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Maternal and Fetal Variants in the TGF-beta3 Gene and Risk of Pregnancy-Induced Hypertension in a Predominantly Latino Population

Melissa L. WILSON, Ph.D., **Daniel H. DESMOND, B.S.**, **T. Murphy GOODWIN, M.D.**, **David A. MILLER, M.D.**, and **Sue Ann INGLES, Dr.P.H.**
Los Angles, California University of Southern California Keck School of Medicine Department of Obstetrics and Gynecology Division of Maternal-Fetal Medicine Department of Preventive Medicine

Abstract

Objective—To determine if polymorphisms in the Transforming Growth Factor Beta-3 (TGF-β3) gene are associated with risk of pregnancy-induced hypertension (PIH) in case-control mother-baby dyads.

Study Design—Cases (N=136) and controls (N=169) were recruited from the Los Angeles County + University of Southern California Women's and Children's Hospital. We genotyped four TGF-β3 polymorphisms and examined association with PIH using logistic regression, adjusting for parity, maternal age, gestational age at delivery, fetal (or maternal) genotypes for the polymorphism in question, and for the three other polymorphisms within the TGF-β3 gene.

Results—Only one of the TGF-β3 polymorphisms (rs11466414) was associated with PIH. Mothers who carried a baby with a minor allele were at decreased risk ($OR_{multi\text{-}locus\ adj}$= 0.32, 95% CI: 0.14, 0.77). Maternal TGF-β3 variants had no effect on risk of PIH.

Conclusion—A fetal TGF-beta3 polymorphism (rs11466414) is associated with pregnancy-induced hypertension in a predominantly Hispanic population.

Keywords

Genetics; Polymorphism; Preeclampsia; Pregnancy-induced hypertension; Transforming Growth Factor Beta-3 (TGF-β3

INTRODUCTION

Failure to form an optimally vascularized and adequately invaded placenta may result in a range of pathologies, including recurrent miscarriage and pregnancy-induced hypertension (PIH) [1,2]. Many aspects of placental development are believed to be genetically determined and therefore genes responsible for placental development are obvious candidates for

Correspondence should be directed to Melissa Wilson Norris Topping Tower #6419 1441 Eastlake Ave. Los Angeles, CA 90033 email: melisslw@usc.edu Phone: 323-865-0472 Fax: 323-865-0473 Home Phone: 310-204-0865.

CONDENSATION: Polymorphisms in the Transforming Growth Factor β-3 gene were examined in case-control mother-baby dyads to determine if they are associated with pregnancy-induced hypertension.

susceptibility to conditions associated with placental dysfunction, such as preeclampsia [2-4]. We hypothesize that genes involved in early placental development and, in particular, trophoblast invasion, will be important regulators of placental invasion and that genetic variations in such genes may play a role in predisposing to the development of PIH.

Adequate placentation requires tight regulation of oxygen (O_2) levels in the developing placenta [5]. Early placentation (trophoblast proliferation) occurs in an environment that is low in O_2. An increase in O_2 and subsequent down-regulation of the Transforming Growth Factor Beta-3 (TGF-β3) gene is needed for trophoblast differentiation to the invasive phenotype [6, 7], and failure to downregulate results in shallow placental invasion [8-10]. Moreover, TGF-β3 has been shown to be overexpressed in villous explants from women with preeclampsia [11] and placental homogenates from women with HELLP syndrome [12] and inhibition of TGF-β3 in placental explants can restore the invasive capacity of trophoblasts [8].

Based on the evidence that TGF-β3 is a crucial regulator of placental development, we examined polymorphic variants in the TGF-β3 gene as potential contributors to PIH susceptibility. We examined TGF-β3 genotypes in women with PIH and women with unaffected pregnancies, and in the offspring of these women.

MATERIALS AND METHODS

Subjects

Cases of clinically-defined preeclampsia (n = 136) and controls (n = 169) were recruited retrospectively from delivery logs at the Los Angeles County (LAC) + University of Southern California (USC) Women's and Children's Hospital (WCH) from 1999-2006 (103 subjects) and during their postpartum hospital stay at the WCH from 2007-2008 (202 subjects). While not matched in the usual sense, care was taken to ensure that the gestational age of each control selected was at least as great as that of a case already in the study to ensure that the controls had as much opportunity to become a case as the cases themselves had.

DNA was collected from both the mothers via blood (n=39), mouthwash (n=27), buccal swabs (n=13) or saliva (n=214) (Oragene, DNA Genotek) and their infants via buccal swabs (n=92) or saliva (n=204) (Oragene, DNA Genotek). There were no differences in genotyping success rates by method of DNA sampling. Information on known and suspected risk factors was obtained by questionnaire, modified for the Hispanic community from a questionnaire used by the University of Pittsburgh (R. Ness, personal communication) and administered by a trained interviewer.

Charts were abstracted to verify case diagnosis and to verify the absence of significant hypertension among controls. Preeclampsia (PE) was defined as blood pressure >=140 (systolic) or >= 90 (diastolic) on two or more occasions at least six hours apart plus proteinuria >= 300 mg/dL in a 24-hour urine collection or +1 on a dipstick in women who were normotensive in early pregnancy (less than 20 weeks gestation). Severe PE was defined as >= 160 (systolic) or >= 110 (diastolic) on two or more occasions at least six hours apart plus proteinuria >=500 mg/dL in a 24-hour urine collection or +3 on a dipstick. Gestational hypertension was defined as elevated blood pressure (as described above) without evidence of proteinuria. Women with lupus, chronic renal disease, multiple gestations, or sickle cell disease/trait were excluded.

This study was approved by the University of Southern California Health Sciences Campus Institutional Review Board. All participants signed an informed consent for both herself and her infant and, for women under the age of 18 at the time of recruitment (n=14), parental permission for participation was also obtained.

Laboratory Methods

Buccal swabs and blood were extracted using QIAamp DNA Mini kits per manufacturer's protocol (Qiagen, Valencia, CA), mouthwash specimens were extracted using a phenyl-chlorform protocol [13], and saliva samples were extracted using ethanol precipitation per manufacturer's protocol (DNA Genotek, Ottawa, Ontario, Canada).

Four single nucleotide polymorphisms (SNPs) in the TGF-β3 gene (rs3917200, rs11466414, rs2268624, and rs2205181) were selected on the basis of likely functionality and were genotyped using TaqMan assays (7900HT Sequence Detection System, Applied Biosystems, Foster City, CA). SNPs were considered to have a high probability of functionality if they met one or more of the following criteria: 1). were known to be functional on the basis of functional data, 2). were found to be associated with any disease in a published study, 3). were located in a regulatory region of the gene, 4). were located in a coding region of the gene and resulted in a nonsynonymous amino acid change, or 5). were in an evolutionarily conserved region of the gene.

Each SNP was amplified in a 5 μl total reaction volume containing 2.5 μl of Taqman Universal Master Mix, 0.125 μl of 40x Assay mix and 2.375 μl of genomic DNA diluted with dH_2O. Thermocycler conditions were as follows (rs3917200 and rs2268624):one 10 min hold at 95 degrees C, and 50 cycles containing a 15 second denaturing cycle at 92 degrees C, followed by a 1 min annealing/extension cycle at 60 degrees C. For rs11466414 and rs2205181, the thermocycler conditions were as before, except that the denaturing cycle was at 95 degrees C.

Statistical Analysis—Mother/baby dyads were included in the analysis if all four genotypes were available for both mother and baby. Three mothers and six babies had missing data for at least one SNP, resulting in a final sample size of 296 dyads (129 cases and 167 controls).

Study subject characteristics were compared using either Pearson's or Fisher's chisquared test (categorical variables) and Student's t-test (continuous variable). A two sided p-value of < 0.05 was considered statistically significant and no adjustments were made for multiple comparisons.

Logistic regression was used to model the association between genotypes and PIH risk, adjusting for maternal age (<29 vs. ≥29 years), gestational age at delivery (<37, 37, 38, 39, ≥40 weeks), parity (nulliparous vs. parous), and fetal (or maternal) genotypes at the same loci. The maternal age cutpoint represents the point at which increased age became modestly associated with PIH risk ($OR_{\geq 29 \text{ vs. } <29} = 1.6$, p=0.11). For all SNPs except rs2205181 the homozygous genotype was sufficiently rare (≤5%), so that the corresponding odds ratio could not be precisely estimated, thus we collapsed the rare homozygotes with the heterozygotes Thus the models presented in Table 3 represent the co-dominant (most general) model for rs2205181 and the dominant model for all other loci. Other genetic models are not shown since model fit was not improved by imposing additional constraints (dominance or recessivity) for rs2205181 nor by imposing additivity for any of the loci. All models were further adjusted for maternal and fetal genotypes at all other loci (multi-locus adjustment).

To test for heterogeneity by disease severity, we fit multinomial logistic models with three outcome categories (control, gestational hypertension, PE), constraining the coefficients for the adjustment variables (age, gestational age, parity) to be constant across severity strata. Likelihood ratio tests were performed to compare models with genetic effects constrained to be equal across strata vs. models with unconstrained genetic effects. All statistical analyses were conducted using Stata SE 10.0 (Statacorp LP, College Station, TX).

RESULTS

Of the cases, 65 met the criteria for mild PE, 24 were classified as severe PE and 40 were considered gestational hypertensives, according to the standard definition [14]. Among the gestational hypertensives, 30 (75%) had either abnormal laboratory values suggestive of more extensive disease (elevated liver enzymes, uric acid, or lactose dehydrogenase, or decreased platelets) (n=16), symptoms of preeclampsia (headache, epigastric pain, right upper quadrant pain, or visual disturbances) (n=16), and/or had a history of PIH in a previous pregnancy (having been normotensive in between pregnancies, providing evidence of recurrent gestational hypertension) (n=8). Among the preeclamptics, 3 (2%) had superimposed preeclampsia, 4 (3%) had eclampsia and 5 (4%) had HELLP or partial HELLP.

Ninety-seven percent of cases and controls were Hispanic, as determined by birthplace, maternal and paternal birthplace, and the birthplace of maternal and paternal grandparents. Cases and controls did not significantly differ with respect to race/ethnicity, maternal age, gravidity, or rates of preexisting or comorbid conditions (Tables 2 and 3). However, cases were statistically significantly more likely than controls to deliver preterm (31% vs. 9%), to be nulliparous (45% vs. 31%), and to have a history of PIH (12% vs. 4%). There was a somewhat increased rate of small for gestational age infants among the cases (12% vs. 6%) compared to controls but this difference did not reach statistical significance.

There were no inconsistencies between maternal and fetal genotypes (no “non-maternity”), and all four SNPs were in Hardy-Weinberg Equilibrium among the controls. Of the four polymorphisms studied, one was statistically significantly associated with PIH (Table 3). Specifically, cases were more likely than controls to carry a fetus with at least one copy of the minor allele for rs11466414 (23% vs. 16%, respectively). The odds of developing PIH were one-third lower among women carrying a fetus with either one or two copies of the T allele compared to those with no T allele ($OR_{\text{multi-locus adj}}$= 0.32, 95% CI: 0.14, 0.77). There was no effect when the mothers themselves carried the T allele for this polymorphism. None of the other polymorphisms were associated with PIH, either in the mothers or the offspring.

When cases were stratified by disease severity (gestational hypertension vs. PE), there was no evidence for heterogeneity in the association between genotype and disease status (p=0.26, 0.24, 0.70, and 0.65 for SNPs rs11466414, rs2205181, rs2268624, and rs3917200, respectively).

Additional analyses were conducted to determine if the results were unduly influenced by co-morbid conditions that might mimic or affect the risk of PIH independent of placental risk factors, disease severity or recruitment method. The results did not change after excluding women with a history of PIH, seizure disorder, thyroid disease, chronic hypertension, or who are HIV positive. While it would be interesting to evaluate a subgroup of women who delivered preterm or who delivered a small-for-gestationalage infant, the numbers of women in these categories were too small to allow a meaningful statistical analysis. Instead, we performed an analysis excluding these women to determine if their exclusion altered the results, which it did not. In this way, we were able to assess, to some degree, if women in these subcategories were substantially different from the remaining population. Last, excluding women with gestational hypertension had no effect on the results, and there were no differences between women who were recruited retrospectively as compared to women who were recruited at the time of delivery.

COMMENT

To our knowledge, this is the first reported study of TGF-β3 genetic variants and PIH. The finding of a decreased risk of PIH when the offspring, but not the mother, carries one or two

copies of the T allele (rs11466414) underscores the importance of fetal genetics in the developing placenta. Fetal genetic variants can currently be evaluated prenatally via amniocentesis, chorionic villi sampling or, in the near future, via DNA or RNA isolated from maternal blood samples.

Since little is known about the functional effects of polymorphism in the TGF-β3 gene, candidate SNPs were chosen based on their location within the gene, as described previously. The rs11466414 polymorphism is located in the upstream regulatory region of the TGF-β3 gene, which overlaps with another gene, hypothetical protein LOC112752 (MGC16028). The effect of this polymorphism, if any, would likely be on protein levels via regulation of gene transcription or translation.

While the significance of genetic polymorphism on TGF-β3 expression has not been previously established, the literature is supportive of a role for TGF-β3 in the development of PIH. Several studies have found increased placental expression of TGF-β3 among women with preeclampsia [11] and HELLP Syndrome [12], though the later study did not find a statistically significant difference in expression between preeclamptic and control placentae [12]. This finding might be explained by the exclusion of PE cases with preterm deliveries and thus, more severe disease, for whom placental factors are likely to be etiologically important.

Gene expression studies in term placentas, however, are of questionable relevance with respect to placental TGF-β3 expression during the relevant time point - early placental formation. TGF-β3 expression levels vary throughout the gestational period, being low in early pregnancy, peaking at 7-8 weeks, and dropping to very low levels in the third trimester [11,15]. In contrast, TGF-β genotype does not change. Thus, compared to expression levels at term, a functionally-significant regulatory variant in the TGF-β3 gene might be a better biomarker of the potential for TGF-β3 up-regulation during placental formation.

Limitations of our study include the relatively small sample size, especially the limited number of confirmed preeclamptics. While all of the study subjects were clinically diagnosed with preeclampsia, upon chart review reliable evidence of proteinuria could not be obtained for 31% of the cases. Notwithstanding, 75% of these cases showed signs of more significant disease, suggesting that they are well within the preeclamptic spectrum of disease and are likely to share many of the same etiologic factors. Additionally, one recent review has demonstrated that proteinuria is not a reliable predictor of maternal or fetal outcomes [16] and therefore, controversy surrounding the use of proteinuria as a criterion for diagnosis of preeclampsia has surfaced. In light of this controversy, we felt that the use of other signs of disease, including, in a minority of cases, the clinical judgment of experienced clinicians should be considered evidence of significant disease. Furthermore, while inclusion of women with less severe disease is expected to bias the results toward the null, in fact, we found that excluding the gestational hypertensives did not substantively alter the effect estimates. Nevertheless, these results should be considered preliminary until they are confirmed in a larger study.

Since the vast majority of women in this study population were of Hispanic ethnicity, these results may not be generalizable to all racial or ethnic groups. However, the polymorphisms studied are not specific to Hispanic ethnicity and the underlying biological rationale for the putative role of TGF-β3 variants in predisposing to PIH remains intact in other ethnic groups. Still, confirmation of these results in other ethnic populations is warranted.

An additional, and important, limitation of this study is that it does not address the entire pathway that is likely to be involved in the development of PIH. This condition is most probably a complex interaction among many genes and environmental factors, of which we address only one in this study. However, we believe that this study makes an important first step by

addressing the fetal/placental contribution to the disease, which itself is likely to involve the interaction of many other genes.

Our study also has several important strengths. First, we were able to perform extensive chart abstractions for nearly all of the women in the study population and were able to perform abstractions sufficient to confirm diagnoses for all study subjects.

This not only allowed us to verify the clinical diagnosis and determine the extent of disease, but also to obtain reliable information on medical history and co-morbid conditions. In addition, the collection of both maternal and fetal DNA samples allowed for the investigation of a candidate gene involved in placental development, for which maternal genotypes should have little relevance, based on our limited understanding of the underlying biology. Furthermore, gene variants, being present from birth, do not change over gestational age, thereby ruling out the possibility that the disease process itself leads to the alterations in TGF-β3 expression observed in preeclamptic placentae.

The four polymorphisms examined here account for less than half of the variation in the TGF-β3 gene [17]. Thus, even though we prioritized SNPs based on likelihood of functionality, it is possible that we have missed some functionally important variation. Furthermore, since it is highly likely that maternal and fetal genomes interact to produce PIH, future studies should evaluate the possibility that different sets of candidate genes are important in the mother than in the offspring [18]. Large numbers of maternal-offspring pairs must be recruited to ensure adequate statistical power to detect complex gene-by-gene interactions.

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REFERENCES

1. Arngrimsson R, et al. Is genetic susceptibility for pre-eclampsia and eclampsia associated with implantation failure and fetal demise? [letter]. Lancet 1994;343(8913):1643–4. [PubMed: 7911952]
2. Knofler M, et al. Novel genes and transcription factors in placental development--a workshop report. Placenta 2000;21(Suppl A):S71–3. [PubMed: 10831126]
3. Cross JC, et al. Genes, development and evolution of the placenta. Placenta 2003;24(23):123–30. [PubMed: 12596737]
4. Rawn SM, Cross JC. The evolution, regulation, and function of placenta-specific genes. Annu Rev Cell Dev Biol 2008;24:159–81. [PubMed: 18616428]
5. Seligman SP, et al. Hypoxia stimulates ecNOS mRNA expression by differentiated human trophoblasts. Ann N Y Acad Sci 1997;828:180–7. [PubMed: 9329838]
6. Genbacev O, et al. Hypoxia alters early gestation human cytotrophoblast differentiation/invasion in vitro and models the placental defects that occur in preeclampsia. J Clin Invest 1996;97(2):540–50. [PubMed: 8567979]
7. Genbacev O, et al. Regulation of human placental development by oxygen tension. Science 1997;277 (5332):1669–72. [PubMed: 9287221]
8. Caniggia I, et al. Hypoxia-inducible factor-1 mediates the biological effects of oxygen on human trophoblast differentiation through TGFbeta(3). J Clin Invest 2000;105(5):577–87. [PubMed: 10712429]
9. Redline RW, Patterson P. Pre-eclampsia is associated with an excess of proliferative immature intermediate trophoblast. Hum Pathol 1995;26(6):594–600. [PubMed: 7774887]
10. Rajakumar A, et al. Impaired Oxygen-dependent Reduction of HIF-1alpha and -2alpha Proteins in Pre-eclamptic Placentae. Placenta 2003;24(23):199–208. [PubMed: 12566247]

11. Caniggia I, et al. Inhibition of TGF-beta 3 restores the invasive capability of extravillous trophoblasts in preeclamptic pregnancies. J Clin Invest 1999;103(12):1641–50. [PubMed: 10377170]
12. Emanuelli M, et al. Placental overexpression of transforming growth factor-beta3 in the HELLP syndrome. Gynecol Obstet Invest 2008;65(1):1–5. [PubMed: 17671384]
13. Lum A, Le Marchand L. A simple mouthwash method for obtaining genomic DNA in molecular epidemiological studies. Cancer Epidemiol Biomarkers Prev 1998;7(8):719–24. [PubMed: 9718225]
14. Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy. Am J Obstet Gynecol 2000;183(1):S1–S22.
15. Lyall F, et al. Transforming growth factor-beta expression in human placenta and placental bed in third trimester normal pregnancy, preeclampsia, and fetal growth restriction. Am J Pathol 2001;159 (5):1827–38. [PubMed: 11696443]
16. Hofmeyr GJ, Belfort M. Proteinuria as a predictor of complications of preeclampsia. BMC Med 2009;7:11. [PubMed: 19317890]
17. Project, IH. Jan. 13. 2009 Available from: <http://www.hapmap.org/cgiperl/gbrowse/hapmap_B36/ >
18. Wilson ML, et al. Molecular epidemiology of preeclampsia. Obstet Gynecol Surv 2003;58(1):39–66. [PubMed: 12544785]

Table 1

Characteristics of the Study Population

Variable	Controls (n=167)	Cases (n=129)	p-value
Maternal Age (mean ± SD)	26.8 (0.54)	27.6 (0.65)	0.38*
Gestational Age (weeks)	range: 28-42	range: 25-41	p<0.01**
<37	15 (9)	40 (31)	
37-38	47 (28)	41 (32)	
>=39	105 (63)	48 (37)	
Parity (%)			0.02**
Nulliparous	52 (31)	58 (45)	
Multiparous	115 (69)	71 (55)	
Race/ethnicity			0.92**
Hispanic white	161 (96)	124 (96)	
Hispanic black	1 (1)	1 (1)	
non-Hispanic black	3 (2)	3 (2)	
Arab	1 (1)	1 (1)	
Phillipino	1 (1)	0 (0)	

Note: Percentages not totaling 100% are due to rounding errors.

* P-value obtained by t-test

** P-value obtained by Pearson's chi-square test

Table 2

Co-morbid Conditions and Blood Pressure Among Study Participants

Variable	Controls (%)	Cases (%)	p-value
Chronic Hypertension	2 (1)	6 (5)	0.08**
Diabetes	18 (11)	7 (6)	0.14**
Fetal Malformations	1 (1)	2 (2)	0.58**
Thyroid Disease	9 (5)	8 (6)	0.81**
Seizure Disorder	4 (2)	1 (1)	0.39**
HIV+	6 (4)	1 (1)	0.14**
History of PIH	6 (4)	1 5(12)	0.01**
SGA	10 (6)	16 (12)	0.06**
Maximum Systolic BP (mmHg)	117 (13.9)	163 (15.2)	<0.01§
Maximum Diastolic BP (mmHg)	69 (9.0)	97 (9.0)	<0.01§

Note: Percentages not totaling 100% are due to rounding errors.

SGA = small for gestational age; IUGR = intrauterine growth restriction, PIH = pregnancy induced hypertension

** P-value obtained by Fisher's Exact chi-squared test

§ P-value obtained by t-test

TABLE 3

TGFB3 maternal and fetal genotypes and risk of PIH

TGFB3 locus	Maternal			Fetal		
	N(%)	OR (95% Cl)		N(%)	OR (95% Cl)	
	Controls	CasesAdjusted*	Multl-locus Adj**	Controls	CasesAdjusted*	MUltl-locus Adj**
rs114664142						
CC	136 (81%)	105 (81%)1.00 (ref)	1.00 (ref)	129 (77%)	109 (85%)1.00 (ref)	1.00 (ref)
CT/TT	31 (19%)	24 (19%)1.66 (0.75, 3.65)	1.68 (0.74, 3.85)	38 (23%)	20 (16%)0.34 (0.15, 0.77)	0.32 (0.14, 0.77)
rs2205181						
CC	42 (25%)	30 (23%)1.00 (ref)	1.00 (ref)	45 (27%)	37 (29%)1.00 (ref)	1.00 (ref)
TC	85 (51%)	72 (56%)1.11 (0.57, 2.14)	1.34 (0.65, 2.79)	86 (52%)	64 (50%)0.87 (0.46, 1.65)	1.17 (0.58, 2.34)
TT	40 (24%)	27 (21%)0.99 (0.43, 2.26)	1.29 (0.48, 3.49)	36 (22%)	28 (22%)0.87 (0.39, 1.94)	1.33 (0.53, 3.36)
rs2268624						
GG	110 (66%)	79 (61%)1.00 (ref)	1.00 (ref)	112 (67%)	81 (63%)1.00 (ref)	1.00 (ref)
GC/CC	57 (34%)	50 (39%)1.24 (0.74, 2.10)	1.27 (0.67, 2.40)	55 (33%)	48 (37%)0.91 (0.52, 1.61)	1.28 (0.68, 2.41)
rs3917200						
GG	146 (87%)	108 (84%)1.00 (ref)	1.00 (ref)	142 (85%)	103 (80%)1.00 (ref)	1.00 (ref)
GA/AA	21 (13%)	21 (16%)1.14 (0.65, 2.01)	1.66 (0.68, 4.01)	25 (15%)	26 (20%)1.28 (0.72, 2.26)	1.33 (0.60, 2.97)
Total	167 (100%)	129 (100%)		167 (100%)	129 (100%)	

* Adjusted for maternal age (<29 vs. ≥29 years), gestational age (<37, 37, 38, 39, ≥40 weeks), parity (nulliparous vs. parous), and fetal (or maternal) genotype at the same locus

** Further adjusted for maternal and fetal genotypes at all other loci