase in *D*-allele[a])	0.95 (0.81–1.10)	0.92 (0.78–1.07)	0.90 (0.77–1.06)
Pairwise comparisons, *ID*[a] versus *II*	0.83 (0.65–1.06)	0.87 (0.67–1.13)	0.85 (10.65–1.12)
Pairwise comparisons, *DD*[a] versus *II*	0.81 (0.60–1.08)	0.83 (0.60–1.16)	0.82 (0.59–1.14)

Model-1: Adjusted by maternal age, ethnicity, recruitment centre, and place of birth. Model-2: Previous variables plus socioeconomic status, infections during pregnancy (urinary or vaginal), and smoking status during pregnancy.
[a]The genotype or allele considered at risk.
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were used. Women with gestational hypertension were excluded from the present meta-analysis in order to improve the homogeneity of phenotype between studies.

Statistical analysis. Data were analysed using Stata 9. The genetic model to be considered as the priori hypothesis was an additive model, following the considerations previously described for the case-control study. Secondary analyses involved ORs for other genetic models of inheritance such as recessive, dominant, and pairwise comparisons of the different genotypes. For all the models used, the *D* allele was considered the one at risk. For the additive or per-allele model, the OR was compared between cases and controls by assigning scores for different genotype groups and calculating ORs by logistic regression. We calculated the random effect summary OR and CIs for each polymorphism. To make some allowance for multiple comparisons 99% CIs were used for individual studies, and 95% CIs were reserved for the combined estimates. The inverse variance-weighted method was used to calculate the summary OR [20]. Heterogeneity was assessed by the DerSimonian and Laird Q test and I^2 was used as a measure to describe the percentage of variability in point estimates that was due to heterogeneity rather than sampling error [20]. Sources of between-study heterogeneity were explored using random effect metaregression models with restricted maximum likelihood estimation. The prespecified characteristics for assessment of sources of inter-study heterogeneity were: Study size (number of cases: <100, 100–200, and ≥ 200); blinding of genotyping staff (blinded, unblinded, or unknown); disease severity (severe versus nonsevere preeclampsia); ethnicity of women evaluated (of European descent, Asian, Hispanic, Afro-Caribbean, and others); preeclampsia definition (adequate versus unclear); and publication language (English– versus non-English–language journals). Funnel plots of the effect estimate against the sample size, Egger regression asymmetry test, and a weighted (by the inverse of the variance of the estimate) linear fixed regression of the log OR against the inverse of the sample size (linear-regression model) were used to evaluate small-study effects [20,21]. To evaluate stability over time of the effect estimate, cumulative meta-analysis using random effect models was conducted [20].

Results

Case-Control Study

Clinical and demographic data of the cases and controls are shown in Table 1. There were no significant differences in maternal age, ethnic background, and socioeconomic position between cases and controls (Table 1). Expected differences in maternal blood pressure, parity, and newborn weight and condition were recorded.

The distribution of the *ACE-I/D* genotypes and allelic frequencies were not significantly different according to the case-control status (Table 1). Genotype frequencies according to the ethnic group and by recruitment centre are reported in "Appendix Table I" in Protocol S1. An additive model (per-*D*-allele) revealed a null association between the *ACE-I/D* variant and preeclampsia risk (crude OR = 0.95 [95% CI, 0.81–1.10]). Adjusting for maternal age, ethnicity, recruitment centre, and place of birth, aimed to minimize the effect of possible population admixture, did not change the estimate of the effect (model-1 OR = 0.92 [95% CI, 0.78–1.07]). A similar result was obtained after further adjustment for additional potential confounders such as socioeconomic position, presence of urinary or vaginal infections during pregnancy, and smoking status during pregnancy (model-2 OR = 0.90 [95% CI, 0.77–1.06]). ORs for other genetic models of inheritance also yielded a null association (Table 2). Prespecified exploratory subgroup analyses indicated that with the exception of family history of preeclampsia (positive-history OR = 1.30 [99% CI, 0.75–2.26] versus negative-history OR = 0.86 [99% CI, 0.68–1.10]; p-value for heterogeneity equal to 0.07), no substantial heterogeneity of the genetic effect size was observed for any of the subgroups (Figure 1). Additionally, stratified analysis by recruitment centre according to the conformity with Hardy-Weinberg equilibrium yielded similar, null results (see "Appendix Table II" in Protocol S1).

Meta-Analysis

A total of 30 genetic association studies, including the present study, evaluating the *ACE-I/D* gene variant and preeclampsia risk were identified [17,22–49]. We excluded eight out of 30 studies for one or more of the following reasons: two in which duplication or partial overlapping of reported data were considered likely [43,44]; three in which the outcome evaluated was solely gestational hypertension [45–47]; one in which relevant data were not reported and could not be obtained from study authors [48]; one that only recruited women with previous preeclampsia [17]; and one in which the sampling frame was based on the *ACE-I/D* genotype [49]. A total of 22 genetic association studies including 2,596 cases and 3,828 controls were included in the present meta-analysis (Tables 3 and 4) [22–42]. Out of the 22 studies, nine were conducted with Asian participants, eight with partic-

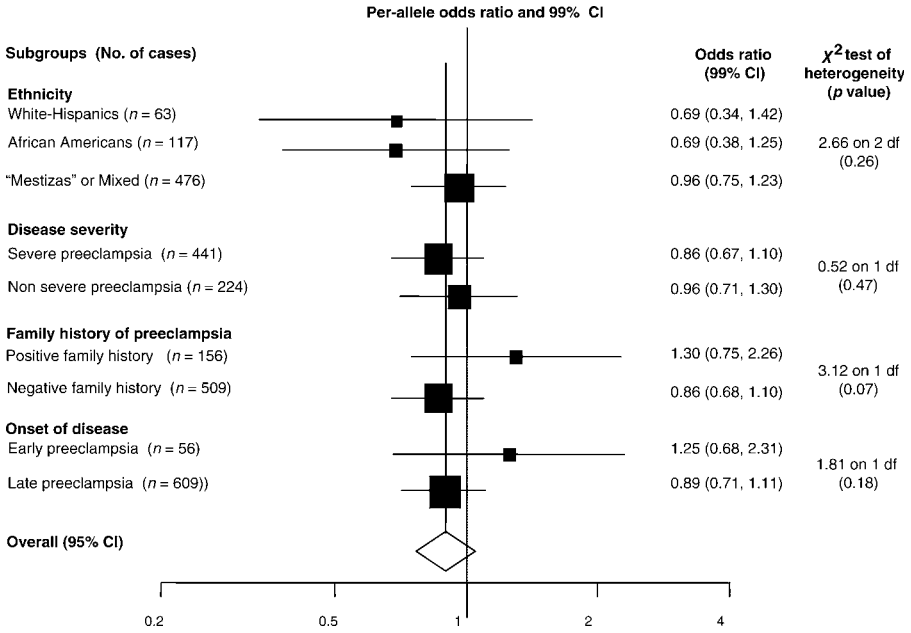

Figure 1. Risk Association of *ACE-I/D* Variant with Preeclampsia in Selected Subgroups within the Current Study
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ipants of European descent, two with African Americans, two with more than one ethnic group, and one with South Asians.

The OR under an additive model for preeclampsia was 1.26 (95% CI, 1.07–1.49; $p = 0.006$) (Figure 2). However, there was evidence of substantial between-study heterogeneity ($I^2 = 75.1\%$, $\chi_{21}^2 = 84.23$, $p_{Het} < 0.0001$). Study characteristics such as blinding of genotyping staff, publication language, preeclampsia definition, and disease severity explained little of the heterogeneity (Figure 3). A stratified analysis by study size, evaluated as the number of cases in each study (<100, 100–200, and ≥200), showed a diminished effect as the study size increased ($\chi_2^2 = 16.95$, $p_{Het} = 0.0002$) (Figure 3). Analogous results were obtained when different cutoff points (< 100, 100–500, and ≥ 500) for the number of cases were used ($\chi_2^2 = 18.51$, $p_{Het} = 0.00009$). Similarly, stratifying by ethnicity indicated that studies conducted in Asian populations tended to have a larger ORs ($\chi_5^2 = 14.4$, $p_{Het} = 0.007$). These findings might be explained by the fact that eight out of the nine studies conducted in Asian populations had fewer than 100 cases in each study. The funnel plot including all studies was asymmetric, and the Egger's test ($p = 0.06$) and the linear regression model ($p = 0.003$) suggested the presence of an excess of small studies with more positive results, predominately of studies published in non-English–language journals (Figure S1; see Protocol S1 for details). A cumulative synthesis of ACE-I/D variants and preeclampsia revealed substantial instability of the genetic effect over time, with studies published between 1996 and 1997 demonstrating the most protective effect, immediately followed by studies published in 1998 and 1999 indicating the most significant results in the opposite direction. Then, the effect size is seen to attenuate gradually over time toward a null association with the accumulation of more data (Figure S2; see Protocol S1 for details). Genotypic ORs under other genetic models of inheritance are outlined in Table 5.

Discussion

The current meta-analysis, which includes new data from the largest case-control study to date, represents the most comprehensive evaluation of the *ACE-I/D* variant in preeclampsia. Although a pooled per-allele OR suggested evidence of an increase in the risk of preeclampsia of 1.26 (95% CI, 1.07–1.49), the robustness of this summary estimate is uncertain. First, our study found a null association of the *ACE-I/D* variant with preeclampsia. Moreover, there was no substantial evidence of a positive effect in any of the subgroups in the prespecified analyses. Second, the meta-analysis revealed diminishing summary risk estimate as study size increased (Figure 3), regardless of the arbitrary cutoff points used to define the categories. This result is concordant with the results of several statistical tests used to evaluate the presence of small-study bias (Egger's test $p = 0.06$ and linear-regression model test $p = 0.003$). Furthermore, since only the published literature was included, it is possible that including unpublished studies (which more often provide evidence of negative or null effects) would have provided additional evidence of small-study bias. Taken together, these findings point to small-study bias as a potential explanation for the results observed in the meta-analysis. Discrepant findings from large and small studies are not new in the field of genetics of complex disorders [50]. When present, discrepancy of genetic effects sizes may be due to multiple causes such as genuine heterogeneity, data manipulation and fabrication, study quality, or publication bias. A form of publication bias relevant to the current report is within-study reporting bias. Because of the facility of measuring multiple genetic markers in a study, significant positive and negative associations (sometimes arising from multiple testing) are more likely to be published early rather than late. Results from the cumulative meta-analysis support this as one possible explanation, which has been referred to as the

Table 3. Characteristics of Published Studies of the Association between the *ACE-I/D* Polymorphism and Preeclampsia Included in the Meta-Analysis

Reference	Year	Total Cases	Total Controls	Total Sample Size	*DD* Genotype Cases, *n*	*ID* Genotype Cases, *n*	*II* Genotype Cases, *n*	*DD* Genotype Controls, *n*	*ID* Genotype Controls, *n*	*II* Genotype Controls, *n*	Design
Bai [22]	2002	81	199	280	8	38	35	31	83	85	Case-control
Bouba [23]	2003	41	102	143	17	19	5	29	52	21	Case-control
Choi H [24]	2004	90	98	188	33	34	23	14	51	33	Case-control
Dizon-Townson [25]	1995	124	200	324	49	42	33	65	98	37	Case-control
Galao [26]	2004	51	71	122	16	23	12	21	33	17	Case-control
Gurdol [27]	2004	95	89	184	47	31	17	31	37	21	Case-control
Heiskanen [28]	2001	133	115	248	43	59	31	31	58	26	Case-control
Kaur [29]	2005	50	50	100	30	14	6	15	26	9	Case-control
Kim [30]	2004	98	110	208	26	36	36	25	52	33	Case-control
Kobashi [31]	2005	122	291	413	19	52	51	35	136	120	Case-control
Levesque [32]	2004	174	306	480	50	92	32	97	151	58	Nested case-contol
Mingwei [33]	1998	35	25	60	23	7	5	2	10	13	Case-control
Morgan [34]	1999	73	83	156	23	31	19	25	36	22	Case-control
Mozgovaia [35]	2000	45	73	118	21	14	10	28	37	8	Case-control
Roberts [36]	2004	391	338	729	183	164	44	152	142	44	Case-control
Roh [37]	1997	36	115	151	4	11	21	14	62	39	Case-control
Seremak-Mrozikiewicz [38]	2000	25	110	135	16	7	2	34	61	15	Case-control
Serrano et al. (present study)	2006	665	1046	1711	140	382	143	230	607	209	Case-control
Tamura [39]	1996	12	179	191	3	6	3	63	82	34	Cohort
Wang [40]	2004	99	54	153	42	37	20	14	16	24	Case-control
Watanabe [41]	2001	96	96	192	13	48	35	10	40	46	Case-control
Zhou [42]	1999	60	78	138	39	12	9	8	32	38	Case-control

[a]Studies on which the reporting of the criteria to define preeclampsia was unclear. Results from these studies were compared against the others in Figure 3.
[b]Definition used from a posterior manuscript from the same authors.
BP, blood pressure; HWE, Hardy-Weinberg equilibrium.
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Table 3. Extended.

Reference	Source of Controls	Matching Variable	Systolic BP Threshold (mm Hg)	Diastolic BP Threshold (mm Hg)	Proteinuria Threshold
Bai [22]	Normal pregnant women	Maternal age	140	90	Not specified
Bouba [23]	Normotensive pregnant women who had undergone at least two pregnancies with no history of preeclampsia	None	140	90	>300 mg/l in a random specimen or an excretion of >300 mg per 24 h after 20 wk of gestation.
Choi H [24]	Normotensive volunteers randomly recruited from the Obstetric Service at the Samsung Cheil Hospital	Maternal age	140	90	> 1 g/l (or 2+ dipstick) in random urine
Dizon-Townson [25]	Referred to only as ''controls''	None	140	90	> 500 mg of protein in 24 h or a new 3 dipstick without infection[b]
Galao [26]	Normotensive pregnancy under medical assistance	None	140	90	> 300 mg/24 h
Gurdol [27]	50 participants were complication-free-pregnancies. The remaining 39 women were normotensive with ≥ 2 pregnancies unaffected by preeclampsia	None	140	90	> 300 mg/l in a 24-h collection
Heiskanen [28]	Women who delivered at Kuopio University Hospital	None	140	90	> 300 mg of urinary protein in 24 h
Kaur [29]	Normotensive primigravidae who were followed till delivery	Gestation and maternal age	140	90	> 0.3 g/l
Kim [30]	Randomly selected nulliparous and parous women who were not affected by preeclampsia in the pregnancy progressing to >20 weeks' gestation	None	140	90	> 300 mg/24 h
Kobashi [31]	Randomly selected from healthy pregnant women	None	140	90	30 mg/dl (1+ on a dipstick) or greater
Levesque [32]	Normotensive nulliparous women attending a tertiary perinatal center	Maternal age, gestational age, body mass index, and month of the year at delivery.	Not specified	90	≥ 0.3 g/l in a 24-h or ≥ 1+, using Albustix
Mingwei [33]	Control population	None	Not specified	Not specified	Not specified
Morgan [34]	Normotensive women recruited during the second half of the pregnancy	None	140	90	> 300 mg/l in 24 h or ≥ 2+ on dipstick in a random urine sample
Mozgovaia [35]	Pregnant women without obvious somatic pathology	None	140	90	≥ 0.3 g/l in a 24 h
Roberts [36]	Healthy pregnant normotensive participants who had delivery normally beyond 37th week of gestation	None	140	90	1+ on dipstick after 34 wk of gestation
Roh [37]	Normal population	None	140	90	> 300 mg/24 h
Seremak-Mrozikiewicz [38]	Healthy pregnant women	None	160	110	> 5 g in 24 h
Serrano et al. (present study)	Healthy normotensive pregnant women at delivery from the same hospitals where cases were recruited	None	140	90	> 0.3 g in 24 h, or > 2+ reading on dipstick in a random sample
Tamura [39]	Pregnant women enrolled in a clinical trial who did not develop preeclampsia or pregnancy induced hypertension	None	140	90	Referred to as proteinuria (not threshold reported)
Wang [40]	Normal pregnant women	None	Not specified	Not specified	Not specified
Watanabe [41]	Healthy pregnant volunteers	Maternal age	160	110	≥ 5 g in a 24-h collection or ≥ 3+ on dipstick testing of two random urine samples collected at least 4 h apart
Zhou [42]	Healthy pregnant women	None	Not specified	Not specified	Not specified

Table 3. Extended.

Reference	Reference Cited to Define Preeclampsia	Main Exclusion Criteria	Country	Ethnicity
Bai [22]	Authors referred only to: "rigorous definition of pre-eclampsia"[a]	Not specified	China	Asian
Bouba [23]	Not specified	Preexisting cardiovascular or renal disease	Greece	European
Choi H [24]	[66]	Cardiac disease, serum creatinine $>$ 150 μmol/l or the presence of other systemic disease	Korea	Asian
Dizon-Townson [25]	Not specified[a]	Not specified	United States	European
Galao [26]	[66]	History of essential or secondary hypertension, multiparous women, diabetes mellitus, renal disease	Brazil	Hispanic (European and American Indian)
Gurdol [27]	Not specified	Preexisting hypertension	Turkey	European
Heiskanen [28]	[67]	Chronic hypertension and multiple pregnancy	Finland	European
Kaur [29]	[66]	Chronic hypertension, diabetes mellitus, hyperthyroidism, sarcoidosis, chronic renal disorders, collagen disorders	India	South Asian
Kim [30]	[66]	No chronic hypertension. No personal or family history of preeclampsia (only for controls)	Korea	Asian
Kobashi [31]	[68]	Preexisting hypertension, renal disease, diabetes mellitus, amniotic fluid abnormalities, fetal anomalies, multiple pregnancies	Japan	Asian
Levesque [32]	[69]	Preexisting secondary hypertension, preexisting hypertension with superimposed preeclampsia, multiple pregnancies	Canada	European
Mingwei [33]	Reported as standard criteria. The authors cited a Chinese textbook of obstetrics and gynecology.[a]	Not specified	China	Asian
Morgan [34]	Not specified	History of essential hypertension, diabetes mellitus, or chronic renal disease	United Kingdom	European
Mozgovaia [35]	Not specified	Not specified	Russia	European
Roberts [36]	Not specified	History of hypertension in a previous pregnancy (only for controls)	South Africa	Afro-Caribbean
Roh [37]	American College of Obstetrics and Gynecology	Not specified	Korea	Asian
Seremak-Mrozikiewicz [38]	Not specified	Multiparous women	Poland	European
Serrano et al. (present study)	[67]	See methods section, manuscript	Colombia	Mixed (European, Hispanic, Afro-Caribbean, and Native American)
Tamura [39]	Not specified	Hypertension, renal disease, diabetes, or other significant medical complications, such as heart disease	United States	Afro-Caribbean
Wang [40]	Not specified [†]	Not specified	China	Asian
Watanabe [41]	[71]	History of hypertension or renal disease	Japan	Asian
Zhou [42]	Reported as standard criteria. The authors cited a Chinese textbook of obstetrics and gynaecology	Not specified	China	Asian

Table 4. Characteristics of Published Studies of the Association between the *ACE-I/D* Polymorphism and Preeclampsia Included in the Meta-Analysis

Study	Mean Maternal Age, Participants	Mean Maternal Age, Controls	Percentage of Nulliparous Cases	Percentage of Nulliparous Controls	Gestational Age, Cases	Gestational Age, Controls	Language of Publication	Blinding	Confirmation of *DD* Genotype	Regenotyping of a Random Subsample	χ^2 Value for HWE	Evidence for Departure from HWE (*p*-Value)
Bai [22]	28	28	Not specified	Not specified	Not specified	Not specified	Non-English	Unknown	Not reported	Not reported	1.971	0.160
Bouba [23]	31	29	Not specified	0	34.32	38.96	English	Yes	Yes	Not reported	0.069	0.793
Choi [24]	30	31	69	67	36.2	39.7	English	Yes	Yes	Not reported	0.650	0.420
Dizon-Townson [25]	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	English	Unknown	Yes	Not reported	< 0.0001	0.995
Galao [26]	21	25	100	Not specified	36.2	39	English	No	Yes	Not reported	0.323	0.569
Gurdol [27]	28	27	Not specified	56	30–38	30–38	English	Yes	Yes	Not reported	2.219	0.136
Heiskanen [28]	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	English	Yes	Not reported	Not reported	0.013	0.909
Kaur [29]	25	24	100	100	35.9	35.8	English	Yes	Not reported	Not reported	0.152	0.696
Kim [30]	31	31	58.6	48.2	36.2	39	English	Yes	Not reported	Not reported	0.270	0.603
Kobashi [31]	29	29	70	57	36.4	39.1	English	Unknown	Yes	Not reported	0.140	0.708
Levesque [32]	26	26	100	100	36.7	37.8	English	Yes	Yes	Not reported	0.003	0.955
Mingwei [33]	Not specified	Not specified	100	Not specified	Not specified	Not specified	Non-English	Unknown	Not reported	Not reported	0.002	0.968
Morgan [34]	29	28	84.7	90	35.5	39.9	English	Yes	Yes	Not reported	1.433	0.231
Mozgovaia [35]	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	English	Yes	Not reported	Not reported	0.672	0.412
Roberts [36]	26	25	0	0	34.4	38.8	English	No	Yes	Not reported	1.394	0.237
Roh [37]	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	Non-English	Unknown	Not reported	Not reported	1.990	0.157
Seremak-Mrozikiewicz [38]	25.2	25.6	100	Not specified	36	38.1	English	Yes	Not reported	Not reported	2.256	0.133
Serrano et al. (present study)	19	18	100	100	37	39	English	Yes	Yes	Yes	27.140	< 0.00001
Tamura [39]	23	23	Not specified	53	Not specified	38	English	Yes	Yes	Not reported	0.625	0.429
Wang [40]	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	Non-English	Unknown	Not reported	Not reported	8.061	0.004
Watanabe [41]	Not specified	Not specified	Not specified	Not specified	Not specified	Not specified	English	No	Not reported	Not reported	0.088	0.766
Zhou [42]	Not specified	Not specified	100	100	Not specified	Not specified	Non-English	Unknown	Yes	Not reported	0.107	0.743

"Blinding" column indicates whether laboratory staff was blind or not to the case-control status. All samples were genotyped by PCR.
HWE, Hardy-Weinberg equilibrium.
doi:10.1371/journal.pmed.0030520.t004

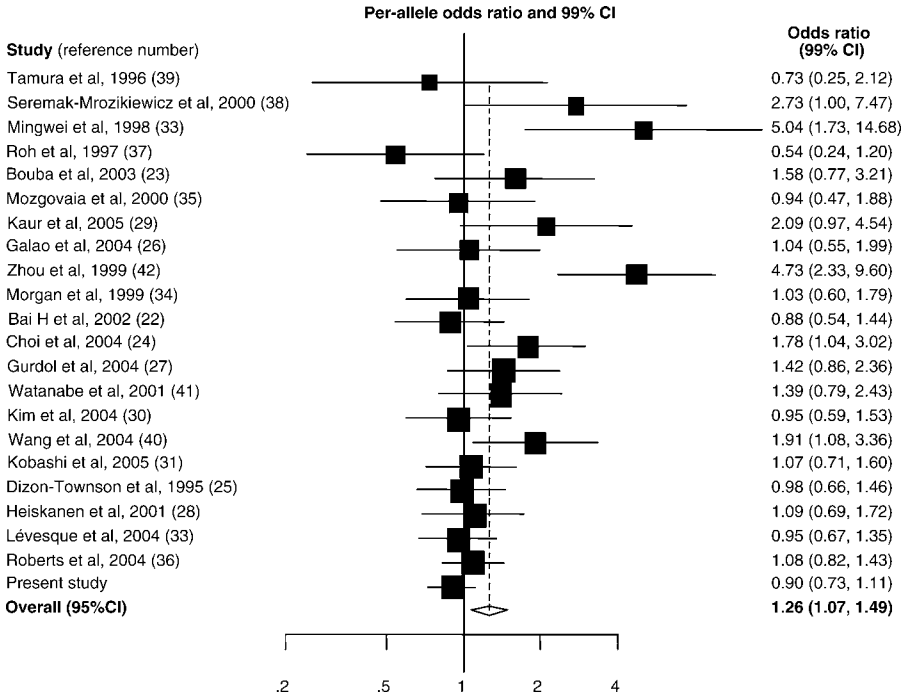

Figure 2. Meta-Analysis of Studies of *ACE-I/D* Polymorphism and Risk of Preeclampsia
doi:10.1371/journal.pmed.0030520.g002

Proteus phenomenon [51]. Further evidence in support of the presence of within-study reporting bias is the fact that studies published in languages other than English, and in the Asian ethnic group, tend to have larger effects, findings consistent with other recent results [52].

Publication bias is increasingly being recognized as one of the main threats to the reliability of conclusions drawn from association studies with common disease outcomes. In the setting of cardiovascular and neurological diseases, several positive gene–disease associations, usually based on meta-analysis of small studies, have been subsequently refuted by large genetic studies [13,53]. As a result, several initiatives are

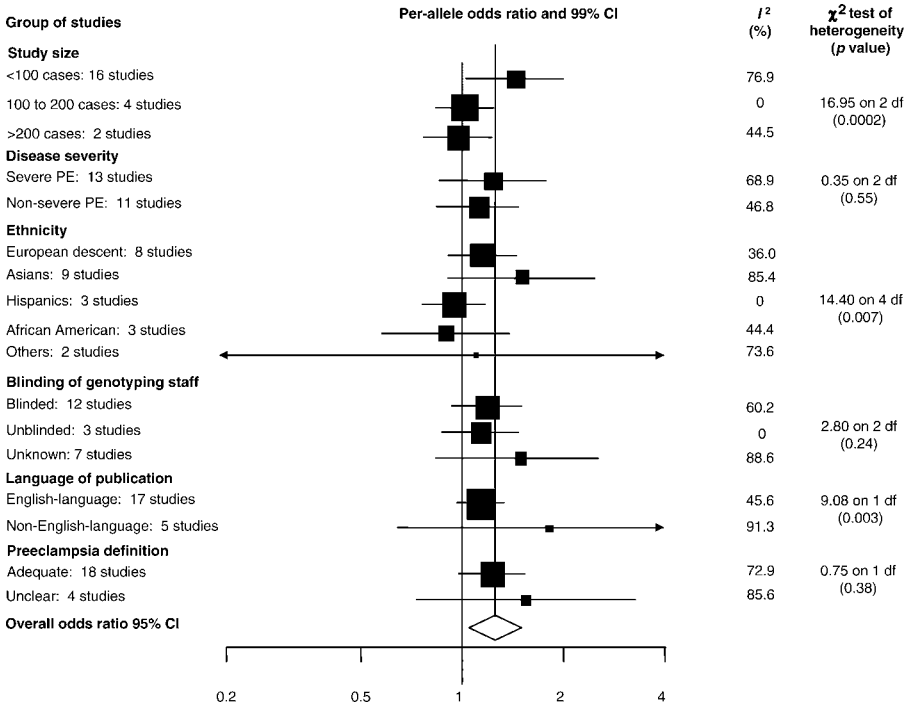

Figure 3. Studies of *ACE-I/D* Polymorphism and Risk of Preeclampsia Grouped by Study Characteristics
doi:10.1371/journal.pmed.0030520.g003

Table 5. Genotypic ORs for Preeclampsia and the *ACE-I/D* Variant.

Model	Comparison	ACE-I/D Variant
Additive model	Random, OR (95% CI), *p*-value	1.26 (1.07–1.49), $p = 0.006$
	I^2 (*p* for heterogeneity)	75.1% ($p < 0.0001$)
	Egger test, *p*-value	$p = 0.06$
Homozygous for rare allele versus homozygous for common allele	Random, OR (95% CI), *p*-value	1.51 (1.09–2.08), $p = 0.01$
	I^2 (*p* for heterogeneity)	71.8% ($p < 0.0001$)
	Egger test, *p*-value	$p = 0.02$
Heterozygous versus homozygous for common allele	Random, OR (95% CI), *p*-value	0.94 (0.78–1.12), $p = 0.50$
	I^2 (*p* for heterogeneity)	31.5% ($p = 0.07$)
	Egger test, *p*-value	$p = 0.76$
Recessive model	Random, OR (95% CI), *p*-value	1.59 (1.22–2.07), $p = 0.001$
	I^2 (*p* for heterogeneity)	74.5% ($p < 0.0001$)
	Egger test, *p*-value	$p = 0.008$
Dominant model	Random, OR (95% CI), *p*-value	1.15 (0.91–1.45), $p = 0.21$
	I^2 (*p* for heterogeneity)	63.1% ($p < 0.0001$)
	Egger test, *p*-value	$p = 0.09$

doi:10.1371/journal.pmed.0030520.t005

now underway to help overcome problems of reporting and publication bias and to help to achieve datasets of appropriate size to detect plausible genetic effects for common disorders, which are likely to require several thousands of cases of the disease (The Wellcome Trust Case Control Consortium, http://www.wtccc.org.uk) [54]. Genetic studies in preeclampsia continue to be somewhat small in size [55,56] and are usually underpowered to detect realistic genotypic relative risks (ORs between 1.15 and 1.4) [14,57]. However, considering the low incidence of preeclampsia (2%–3% in developed countries), it is highly unlikely that a single centre will be able to amass the large number of cases required, and the development of networks of interested investigators may be essential [54]. Therefore, international collaborations, particularly among those countries with a high incidence of preeclampsia, may make recruitment more efficient and help to include participants with different cultural and genetic backgrounds, which can provide further insight into the aetiology of the disease both genetic and/or environmental.

Despite these obstacles, the investment in adequate resources to study the genetics of preeclampsia is an important priority. Observational studies and the randomised trials of interventions that have followed have been unsuccessful thus far in identifying causal pathways in preeclampsia amenable to preventive therapies, a clear example of which are the recently failed clinical trials using either antioxidant vitamins or calcium supplements [58–61]. A genetic approach that is less prone to confounding and reverse causation than nongenetic observational studies, may be more likely to identify causal pathways and may help to prioritise therapeutic targets that require evaluation in large and expensive randomised clinical trials [62,63]. The challenge is in how to make better use of the genetic approach in complex diseases such as preeclampsia, in particular to overcome random errors in risk estimates from small studies as well as publication bias. A suggested approach is to establish a collaborating consortium of investigators from existing studies in genetics of preeclampsia to reduce the multiple existing problems such as: (1) inadequate selection of candidate gene variants to be evaluated, (2) biased analyses, and selective reporting of positive results; (3) to promote access to unpublished data; (4) to overcome inadequate outcome definitions; and (5) to provide guidance for developing new large studies [54]. Until such measures are established, it will be important for both authors and journal editors to embrace the publication of both positive and negative results from "well-designed case-control" studies to diminish the problem of publication bias [64]. This approach has recently become a reality for clinical trials [65], and it might help in reducing the temptation of researchers to explore multiple hypotheses in subgroup analyses to obtain one finding of nominal statistical significance that might help acceptance of the paper.

Investigating the aetiology of preeclampsia, one of the main causes of maternal and neonatal mortality and morbidity worldwide, should be a health research priority. A genetic approach may indeed be useful, but large collaborative studies will also be needed.

Supporting Information

Figure S1. Funnel Plot of Studies of *ACE-I/D* Polymorphism and Preeclampsia

ORs for outcome using a per-allele model. Studies in bold are those published in non-English-language journals.

Found at doi:10.1371/journal.pmed.0030520.sg001 (38 KB PPT).

Figure S2. Cumulative Synthesis of Studies of *ACE-I/D* Polymorphism and Preeclampsia

OR (random effect model) for outcome using a per-allele model.

Found at doi:10.1371/journal.pmed.0030520.sg002 (36 KB PPT).

Protocol S1. *ACE-I/D* Polymorphism and Preeclampsia Risk: Evidence of Small-Study Bias

Found at doi:10.1371/journal.pmed.0030520.sd001 (68 KB DOC).

Alternative Language Abstract S1. Translation of the Abstract into Spanish

Translation by N. C. Serrano, Universidad Autónoma de Bucaramanga, Bucaramanga, Colombia.

Found at doi:10.1371/journal.pmed.0030520.sd002 (25 KB DOC).

Alternative Language Abstract S2. Translation of the Abstract into Chinese

Translation by D. Wang, Medical Statistics Unit, London School of Hygiene and Tropical Medicine, London, United Kingdom.

Found at doi:10.1371/journal.pmed.0030520.sd003 (33 KB DOC).

Accession Numbers

The SNP database (http://www.ncbi.nlm.nih.gov/SNP/) single-nucleotide polymorphism discussed in this paper is an insertion/deletion variant (rs1799752) referenced to the *ACE* gene with the GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) accession number J04144.

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Author contributions. NCS, LAD, MCP, RC, AM, LS, ADH, and JPC designed the study. NCS, LAD, MCP, CMM, AM, AG, LS, and JPC analyzed the data. NCS, MCP, CMM, RC, AM, AG, and LS enrolled patients. NCS, LAD, MCP, CMM, AM, AG, LS, ADH, and JPC contributed to writing the paper. MCP genotyped the insertion/deletion within the angiotensin-converting enzyme gene and collected the data for the meta-analysis.

References

1. World Health Organization International Collaborative Study of Hypertensive Disorders of Pregnancy (1988) Geographic variation in the incidence of hypertension in pregnancy. Am J Obstet Gynecol 158: 80–83.
2. Khan KS, Wojdyla D, Say L, Gulmezoglu AM, Van Look PF (2006) WHO analysis of causes of maternal death: A systematic review. Lancet 367: 1066–1074.
3. Sibai B, Dekker G, Kupferminc M (2005) Pre-eclampsia. Lancet 365: 785–799.
4. Villar J, Abalos E, Nardin JM, Merialdi M, Carroli G (2004) Strategies to prevent and treat preeclampsia: Evidence from randomized controlled trials. Semin Nephrol 24: 607–615.
5. Duckitt K, Harrington D (2005) Risk factors for pre-eclampsia at antenatal booking: Systematic review of controlled studies. BMJ 330: 565–567.
6. Ray JG, Vermeulen MJ, Schull MJ, Redelmeier DA (2005) Cardiovascular health after maternal placental syndromes (CHAMPS): Population-based retrospective cohort study. Lancet 366: 1797–1803.
7. Zaman MA, Oparil S, Calhoun DA (2002) Drugs targeting the renin-angiotensin-aldosterone system. Nat Rev Drug Discov 1: 621–636.
8. Shah DM (2006) The role of RAS in the pathogenesis of preeclampsia. Curr Hypertens Rep 8: 144–152.
9. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, et al. (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 86: 1343–1346.
10. Agerholm-Larsen B, Nordestgaard BG, Tybjaerg-Hansen A (2000) ACE gene polymorphism in cardiovascular disease: Meta-analyses of small and large studies in whites. Arterioscler Thromb Vasc Biol 20: 484–492.
11. Kammerer CM, Gouin N, Samollow PB, VandeBerg JF, Hixson JE, et al. (2004) Two quantitative trait loci affect ACE activities in Mexican-Americans. Hypertension 43: 466–470.
12. Sayed-Tabatabaei FA, Houwing-Duistermaat JJ, van Duijn CM, Witteman JC (2003) Angiotensin-converting enzyme gene polymorphism and carotid artery wall thickness: A meta-analysis. Stroke 34: 1634–1639.
13. Keavney B, McKenzie C, Parish S, Palmer A, Clark S, et al. (2000) Large-scale test of hypothesised associations between the angiotensin-converting-enzyme insertion/deletion polymorphism and myocardial infarction in about 5,000 cases and 6,000 controls. International Studies of Infarct Survival (ISIS) Collaborators. Lancet 355: 434–442.
14. Casas JP, Hingorani AD, Bautista LE, Sharma P (2004) Meta-analysis of genetic studies in ischemic stroke: Thirty-two genes involving approximately 18,000 cases and 58,000 controls. Arch Neurol 61: 1652–1661.
15. Bonnici F, Keavney B, Collins R, Danesh J (2002) Angiotensin converting enzyme insertion or deletion polymorphism and coronary restenosis: Meta-analysis of 16 studies. BMJ 325: 517–520.
16. Ng DP, Tai BC, Koh D, Tan KW, Chia KS (2005) Angiotensin-I converting enzyme insertion/deletion polymorphism and its association with diabetic nephropathy: A meta-analysis of studies reported between 1994 and 2004 and comprising 14,727 subjects. Diabetologia 48: 1008–1016.
17. Mello G, Parretti E, Gensini F, Sticchi E, Mecacci F, et al. (2003) Maternal-fetal flow, negative events, and preeclampsia: Role of ACE I/D polymorphism. Hypertension 41: 932–937.
18. Report of the National High Blood Pressure Education Program (2000) Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 183: S1–S22.
19. Shanmugam V, Sell KW, Saha BK (1993) Mistyping ACE heterozygotes. PCR Methods Appl 3: 120–121.
20. Little J, Higgins JPT editors. The HuGENet™ HuGE Review Handbook, version 1.0. Available: http://www.hugenet.ca. Accessed: 28 February 2006.
21. Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L (2005) Performance of tests and adjustments for publication bias in the presence of heterogeneity: Technical report 05–01. Leicester, England: Department of Health Sciences, University of Leicester. Report Number 05–01. pp. 1–31.
22. Bai H, Liu X, Liu R, Liu Y, Li M, et al. (2002) Angiotensinogen and angiotensin-I converting enzyme gene variations in Chinese pregnancy induced hypertension. Hua Xi Yi Ke Da Xue Xue Bao 33: 233–237.
23. Bouba I, Makrydimas G, Kalaitzidis R, Lolis DE, Siamopoulos KC, et al. (2003) Interaction between the polymorphisms of the renin-angiotensin system in preeclampsia. Eur J Obstet Gynecol Reprod Biol 110: 8–11.
24. Choi H, Kang JY, Yoon HS, Han SS, Whang CS, et al. (2004) Association of Angiotensin-converting enzyme and angiotensinogen gene polymorphisms with preeclampsia. J Korean Med Sci 19: 253–257.
25. Dizon-Townson D, Lompe I, Hastings S, Nelson LM, Varner M, et al. (1995) A common genetic variant of the angiotensin converting enzyme is associated with both preeclampsia and chronic hypertension [abstract]. Am J Obstet Gynecol 172: 374.
26. Galao AO, de Souza LH, da Costa BE, Scheibe RM, de Figueiredo CE (2004) Angiotensin-converting enzyme gene polymorphism in preeclampsia and normal pregnancy. Am J Obstet Gynecol 191: 821–824.
27. Gurdol F, Isbilen E, Yilmaz H, Isbir T, Dirican A (2004) The association between preeclampsia and angiotensin-converting enzyme insertion/deletion polymorphism. Clin Chim Acta 341: 127–131.
28. Heiskanen JT, Pirskanen MM, Hiltunen MJ, Mannermaa AJ, Punnonen KR, et al. (2001) Insertion-deletion polymorphism in the gene for angiotensin-converting enzyme is associated with obstetric cholestasis but not with preeclampsia. Am J Obstet Gynecol 185: 600–603.
29. Kaur R, Jain V, Khuller M, Gupta I, Sherawat BS (2005) Association of angiotensin-converting enzyme gene polymorphism with pregnancy-induced hypertension. Acta Obstet Gynecol Scand 84: 929–933.
30. Kim YJ, Park MH, Park HS, Lee KS, Ha EH, et al. (2004) Associations of polymorphisms of the angiotensinogen M235 polymorphism and angiotensin-converting-enzyme intron 16 insertion/deletion polymorphism with preeclampsia in Korean women. Eur J Obstet Gynecol Reprod Biol 116: 48–53.
31. Kobashi G, Hata A, Shido K, Ohta K, Yamada H, et al. (2005) Insertion/deletion polymorphism of the angiotensin-converting enzyme gene and preeclampsia in Japanese patients. Semin Thromb Hemost 31: 346–350.
32. Levesque S, Moutquin JM, Lindsay C, Roy MC, Rousseau F (2004) Implication of an AGT haplotype in a multigene association study with pregnancy hypertension. Hypertension 43: 71–78.
33. Mingwei Z, Yamping Z, Wehwei Ch (1998) Study on a deletion polymorphism of the angiotensin converting enzyme gene in pregnancy induced hypertension. Chin J Obs Gynecol 33: 83–85.
34. Morgan L, Foster F, Hayman R, Crawshaw S, Baker PN, et al. (1999) Angiotensin-converting enzyme insertion-deletion polymorphism in normotensive and pre-eclamptic pregnancies. J Hypertens 17: 765–768.
35. Mozgovaia EV, Malysheva OV, Ivashchenko TE, Branov VS (2002) Genetic predisposition to pre-eclampsia: Polymorphism of genes involved in regulation of endothelial functions. Balkan J Med Genetics 5: 19–26.
36. Roberts CB, Rom L, Moodley J, Pegoraro RJ (2004) Hypertension-related gene polymorphisms in pre-eclampsia, eclampsia and gestational hypertension in Black South African women. J Hypertens 22: 945–948.
37. Roh CR, Kim DK, Yoon BK, Yang SH, Chung JH, et al. (1997) A common genetic variant of the angiotensin converting enzyme (ACE) gene and pregnancy induced hypertensive disorders. Korean J Obstet Gynecol 40: 1189–1199.
38. Seremak-Mrozikiewicz A, Drews K, Malewski Z, Slomko Z, Mrozikiewicz A (2000) Polymorphic genotypes of the angiotensin-converting enzyme in severe pregnancy-induced hypertension. Arch Perinat Med 6: 22–24.
39. Tamura T, Johanning GL, Goldenberg RL, Johnston KE, DuBard MB (1996) Effect of angiotensin-converting enzyme gene polymorphism on pregnancy outcome, enzyme activity, and zinc concentration. Obstet Gynecol 88: 497–502.
40. Wang HY, Li CM, Wang Z, Yang F (2004) Relationships between polymorphisms of angiotensin-converting enzyme and methylenetetrahydrofolate reductase genes and genetic susceptibility to pregnancy-induced hypertension. Zhonghua Fu Chan Ke Za Zhi 39: 369–372.
41. Watanabe H, Hamada H, Yamakawa-Kobayashi K, Yoshikawa H, Arinami T (2001) Evidence for an association of the R485K polymorphism in the coagulation factor V gene with severe preeclampsia from screening 35 polymorphisms in 27 candidate genes. Thromb Haemost 86: 1594–1595.
42. Zhou N, Yu P, Chen J, Huang H, Jiang S (1999) Detection of insertion/deletion polymorphism of angiotensin converting enzyme gene in preeclampsia. Chin J Med Genet 16: 29–31.
43. Kim YJ, Pang MG, Park MY, Park MH, Kim YW, et al. (2001) A study on the association between angiotensinogen gene and angiotensin-converting-enzyme gene and pregnancy-induced hypertension in Korean women. Korean J Obstet Gynecol 44: 1072–1077.
44. Seremak-Mrozikiewicz A, Drews K, Chmara E, Mrozikiewicz A (2001) Polymorphism of gene angiotensin converting enzyme in pregnancy induced hypertension. Ginekol Pol 72: 605–610.
45. Nalogowska-Glosnicka K, Lacka BI, Zychma MJ, Grzeszczak W, Zukowska-Szczechowska E, et al.PIH Study Group (2000) Angiotensin II type 1 receptor gene A1166C polymorphism is associated with the increased risk of pregnancy-induced hypertension. Med Sci Monit 6: 523–529.

46. Nalogowska-Glosnicka K, Lacka B, Zychma M, Grzeszczak W, Michalski B, et al. (1998) Lack of relationship between angiotensinogen gene m235t polymorphism and gene insertion/deletion (I/D-intron 16) and Pst I RFLP (P/M-intron 7) polymorphisms of the angiotensin I converting enzyme (ACE) gene and the development of H-gestosis. Preliminary results. Pol Arch Med Wewn 100: 19–26.
47. Zhu M, Xia Y, Cheng W (1998) Study on a deletion polymorphism of the angiotensin converting enzyme gene in pregnancy induced hypertension. Zhonghua Fu Chan Ke Za Zhi 33: 83–85.
48. Huang Y, Liao B, Sun X (2001) Study on the relation between the angiotensin converting enzyme gene and pregnancy induced hypertension. Zhonghua Fu Chan Ke Za Zhi 36: 15–17.
49. Mello G, Parretti E, Fatini C, Riviello C, Gensini F, et al. (2005) Low-molecular-weight heparin lowers the recurrence rate of preeclampsia and restores the physiological vascular changes in angiotensin-converting enzyme DD women. Hypertension 45: 86–91.
50. Ioannidis JP, Trikalinos TA, Ntzani EE, Contopoulos-Ioannidis DG (2003) Genetic associations in large versus small studies: An empirical assessment. Lancet 361: 567–571.
51. Ioannidis JP, Trikalinos TA (2005). Early extreme contradictory estimates may appear in published research: The Proteus phenomenon in molecular genetics research and randomized trials. J Clin Epidemiol 58: 543–549.
52. Pan Z, Trikalinos TA, Kavvoura FK, Lau J, Ioannidis JP (2005). Local literature bias in genetic epidemiology: An empirical evaluation of the Chinese literature. PLoS Med 2: e334. doi:10.1371/journal.pmed.0020334
53. Healy DG, Abou-Sleiman PM, Casas JP, Ahmadi KR, Lynch T, et al. (2006) UCHL-1 is not a Parkinson's disease susceptibility gene. Ann Neurol 59: 627–633.
54. Ioannidis JP, Gwinn M, Little J, Higgins JP, Bernstein JL, et al. Human Genome Epidemiology Network and the Network of Investigator Networks (2006) A road map for efficient and reliable human genome epidemiology. Nat Genet 38: 3–5.
55. Yu CK, Casas JP, Savvidou MD, Sahemey MK, Nicolaides KH, et al. (2006) Endothelial nitric oxide synthase gene polymorphism (Glu298Asp) and development of pre-eclampsia: A case-control study and a meta-analysis. BMC Pregnancy Childbirth 6: 7.
56. Lin J, August P (2005) Genetic thrombophilias and preeclampsia: A meta-analysis. Obstet Gynecol 105: 182–192.
57. Cardon LR, Bell JI (2001) Association study designs for complex diseases. Nat Rev Genet 2: 91–99.
58. Rumbold AR, Crowther CA, Haslam RR, Dekker GA, Robinson JS, ACTS Study Group (2006) Vitamins C and E and the risks of preeclampsia and perinatal complications. N Engl J Med 354: 1796–1806.
59. Poston L, Briley AL, Seed PT, Kelly FJ, Shennan AHVitamins in Pre-eclampsia (VIP) Trial Consortium (2006) Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): Randomised placebo-controlled trial. Lancet 367: 1145–1154.
60. Villar J, Abdel-Aleem H, Merialdi M, Mathai M, Ali MM, et al. World Health Organization Calcium Supplementation for the Prevention of Preeclampsia Trial Group (2006) World Health Organization randomized trial of calcium supplementation among low calcium intake pregnant women. Am J Obstet Gynecol 194: 639–649.
61. Levine RJ, Hauth JC, Curet LB, Sibai BM, Catalano PM, et al. (1997) Trial of calcium to prevent preeclampsia. N Engl J Med 337: 69–76.
62. Hingorani AD, Shah T, Casas JP (2006) Linking observational and genetic approaches to determine the role of C-reactive protein in heart disease risk. Eur Heart J 27: 1261–1263.
63. Hingorani A, Humphries S (2005) Nature's randomised trials. Lancet 366: 1906–1908.
64. Ioannidis JP (2006) Journals should publish all "null" results and should sparingly publish "positive" results. Cancer Epidemiol Biomarkers Prev 15: 186.
65. Veitch E (2006) Introducing PLoS Clin trials 1: e8. doi:0.1371/journal.pctr.0010008
66. (2000) Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 183: S1–S22.
67. Brown MA, de Swiet M (1999) Classification of hypertension in pregnancy. Baillieres Best Pract Res Clin Obstet Gynaecol 13: 27–39.
68. National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy (1990) Am J Obstet Gynecol 163: 1691–1712.
69. Helewa ME, Burrows RF, Smith J, Williams K, Brain P, et al. (1997) Report of the Canadian Hypertension Society Consensus Conference: 1. Definitions, evaluation and classification of hypertensive disorders in pregnancy. Can Med Assoc J 157: 715–725.
70. Committee on Technical Bulletins of the American College of Obstetricians and Gynecologists (1996) ACOG technical bulletin. Hypertension in pregnancy. Number 219—January 1996 (replaces number 91, February 1986). Int J Gynaecol Obstet 53: 175–183.

Editors' Summary

Background. Preeclampsia is a common condition affecting pregnant women worldwide; it is defined as the presence of increased blood pressure, together with protein in the urine. Although in many women preeclampsia may never result in symptoms, other women may experience headaches, problems with their vision, swollen ankles and feet, and other problems. Sometimes, preeclampsia progresses to eclampsia, in which potentially life-threatening seizures result. The causes of preeclampsia are not well understood, but several factors are known to contribute to the risk. These factors include diabetes, high blood pressure prior to pregnancy, obesity, and first pregnancy. There is also the possibility that preeclampsia has, at least in part, a genetic basis; the condition is more likely among women whose relatives have also had it. However, no definite genetic cause has yet been confirmed.

Why Was This Study Done? A common variant in one particular gene, *ACE,* which codes for the angiotensin-1 converting enzyme, has been linked with preeclampsia in a number of different studies. The protein encoded by *ACE* is involved in controlling blood pressure and the balance of fluid and salts in the blood. However, many of the studies supposedly linking *ACE* and preeclampsia were done on very few participants. Small studies are more likely to generate "false positive" findings. Therefore, a group of investigators from Colombia and the UK wanted to find out whether they could reproduce the supposed link between the *ACE* gene variant and preeclampsia in a large study, and also to see whether the previous studies could have been "false positives."

What Did the Researchers Do and Find? These investigators carried out a case-control study. This means that women with preeclampsia ("cases") were recruited, and compared with women similar in all other respects but who did not have preeclampsia ("controls"). In total 1,711 pregnant women from five Colombian cities were studied, of whom 665 had preeclampsia and 1,046 did not. Blood was taken from each participant and used for DNA sequencing of the *ACE* gene. The investigators then did a statistical comparison to see whether there was any association between preeclampsia and possession of a particular variant of the *ACE* gene. The results showed that there was no such association. Then, the investigators did a literature search to find all previous studies that had examined a possible link between variants of the *ACE* gene and preeclampsia. They found 22 studies reporting data obtained from 6,424 women (these figures include the results from the investigators' own case-control study described here). The data from all of these studies were then put together into a combined analysis. This combined analysis did suggest a small increase in the risk of preeclampsia in women with one particular variant in the *ACE* gene. However, this result was more likely in studies with small numbers of participants. Furthermore, the earliest studies done were most likely to show an effect, with the supposed link disappearing as more and more data were collected.

What Do These Findings Mean? The findings presented here suggest that "small study bias" may explain the discrepancy between the results of the case-control study and the combined analysis. That is, studies involving few participants are less reliable and more likely to produce false-positive results. Therefore, it is possible that the proposed link between *ACE* gene variants and preeclampsia is a spurious one. The investigators propose that in future, collaborative research networks will be needed to carry out rigorous research on the genetics of preeclampsia. Such initiatives will help to overcome the problem of bias that can arise from small studies.

Additional Information. Please access these Web sites via the online version of this summary at http://dx.doi.org/10.1371/journal.pmed.0030520.

- Information for patients from NHS Direct (UK National Health Service) about preeclampsia
- Medical encyclopedia entry on preeclampsia from MedLine Plus, supplied by the US National Library of Medicine
- Information from the World Health Organization and Pan American Health Organization on maternal health in the Americas