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Effect of Aspirin Response Signature Gene Expression on Preterm Birth and Preeclampsia among Women with Lupus: A Pilot Study

Amanda M. Eudy[1,*], **Deepak Voora**[2], **Rachel A. Myers**[2], **Megan E. B. Clowse**[1]

[1]Division of Rheumatology & Immunology, Duke University School of Medicine, Durham, NC, USA

[2]Duke Center for Applied Genomics & Precision Medicine, Duke University, Durham, NC, USA

Abstract

Background—Women with lupus have an increased risk of preeclampsia and preterm birth, and aspirin 81mg/day is recommended as a preventative measure for preeclampsia. This pilot study quantified the association between a 60-gene aspirin response signature (ARS) gene expression with preterm birth and preeclampsia risk among women with lupus taking aspirin.

Methods—The analysis included 48 RNA samples from 23 pregnancies in the Duke Autoimmunity Pregnancy Registry. RNA was isolated from peripheral blood, and quantitative PCR was performed for ARS genes. The primary outcome was poor pregnancy outcome (preeclampsia or preterm birth). Gene expression was modeled as a response to presence or absence of a poor pregnancy outcome using linear regression models, stratified by trimester.

Results—Of the 23 pregnancies, 9 delivered preterm and 4 had preeclampsia. Expression of *PBX1* and *MMD* was higher in the 2nd trimester among patients who experienced a poor pregnancy outcome compared to those who did not. However, in a global test of all ARS genes, we identified no association between expression of ARS genes with poor pregnancy outcomes.

Conclusion—Our pilot study identified two candidate genes that are reflective of the platelet function response to aspirin. Further work is needed to determine the role of these genes in identifying women with lupus at high risk for preeclampsia and preterm delivery despite aspirin therapy.

Keywords

systemic lupus erythematosus; pregnancy; preterm birth; preeclampsia; aspirin; platelet function

*Corresponding Author Duke University, Division of Rheumatology and Immunology, DUMC 3490, Durham, NC 27710, amanda.eudy@duke.edu.

Ethics Approval: This study was approved by the by the Duke Health Institutional Review Board according to standards indicated by the Declaration of Helsinki (Pro00000756).

Introduction

Systematic lupus erythematosus (lupus) is a rheumatic disease predominately affecting women, with onset typically during childbearing years. Women with lupus experience pregnancy complications at a higher rate than women in the general population, with increased risk of preeclampsia and preterm birth.[1–4] In a recent analysis of our pregnancy cohort, we found that 70% of preterm births were medically indicated, most commonly for maternal preeclampsia or hypertension.[5]

Preeclampsia, characterized by 3rd trimester hypertension and proteinuria, occurs in 3–5% of all pregnancies, but in 10–25% of pregnancies in women with rheumatic disease.[6] The U.S. Preventive Services Task Force recommends that women at high risk for preeclampsia take aspirin 81mg a day, including all women with lupus.[7] Projections suggest that compliance with this recommendation would reduce the national rate of preeclampsia from 4.2% to an estimated 3.8%, saving over $350 million/year.[8]

The pathophysiology of preeclampsia starts early in pregnancy, as the maternal spiral arteries enlarge to nourish the fetus throughout development. In preeclampsia, the spiral arteries remain small, leading to ischemia within the placenta, release of placental products into the maternal circulation, and endothelial changes that prompt maternal hypertension and proteinuria. The only proven preventive measure is low-dose aspirin. Large, randomized trials have demonstrated that low-dose (50–200mg) aspirin can decrease the risk of preeclampsia by an estimated 20%.[9] Although effective, a significant proportion of women develop preeclampsia despite low-dose aspirin.

We hypothesized that an insufficient platelet response to aspirin may underlie a higher risk of preeclampsia despite taking aspirin. Aspirin is well-known to inhibit platelet COX-1, preventing the conversion of arachidonic acid to thromboxane, a potent vasoconstrictor. By decreasing thromboxane A2 production and promoting vasodilation in the placenta, aspirin improves blood flow to the developing fetus and decreases the endothelial dysfunction that drives preeclampsia.[10] While low-dose aspirin very effectively suppresses COX-1 pathways, its impact on non-COX-1 dependent platelet function can be more variable, leaving some patients with high residual platelet function.[11]

A recent meta-analysis suggested a dose-related impact of aspirin, with 60mg least effective and 150mg most effective in preventing preeclampsia.[12] High residual platelet function, despite the recommended aspirin dose, may be a cause for the low efficacy of aspirin in up to 40% of people.[13,14] The meta-analysis authors concluded that "… in a high proportion of women a dose of <100mg/day is not sufficient to affect platelet function or reduce preeclampsia."[7,13,14] A single retrospective observational study has suggested that tailoring the dose of aspirin to measures of platelet function may be associated with significant decreases in preeclampsia (30% with tailored therapy vs 50%) and severe preeclampsia (3.6% with tailored therapy vs 15%).[15] No further studies appear to be underway, however, for this promising approach.

We have previously described the discovery and validation of a 60-gene Aspirin Response Signature (ARS) in peripheral blood RNA that is correlative of platelet function in response

to aspirin and cardiovascular events in patients taking aspirin.[16] Higher levels of ARS genes are correlated with higher risks for death/myocardial infarction despite aspirin therapy. The objective of the present pilot study was to quantify the effect of ARS gene expression on preterm birth and preeclampsia risk among women with lupus taking aspirin during pregnancy, with a specific focus on differences in gene expression by trimester.

Materials and Methods

Duke Autoimmunity in Pregnancy Cohort

Through the Duke Autoimmunity in Pregnancy (DAP) Registry, over 400 pregnancies to women with rheumatic diseases, including lupus, have been followed with repeated collection of blood samples, clinical data, medications, labs, and pregnancy outcomes. The DAP Registry prospectively collected data from December 2007 to 2017, with RNA samples collected between 2008 and March 2012. The registry was approved by the Duke Health Institutional Review Board according to standards indicated by the Declaration of Helsinki (Pro00000756). All patients provided written consent for participation in the study.

This study was limited to a subgroup of the larger cohort meeting the following criteria:

1. A diagnosis of SLE by the ACR SLE criteria.[17,18]
2. Pregnancies with known outcomes. Miscarriages were excluded, as the study focused on gene expression in the 2nd and 3rd trimesters.
3. Taking low dose aspirin (81 mg/day) during any trimester of pregnancy.
4. Provided informed consent for the collection and storage of RNA for future research.
5. At least one visit with collection of a PAXgene tube for RNA. These were collected between 2008 and 2012.

Between 2008 and March 2012, 147 women enrolled in the registry, of which 70 had a diagnosis of lupus. Consent for RNA collection was given by 60, and 46 of these pregnancies had an outcome of live birth or stillbirth with data available on the outcomes of preterm birth and preeclampsia. Aspirin was taken during 34 of these pregnancies, of which 23 were included in the final analysis. Of the 11 excluded: 1 had only a postpartum sample, 2 had only a 1st trimester sample, 2 were twin pregnancies, 4 reported taking aspirin inconsistently, and 2 did not have RNA samples collected despite providing consent.

RNA Purification and qPCR

Methods for RNA purification and qPCR were performed as previously described.[16] Briefly, RNA was isolated from peripheral blood collected in PAX gene tubes. All samples had high quality RNA (RIN > 7) and cDNA synthesized using 500ng of RNA. Quantitative PCR was performed using a previously designed, custom Taqman low-density array (TLDA) for ARS genes. *FPGS* and *TRAP1* were selected as the reference genes from the following pool of candidate reference genes: *FPGS, TRAP1, PPIB, ACTB,* and *GAPDH.*

Statistical Analysis

The primary clinical outcome, a poor pregnancy outcome, was defined as preeclampsia (collected from the medical record) or preterm birth (delivery prior to 37 weeks gestation). Preeclampsia was defined by the treating obstetrician at delivery based on the criteria of hypertension (BP>140/90) and proteinuria (>300mg). As a sensitivity, we analyzed the effect of ARS genes on preterm birth, excluding patients with preeclampsia. A secondary outcome included placental insufficiency, defined as spontaneous preterm birth, preeclampsia, or intrauterine growth restriction (IUGR).

The qPCR gene expression data was normalized, as described previously,[16] and log2 transformed. Gene expression was modeled as a response to presence or absence of a poor pregnancy outcome and log2 fold changes were estimated using linear regression.

Differences in maternal and clinical characteristics by pregnancy outcome were compared by Fisher's exact test, t-tests, and Wilcoxon signed-rank test. Only pregnancy visits were included (no postpartum visits), and multiple samples per pregnancy were allowed. Models were unadjusted and adjusted for platelet count and stratified by trimester. Due to the limited number of 1st trimester samples (n=9), the analysis focused on gene expression in the 2nd and 3rd trimesters. For patients with more than one sample per trimester, the average expression of each gene in the ARS was calculated. P-values were adjusted for multiple comparisons by the false discovery rate (FDR) method.

The Aspirin Response Signature (ARS) was discovered as a set of co-expressed genes where their aggregate expression was correlative of platelet function outcomes on aspirin.[16] In our prior work, we defined the ARS as a linear combination of the expression of 60 co-expressed genes in peripheral blood RNA measured using Affymetrix microarrays. We have previously validated the TLDA platform for quantifying individual ARS gene expression using PCR.[19] However, we have not yet translated the ARS defined using microarrays to a PCR based platforms. Therefore, in addition to testing each ARS gene individually, we also used a global test to simultaneously assess ARS genes for differential expression by adverse pregnancy outcome. The global test provided an estimate of how the ARS genes perform in aggregate in patients with and without a poor pregnancy outcome. Analyses were conducted in R version 3.3 (http://www.r-project.org) and SAS 9.4 (Cary, North Carolina).

Results

The present analysis included a subset of 23 pregnancies among women with lupus from whom 48 RNA samples were collected. Of these pregnancies, n=9 (39%) were delivered preterm and n=4 (17%) had preeclampsia (Table 1). Three pregnancies were delivered preterm and complicated by preeclampsia (13%). Among the 9 preterm deliveries, 6 (67%) were medically-indicated. Reasons for induction of labor included the infant not being well (n=1), SLE activity (n=1), or placental insufficiency, preeclampsia, or maternal hypertension (n=4). The majority of patients started low dose aspirin during the 1st trimester, and all but two patients started prior to 16 weeks. Two stillbirths occurred during the study period, but did not meet inclusion criteria because the patient did not provide consent for RNA (n=1) or the patient was not taking aspirin (n=1).

Platelet count was normal in all patients (range: 165–400) and did not vary by trimester. Platelet count in the 2nd trimester appeared to vary by poor pregnancy outcome; however, this was driven by one outlier with a very early delivery and a high-normal platelet count. In the 2nd trimester, the median platelet count for patients with no preterm birth or preeclampsia was 237 (IQR: 201–289) compared to 291 (IQR: 227–330) in patients with preterm birth or preeclampsia (p=0.08).

In analyses of individual ARS genes, expression of *PBX1* and *MMD* was upregulated in the 2nd trimester among patients who experienced a preterm birth or preeclampsia in unadjusted models, but p-values were >0.05 after adjustment for multiple comparisons (Table 2). Of note, all but two of the β coefficients were greater than 0, suggesting a consistently positive association of the ARS genes with a poor pregnancy outcome in the 2nd trimester. In the 3rd trimester, we found no associations between ARS genes and the outcome of preterm birth or preeclampsia (Figure 1). Results were consistent in a sensitivity analysis that excluded the 4 pregnancies complicated by preeclampsia.

The expression of *MMD* was upregulated in the 2nd trimester among patients who experienced placental insufficiency in unadjusted models, as well as models adjusted for platelet count. In the 3rd trimester, there was a trend for upregulated expression of *PBX1* (p=0.1) among patients with placental insufficiency, adjusted for platelet count.

The aspirin response genes are a tightly co-expressed set of genes with synchronized variations in gene expression that are indicative of an aspirin response pathway: higher levels correlate with poorer platelet function inhibition and higher cardiovascular risk. As described above, in order to simultaneously assess the behavior of ARS genes we used a global test, comparing pregnancies with and without preterm birth or preeclampsia (Table 3). In the 2nd trimester, the global test for the ARS genes alone was not associated with poor pregnancy outcomes (p=0.411). When adjusted for platelets, there was a trend toward an association (p=0.08). However, the association of platelets alone on poor pregnancy outcome was similar (p=0.08), suggesting that the expression of ARS genes is not the driver in poor pregnancy outcomes in this cohort. There was no association in global test for the ARS genes with the outcome of placental insufficiency in either trimester.

Discussion

Preeclampsia, characterized by hypertension and proteinuria, is a leading cause of maternal and infant morbidity. It occurs in an estimated 5% of pregnancies, but in up to 25% of pregnancies in women with lupus.[1–4] It often prompts a preterm delivery, which can lead to short-term and life-long complications for the offspring. The pathophysiology of preeclampsia starts early in pregnancy, as the fetal placental cells migrate into the uterine wall, causing the maternal spiral arteries to enlarge to feed the fetus throughout development. In preeclampsia, this migration is stunted and the spiral arteries remain small, leading to ischemia within the placenta and endothelial changes that prompt preeclampsia. Among pregnancies in women with SLE, changes in the angiogenic factors Flt-1, placental growth factor are noted between 18–21 weeks of gestation among pregnancies destined for preeclampsia.[20]

The only treatment for preeclampsia is delivery of the pregnancy. The only proven preventive measure is low-dose aspirin. Large, randomized trials have demonstrated that low-dose (50–200mg) aspirin can decrease the risk of preeclampsia by an estimated 20%.[9] Therefore, although effective, a significant proportion of women develop preeclampsia despite aspirin use. Aspirin is well-known to inhibit platelet COX-1 which prevents the conversion of arachidonic acid to thromboxane, a potent vasoconstrictor. Aspirin may have additional effects on platelet function beyond platelet COX-1 that are not well described but are characterized, in part, through the expression of genes in the Aspirin Response Signature (ARS).

While aspirin decreases the risk of preeclampsia and preterm birth by an average of 20%, many women at high risk for these complications continue to experience them, despite therapy. This suggests that a subpopulation of women may not respond to aspirin as effectively as others. Several studies have pursued this issue, with mixed results. A study of 87 pregnant women on aspirin identified 29% of women as being aspirin non-responders to 81mg per day, based on the results of the PFA-100®.[13] This group then looked in a larger retrospective study of 270 pregnant women taking aspirin: 159 on standard aspirin dosing were compared to 111 women who underwent platelet function testing with PFA-100® followed by aspirin dose adjustment.[15] Of those with platelet function testing, 39% had their aspirin dose increased to 162mg/day. The rate of preeclampsia and severe preeclampsia were both 2–4-fold lower in the women with aspirin response assessment than women on standard aspirin dosing without testing. On the other hand, a recently published study of 180 pregnant women did not find a population of women with consistent aspirin resistance. This study assessed for aspirin resistance only using Cox-1 dependent platelet function measures, including Multiplate™ impedance aggregometry, VerifyNow™, and urinary 11-dehydrothromboxane B_2. Women were inconsistently non-responsive to aspirin; all 34 women non-responsive in the first half of pregnancy were responsive or ‘indeterminate’ in the 3rd trimester. The 34% of pregnancies in women with variable aspirin response in pregnancy had similar pregnancy outcomes to women who were consistent aspirin responders.[21]

While the data are somewhat variable about aspirin response in pregnancy, it has been extensively explored in cardiovascular disease. A systematic review of 108 studies found some prognostic utility of platelet function tests to identify aspirin resistance, including a correlation between this condition and adverse cardiovascular outcomes.[22] For example, a study of 78 patients with end-stage renal found more than twice the rate of death, myocardial infarction or stroke in the 44% of patients with aspirin resistance defined by arachidonic acid–induced aggregometry (ASPl test).[23] We can locate only one prior study of aspirin resistance in SLE, and it suggested that aspirin resistance defined using a Multiplate impedance aggregometer might be present in up to 20% of women with SLE, based on a study of 26 women with SLE taking aspirin.[24]

Genome wide association studies of the response to aspirin have identified a single genetic variant near *PEAR1* as being associated with the non-COX-1 platelet function response to aspirin.[25] Although functional variants in COX-1 have been identified,[26] its relationship to aspirin response has been inconsistent.[27] There have been no variants in COX-1 that have

been identified. This single variant, however, only explains a small proportion of the overall variation in response to aspirin. Future work could incorporate the results of this variant with the ARS to predict pregnancy outcomes.

The current study did not use platelet function testing to assess aspirin resistance, as fresh blood samples were not available for this retrospective study. Instead, we used a surrogate for the effects of aspirin on platelet function through the ARS genes that we previously identified and validated as biomarkers of aspirin's effects on platelets. The ARS genes reflect the non-COX-1 effects of aspirin on platelets and includes 60 co-expressed genes in peripheral blood RNA that are associated with platelet function in response to aspirin.[16] Aspirin therapy lowers the ARS; individuals with abnormally elevated ARS levels despite aspirin use remain at high risk for cardiovascular events.[16] Our study demonstrates that, in aggregate, aspirin response signature genes do not appear to be predictive of pregnancy outcomes. Altered levels of 2 specific genes within the aspirin-resistance signature, *PBX1* and *MMD*, in the 2nd trimester may correlate with these poor pregnancy outcomes, but these correlations were not statistically significant when corrected for multiple comparisons. Further work will require independent validation in larger sample sizes and assessment of alternative approaches (i.e., higher aspirin dose or alternate inhibitors of platelet function) to improve pregnancy outcomes.

The current analysis is limited by small sample size. More samples would provide additional power to determine an association between adverse outcomes and gene expression. RNA samples were only collected over a 4-year period at the initiation of the DAP Registry, therefore, we were unable to include more recent samples. Four of the 23 pregnancies were complicated by preeclampsia, which limited our ability to analyze this adverse pregnancy outcome as a single outcome or determine differences between patients based on the severity of preeclampsia. Additionally, only two of the births were early preterm (<34 weeks), therefore, we were unable to analyze this outcome. We were not able to assess patient adherence with aspirin dosing as part of this retrospective study. Measurements of aspirin metabolites and platelet function both require fresh samples and these tests were not conducted during data collection. Patients were asked about aspirin use by the nurse at clinic check-in and by the rheumatologist during the office visit; questionnaires did not include a self-assessment of compliance. Randomized trials of aspirin in pregnancy have reported non-adherence rates between 3–37%, suggesting that some of the women in this study were likely not taking aspirin at the time blood samples were taken.[28]

This pilot study has identified two candidate genes that may be associated with platelet function in response to aspirin that possibly play a role in the high rate of preeclampsia and preterm birth among women with lupus, despite aspirin therapy. This study opens the door for further research, both to confirm these findings with a different cohort, and to further explore aspirin response through functional platelet measures in women with lupus in pregnancy. Additionally, it would be interesting to understand whether aspirin response changes in pregnancy and could be assessed pre-pregnancy to determine therapy. While the US Public Health Task Force and American College of Obstetrics and Gynecology have included women with rheumatic disease, and in particular women with lupus, in their guidelines for pregnant women who should take aspirin, there is not clinical data to support

this recommendation. Given the high rate of complications in this cohort, identifying ways to optimize this preventive therapy may be an approach to significantly decrease preeclampsia and preterm birth in this population.

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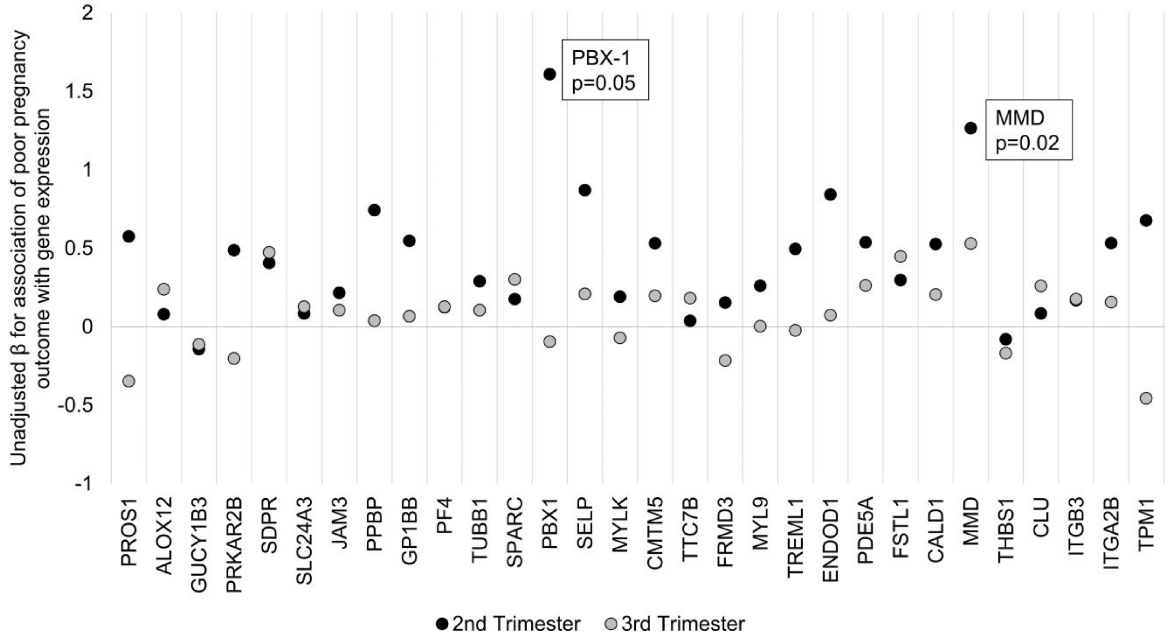

Figure 1.

Unadjusted β for the association of poor pregnancy outcome with gene expression by trimester.

Table 1.

Cohort Characteristics.

	Overall	Preterm Birth and/or Preeclampsia	Term Birth and No preeclampsia	
	n (%)	n (%)	n (%)	p-value
Characteristics per Pregnancy	**n=23**	**n=10**	**n=13**	
Year of Pregnancy				0.5
2008	5 (22%)	3 (30%)	2 (15%)	
2009	8 (35%)	5 (50%)	3 (23%)	
2010	5 (22%)	1 (10%)	4 (31%)	
2011	3 (13%)	1 (10%)	2 (15%)	
2012	2 (9%)	0 (0%)	2 (15%)	
Race				0.3
Black or African American	5 (22%)	3 (30%)	2 (15%)	
White or Caucasian	15 (65%)	7 (70%)	8 (62%)	
Other	3 (13%)	0 (0%)	3 (23%)	
Medications				
Low-dose aspirin	23 (100%)	10 (100%)	13 (100%)	--
Hydroxychloroquine	20 (87%)	10 (100%)	10 (77%)	0.2
Prednisone	14 (61%)	9 (90%)	5 (38%)	0.03
Azathioprine	3 (13%)	3 (30%)	0 (0%)	0.07
Co-Morbidities				
Lupus Nephritis	2 (9%)	1 (10%)	1 (8%)	1.0
Antiphospholipid Syndrome	0 (0%)	0 (0%)	0 (0%)	--
Positive Lupus Anticoagulant	3 (13%)	1 (10%)	2 (15%)	1.0
Anticardiolipin IgG or IgM >40	0 (0%)	0 (0%)	0 (0%)	--
Anti-Beta-2-glycoprotein IgG or IgM >40	0 (0%)	0 (0%)	0 (0%)	--
Mean maternal age (SD)	30.0 (4.4)	29.2 (3.2)	30.6 (5.1)	0.4
Median Platelet Count (IQR)	239 (203–315)	267 (222–335)	237 (197–295)	0.2
2nd Trimester	244 (210–304)	291 (227–330)	237 (201–289)	0.08
3rd Trimester	235 (179–327)	225 (208–366)	230 (179–296)	0.6

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Table 2.

Association of poor pregnancy outcome (preterm birth or preeclampsia) with gene expression in the 2nd trimester.

	Unadjusted Model				Adjusted Model[a]			
Gene	**Model Estimate**	**SE**	**Unadjusted p-value**	**FDR Adjusted p-value**	**Model Estimate**	**SE**	**Unadjusted p-value**	**FDR Adjusted p-value**
MMD	1.2660	0.5002	0.0215	0.6459	1.5162	0.5297	0.0113	0.3387
PBX1	1.6091	0.7666	0.0511	0.7660	1.3846	0.8304	0.1149	0.9940
TPM1	0.6766	0.4713	0.1693	0.9399	0.6683	0.5164	0.2140	0.9940
ENDOD1	0.8428	0.6001	0.1782	0.9399	0.9984	0.6517	0.1450	0.9940
SELP	0.8707	0.7199	0.2430	0.9399	0.8813	0.7887	0.2803	0.9940
GP1BB	0.5471	0.5313	0.3176	0.9399	0.4198	0.5777	0.4780	0.9940
PPBP	0.7423	0.7508	0.3367	0.9399	0.5583	0.8161	0.5037	0.9940
CMTM5	0.5319	0.5526	0.3493	0.9399	0.3631	0.5980	0.5522	0.9940
PRKAR2B	0.4874	0.5109	0.3535	0.9399	0.4536	0.5594	0.4294	0.9940
ITGA2B	0.5324	0.6139	0.3979	0.9399	0.4744	0.6718	0.4902	0.9940
TREML1	0.4959	0.5811	0.4053	0.9399	0.3232	0.6292	0.6145	0.9940
PROS1	0.5759	0.6865	0.4132	0.9399	0.5232	0.7516	0.4963	0.9940
SDPR	0.4066	0.4952	0.4229	0.9399	0.6214	0.5289	0.2572	0.9940
PDE5A	0.5378	0.7136	0.4614	0.9399	0.4709	0.7809	0.5549	0.9940
CALD1	0.5269	0.7871	0.5122	0.9399	0.2899	0.8520	0.7381	0.9940
JAM3	0.2158	0.4134	0.6084	0.9399	0.1708	0.4522	0.7106	0.9940
TUBB1	0.2893	0.5727	0.6199	0.9399	0.1979	0.6253	0.7558	0.9940
FSTL1	0.2979	0.5935	0.6221	0.9399	0.3246	0.6501	0.6244	0.9940
FRMD3	0.1547	0.3484	0.6625	0.9399	0.3763	0.3609	0.3125	0.9940
MYL9	0.2606	0.5965	0.6677	0.9399	−0.0202	0.6342	0.9750	0.9940
MYLK	0.1913	0.4396	0.6689	0.9399	0.0967	0.4786	0.8424	0.9940
SPARC	0.1764	0.5739	0.7622	0.9399	0.0203	0.6226	0.9744	0.9940
GUCY1B3	−0.1426	0.5060	0.7814	0.9399	−0.1950	0.5536	0.7292	0.9940
ITGB3	0.1691	0.7431	0.8227	0.9399	−0.0212	0.8071	0.9793	0.9940
PF4	0.1243	0.6906	0.8592	0.9399	0.0632	0.7558	0.9344	0.9940
SLC24A3	0.0848	0.4830	0.8626	0.9399	0.0070	0.5273	0.9895	0.9940
ALOX12	0.0794	0.5103	0.8782	0.9399	−0.0868	0.5512	0.8768	0.9940

	Unadjusted Model				Adjusted Model[a]			
Gene	**Model Estimate**	**SE**	**Unadjusted p-value**	**FDR Adjusted p-value**	**Model Estimate**	**SE**	**Unadjusted p-value**	**FDR Adjusted p-value**
CLU	0.0843	0.5631	0.8828	0.9399	−0.0354	0.6133	0.9548	0.9940
THBS1	−0.0800	0.8299	0.9244	0.9399	0.0855	0.9045	0.9259	0.9940
TTC7B	0.0373	0.4872	0.9399	0.9399	0.0041	0.5335	0.9940	0.9940

[a] adjusted for platelet count

Table 3.

Global test for the association of poor pregnancy outcome (preterm birth or preeclampsia) with gene expression.

	2nd Trimester				3rd Trimester			
	p-value	Statistic	Expected	SD	p-value	Statistic	Expected	SD
Gene Expression	0.411	4.46	5.56	5.75	0.969	1.34	5.56	4.75
Gene Expression + Platelet Count	0.081	16.9	5.56	7.63	0.506	2.65	5.56	7.63
Platelet Count *	0.083	16.7	5.56	7.64	0.507	2.63	5.56	7.64

* This is equivalent to an ANOVA F-test from a linear model with 1 independent variable