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Epigenetic regulation of the nitric oxide pathway, 17-alpha hydroxyprogesterone caproate, and recurrent preterm birth

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Abstract

OBJECTIVE—We sought to evaluate nitric oxide pathway placental gene expression and the epigenome (CpG methylation) among women receiving 17-alpha hydroxyprogesterone caproate (17-OHPC) with and without recurrent preterm birth (PTB).

STUDY DESIGN—Case-control study. We prospectively recruited women with ≥ 1 prior singleton spontaneous PTB <34 weeks receiving 17-OHPC. DNA and RNA were isolated from placentas. RNA abundance (gene expression) and the methylome were analyzed for 84 genes in nitric oxide pathways. Women with recurrent PTB <34 weeks (cases) were compared to those delivering at term (controls). Statistical analysis included multivariable models with Bonferroni corrected p-values.

RESULTS—17 women met inclusion criteria; 7 preterm cases (delivered at 22.6 +/− 2.9 weeks) and 10 term controls (delivered at 38.5 +/− 0.8 weeks). Groups had similar PTB history, race/ethnicity, and socioeconomic risk factors for PTB. Twenty-seven nitric oxide genes displayed differential expression ($p<0.05$ and $q<0.10$) when comparing placentas from preterm cases and term controls; all were down-regulated in preterm cases. 860 corresponding CpG sites were differentially methylated between the preterm cases and term controls (Bonferroni p-value <0.05).

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CONCLUSIONS—CpG methylation and gene expression patterns in nitric oxide pathway genes differ among placentas from recurrent PTB compared to term birth following 17-OHPC exposure.

Keywords

17-alpha hydroxyprogesterone carporate; epigenetics; gene expression; nitric oxide; recurrent preterm birth

INTRODUCTION

Despite a recent modest decrease in the rate of prematurity in the US from 2006–2014, rates of prematurity in the US rose in 2015, and hundreds of thousands of babies are delivered too soon every year. A prior spontaneous preterm birth (PTB) is the greatest risk factor for recurrence.[1] Supplementation with 17-alpha hydroxyprogesterone caproate (17-OHPC) reduces the risk of recurrent PTB by one-third, and offering 17-OHPC to women with a history of a prior spontaneous PTB is the standard of care in the US.[2]

Unfortunately, 17-OHPC is only effective for some women, as 18–36% of women experience a recurrent spontaneous PTB despite therapy. The reasons for this variation in response are poorly understood. Pregnancy outcomes among women administered 17-OHPC may be influenced by historical, clinical, pharmacologic, and genetic factors.[3–6] We and others have recently reported that non-Hispanic black women, as well as those with vaginal bleeding or abruption, a history of abruption in the prior pregnancy, or with a family history of PTB were less likely to respond to 17-OHPC.[3–6] Caritis and colleagues also demonstrated that those with the lowest 17-OHPC concentrations have the highest likelihood of recurrent spontaneous PTB.[7] Pharmacogenetics studies of prematurity indicate that maternal genotype may explain some of the variable clinical response to 17-OHPC for recurrent PTB prevention; genes in nitric oxide pathways have been implicated in two separate cohorts.[6,8]

Importantly, no data regarding the influence of placental epigenetics are available to predict clinical outcomes among women receiving 17-OHPC. We hypothesized that the placentas of women delivering at term following exposure to 17-OHPC for the prevention of recurrent spontaneous PTB will have detectable changes in nitric oxide pathway gene expression and site-specific CpG methylation (a mediator of gene transcription) during the mid-trimester compared to the placentas of women experiencing a recurrent spontaneous PTB.

MATERIALS AND METHODS

This was a case-control epigenetic and gene expression association study. Women with a history of one or more singleton non-anomalous spontaneous PTB between 16 and 34 weeks' who received 17-OHPC were recruited prospectively at the University of North Carolina-Chapel Hill (Chapel Hill, NC) from 2015–2016 into the UNC PTB Biobank. We defined spontaneous PTB as delivery following preterm premature rupture of membranes, cervical insufficiency (defined as asymptomatic cervical dilation or effacement in the second trimester of pregnancy), or idiopathic spontaneous preterm labor (uterine contractions leading to cervical dilation). Pregnancies were dated by a combination of last menstrual period (if available) and ultrasound using standard ACOG criteria.[9] Pregnancy management,

including decisions regarding whether to prescribe 17-OHPC and the timing of 17-OHPC administration, were at the discretion of the primary obstetric provider.

After delivery, three full thickness placental biopsies were obtained from the fetal side, 2cm from the placental cord insertion site using a standard 3mm punch biopsy tool, placed in AllProtect solution (Qiagen, Valencia, CA), and frozen at −80°C within 24 hours of delivery until analysis. Care was taken to avoid biopsy of placental sites with obvious gross abnormalities. Labor and delivery course, indication for delivery, and neonatal outcomes were collected.

For this study, we selected women with recurrent early PTB <34 weeks' gestation who were enrolled in the UNC PTB Biobank, received 17-OHPC, had placental samples available, and were self-identified as non-Hispanic black, non-Hispanic white, or Hispanic race/ethnicity. The control group was comprised of women enrolled in the UNC PTB Biobank who also had a history of a prior PTB and received 17-OHPC but delivered at term (at or beyond 37 weeks' gestation). Controls were selected at random to match the composition of the case group with regards to maternal race/ethnicity and history of cervical insufficiency. We excluded women carrying fetuses later diagnosed with major congenital anomalies or aneuploidy and those with known Mullerian anomalies.

All clinical data were collected using standardized questionnaires, and interviews were performed by trained research assistants. Study data were collected and managed using REDCap (Research Electronic Data Capture) tools, a secure, web-based application designed to support data capture for research studies, hosted at the University of North Carolina-Chapel Hill.[10] The electronic medical record was also reviewed to supplement and verify clinical data provided by participants during interviews. This study was approved by the Institutional Review Board at the University of North Carolina-Chapel Hill, and all women provided written, informed consent prior to participation.

DNA and RNA were isolated from placental tissue. Placental biopsies were blotted to remove residual Allprotect reagent and homogenized in Buffer RLT Plus with B-mercaptoethanol (Qiagen, Valencia, CA). DNA and RNA sequences greater than 18 nucleotides in length were extracted from placental tissue using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen, Valencia, CA) according to manufacturer's instructions. Isolated DNA was then bisulfite-converted using the EZ DNA methylation kit (Zymo Research, Irvine, CA). RNA abundance (gene expression) was analyzed using the Human Nitric Oxide Signaling Pathway RT^2 Profiler PCR Array, version 4.0 (SABiosciences, Qiagen, Valencia, CA) per manufacturer's instructions.

CpG methylation was assessed by hybridizing bisulfite converted DNA onto the Illumina MethylationEPIC BeadChip array (Illumina, Inc., San Diego, CA). This platform assesses DNA methylation levels of 850,000 probes at a single nucleotide resolution. Methylation levels were calculated and expressed as β-values (β = intensity of the methylated allele (M) / (intensity of the unmethylated allele (U) + intensity of the methylated allele (m) +100). Probes with poor detection ($p<0.01$) were removed prior to analysis. Quantile normalization was then performed to ensure comparability across samples. Probes representing Single

Nucleotide Polymorphisms (SNPs) were removed as variability at these loci arise from either allelic composition or altered methylation profiles. Finally, methylation data for the 84 genes represented on the nitric oxide pathway RNA expression array (detailed above), represented by 3672 probes, were selected for further analysis.

Bivariate analyses were conducted to compare recurrent early PTB cases to term controls using t-test and Fisher's exact test as appropriate using STATA statistical software (version 13.1, College Station, TX). We compared the relative expression of 84 nitric oxide pathway genes between placentas from preterm birth cases and term controls. Probes corresponding to all genes significant ($p<0.05$ and $q<0.10$) in the initial gene expression analysis were then evaluated for differences in CpG methylation, in relation to PTB case status using an Analysis of Covariance (ANCOVA) model. We controlled for several covariates, selected *a priori* due to known association with PTB, including maternal age, race, prepregnancy body mass index, household income, and earliest prior PTB. The ANCOVA analysis was conducted with Partek Genomic Suite 6.4 (St Louis Missouri). CpG sites corresponding with the significant genes on the nitric oxide signaling pathway gene array were evaluated. To determine if CpG methylation was associated with the expression of genes involved in the nitric oxide pathway, Pearson correlation coefficients and associated p-values were calculated, comparing the placental methylome between early recurrent PTB cases and term controls. Statistical significance was set at Bonferroni-corrected p-value threshold of 0.05.

RESULTS

A total of 17 women met the inclusion criteria for the study. There were 7 preterm cases (delivered at 23.9 +/− 5.1 weeks, range 17.9–33.4 weeks) and 10 term controls (delivered at 38.5 +/− 0.8 weeks). The groups were similar with regards to PTB history, race/ethnicity, and socioeconomic risk factors for PTB (Table 1). All were self-reported non-smokers during pregnancy. Placentas were processed and frozen at similar times post-delivery for cases and controls (13.0 +/− 13.7 hours for cases and 24.0 +/− 6.2 hours for controls, $p=0.23$).

The expression of 84 genes in the nitric oxide pathway was compared between preterm and term placentas. Twenty-seven nitric oxide genes were differentially expressed ($p<0.05$ and $q<0.10$) when comparing placentas from preterm cases and term controls; all were down-regulated in preterm cases (Table 2). The five most significant differentially expressed genes in the preterm placentas relative to term placentas were *PRDX2* (Peroxiredoxin 2; $p=0.0012$, $q=0.024$), *SIRT2* (Sirtuin 2; $p=0.0015$, $q=0.024$), *CAT* (catalase; $p=0.0033$, $q=0.031$), *PNKP* (polynucleotide kinase 3′ phosphatase; $p=0.0059$, $q=0.031$), and *CCS* (coper chaperone for superoxide dismutase; $p=0.0065$; $q=0.031$). Differentially expressed genes were compared to a list of 506 genes published by Winn et al, known to have differential expression in placenta between mid-pregnancy and term.[11] We found 3 of the 27 differentially expressed genes overlapped with those found by Winn et al to be associated with gestational age, including *PRDX2* (Peroxiredoxin 2), *EGFR* (epidermal growth factor receptor), and *GLRX2* (glutaredoxin 2).

The 27 differentially expressed genes in the placenta were subsequently analyzed for association with changes in CpG methylation in the placenta. A total of 860 CpG sites corresponding to the 27 genes were found to be differentially methylated between the preterm cases and term controls (Bonferroni p-value <0.05). A nearly equal proportion of differentially methylated sites were hypomethylated (decreased, 47%) as were hypermethylated (increased, 53%) in progesterone responders compared to progesterone non-responders. The number of differentially methylated sites in each of these 27 genes is displayed, by gene, in Table 3. The mean fold change for progesterone responders vs. non-responders is also shown in Table 3. Finally, the leading CpG methylation results (according to the Bonferroni corrected p-value) for each of the 27 genes is shown in Table 4.

COMMENT

We found differences in the methylation and gene expression patterns in nitric oxide pathway genes in term vs. preterm placentas following exposure to 17-OHPC. Gene expression changes in the placenta at delivery are associated with changes in CpG methylation in the placenta at delivery. These data provide further evidence implicating nitric oxide pathways in recurrent PTB among women on 17-OHPC. Since all women received 17-OHPC, we are unable to compare placentas among those with recurrent PTB with and without 17-OHPC exposure. We speculate that the observed differences are not a direct effect from 17-OHPC exposure, but may reflect a certain pattern of methylation and/or gene expression changes in placentas of those destined to respond or not respond to progesterone therapy. However, our methodology does not allow us to directly understand the interaction between 17-OHPC exposure, gene expression, CpG methylation, and birth outcomes.

Our finding of differential gene expression within the nitric oxide pathway is biologically plausible. Specifically, progesterone and nitric oxide are tightly linked, beginning at the establishment of pregnancy.[12] Progesterone is known to stimulate nitric oxide synthesis via transcriptional and non-transcriptional pathways in human endothelial cells.[13–16] Nitric oxide expression generally increases during pregnancy and decreases before parturition,[17] and nitric oxide is known to regulate vasodilation, smooth muscle relaxation, and the inflammatory response during pregnancy. However, myometrial relaxation occurs in a cGMP-independent manner.[18] In other areas of the body (e.g., blood vessels), changes may be activated in a non-genomic manner by estrogen, though there are no specific studies in pregnancy or in the myometrium.[19,20] The bactericidal, viricidal, and fungicidal activity of macrophages is determined by nitric oxide pathways.[21] Inflammation and infection are established etiologies of PTB, particularly early PTB as examined in the current analysis. Though previously thought to be associated only with iatrogenic PTB (e.g., due to pre-eclampsia), recent evidence implicates abnormal placentation, including malperfusion, in spontaneous PTB.[22,23] Finally, nitric oxide influences uterine activity by increasing cyclic guanosine monophosphate, which in turn relaxes uterine myometrium.[24–26]

In our previous work evaluating 17-OHPC pharmacogenomics, we found a relationship between maternal NOS1 genotype and NO pathway genes and 17-OHPC responder status. However, it is difficult to compare genotype results (DNA) with gene expression results

(RNA) and epigenetics (CpG methylation), particularly across subjects (maternal vs. fetal genetics). In one of the few studies of the contribution of fetal gene expression to PTB, Vora, et al found higher fetal gene expression of nitric oxide synthase 1 and d-aspartate oxidase in mid-pregnancy amniotic fluid supernatant among pregnancies destined to deliver preterm compared to term.[27] However, in the Vora study, the focus was not on pharmacogenomics and no information is available regarding progesterone supplementation. Further, the NOS3 gene was not one of the 65 genes evaluated on their custom panel.

Many of the evaluated genes also have biologic plausibility for PTB. Space limitations preclude in-depth discussion of all genes of interest. Notably, however, two genes in the peroxiredoxin family of genes (*PRDX2* and *PRDX5*) were downregulated in preterm placentas compared with term placentas. The family of peroxiredoxin genes has previously been implicated in cervical ripening[28,29] and the presence of *PRDX2* is considered crucial to regulation of oxidative stress at the placental level.[30,31] We speculate that loss of this protective mechanism to counteract oxidative stress in preterm mothers and placentas may have contributed to recurrent PTB. However, since Winn, et al. found gestational age variation in *PRDX2*,[11] we cannot exclude this as a possible explanation for our result. We also found that expression of SIRT2 was downregulated in preterm placentas. In a study of women with severe, early onset pre-eclampsia, Hannan and colleagues reported that *SIRT2* localizes to the syncytiotrophoblast, villous leukocytes, and vasculature in preterm placentas, and that those with severe pre-eclampsia and fetal growth restriction have reduced *SIRT2* protein expression.[32] Further, in a study of the relationship between transcriptomic profiles of chorioamniotic membranes of preterm neonates with and without neurologic impairment at age 2, *SEPP1* (Selenoprotein P, plasma 1)

This study should be interpreted with several limitations in mind. We were limited by the overall small size of the cohort. Despite our sample size, however, the results were significant even when performing Bonferroni correction for the large number of tests. Nonetheless, these findings should be confirmed in larger studies. Further, it is possible that our findings were confounded by gestational age. However, this confounding was mitigated somewhat by comparing differentially expressed genes to a known database of genes with changes in expression associated with gestational age and finding little overlap. Additionally, the majority of women in this cohort had a history of one or more pre- or peri-viable deliveries. It is uncertain whether these results would therefore apply to a more generalized PTB population with less severe phenotypes. Due to logistic considerations, placentas were processed up to 48 hours post-delivery. Traditionally, immediate processing to stabilize RNA has been considered the gold standard, but recent research has shown that storage at room temperature (4 degrees C) for up to 48 hours prior to dissection and freezing does not alter RNA quality.[33] Finally, it is possible that differences in exposure to progesterone may have impacted our results. However, though some women received both 17-OHPC combined with vaginal progesterone, the exposure to vaginal progesterone is equal in both groups and therefore not expected to alter results. Women who delivered at term most likely received their last 17-OHPC injection 1–3 weeks prior to delivery, whereas those who delivered preterm most likely received their last 17-OHPC injection within a week of delivery. The half-life of 17P is approximately 10 days,[34] so even those women who received their last injection at 36 weeks 0 days gestation would still be expected to have 25%

of their circulating 17-OHPC at 39 weeks (2 half-lives), and we do not anticipate that gene expression and methylation changes occur that rapidly following subsequent “17P withdraw.” Further, as described above, we posit that placental methylation and gene expression changes may not be in direct ‘response’ to 17P but that a certain pattern of methylation and gene expression may be associated with response or non-response.

This study also had several strengths. Our findings provide consistent evidence of a relationship between genetic variation in nitric oxide pathways and recurrent PTB among women exposed to 17-OHPC, a biologically plausible hypothesis. By linking CpG methylation and gene expression in the same samples, we provide a possible mechanism by which genes are turned ‘on’ or ‘off,’ given that methylation within promotor regions of genes typically represses gene transcription. Our study is strengthened by the exclusion of women who delivered between 34–37 weeks’ gestation (“late preterm”), a time period when the etiology for PTB is traditionally very heterogenous, and may include non-spontaneous indications. Our approach, evaluating placental tissue and then examining both gene expression and CpG methylation is integrative, evaluating not only the end result of gene expression changes but the likely biologic changes contributing to them.

In conclusion, we found distinct changes in placental nitric-oxide pathway gene expression and CpG methylation in women with recurrent PTB on 17-OHPC. In the future, evaluation of whether these epigenetic changes are also present in the mid-trimester of women at highest risk of PTB may provide the basis for development of diagnostic tests to identify women at risk for recurrent PTB despite 17-OHPC prophylaxis. Future studies might also investigate whether there are changes at the level of the maternal decidua and/or uterus from women delivering by cesarean section. This has the potential to lead to the testing of alternative therapies for women who are likely to deliver preterm despite 17-OHPC.

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Table 1

Baseline characteristics of recurrent preterm birth cases and term controls. Data are presented as n(%) unless specified.

Characteristic	Recurrent Preterm Birth – Cases N=7	Term delivery – Controls N=10	p-value
Maternal age (mean years, ± SD)	34.9 ± 5.2	32.4 ± 7.1	0.45
Race/ethnicity, n(%)			
Non-Hispanic Black	3 (43)	4 (40)	0.955
Non-Hispanic White	1 (14)	2 (20)	
Hispanic	3 (43)	4 (40)	
Maternal prepregnancy body mass index (median kg/m^2, IQR)	28.9 (23.2, 45.6)	32.0 (24.6, 33.1)	0.412
High school education or greater, n(%)	4 (57)	9 (90)	0.25
History of abnormal pap smear, n(%)	4 (57.1)	3 (30.0)	0.350
History of excisional cervical procedure, n(%)	2 (28.6)	1 (10.0)	0.537
Married, n(%)	6 (85.7)	9 (90.0)	>0.99
Subject herself was born preterm or has first degree relative with history of preterm delivery, n(%)	2 (28.6)	3 (30.0)	>0.99
Gestational age of earliest prior preterm birth (median weeks, IQR)	20.0 (17.3, 32.1)	22.1 (19.0, 27.1)	0.843
Diagnosed with sexually transmitted vaginal infection (gonorrhea, chlamydia, or trichomonas) this pregnancy, n(%)	1 (14.3)	2 (20.0)	>0.99
Used vaginal progesterone >14 weeks gestation, n (%)	3 (33)	1 (10)	0.30
Cerclage this pregnancy	5 (56)	4 (40)	0.656
Cervical length assessed by transvaginal ultrasound this pregnancy, n(%)	6 (85.7)	10 (100)	0.412
Shortest measured cervical length (median mm, IQR)	8.5 (3–24)	36 (33–45)	0.106
Gestational age at initiation of 17-alpha hydroxyprogesterone caproate (median weeks, IQR)	16.7 (16.4–18.4)	16.9 (16.0–18.0)	0.601

Table 2

Significant placenta nitric oxide pathway gene expression results

Gene Symbol	Gene Name	p-value	q-value	Fold change (preterm vs. term)
PRDX2	Peroxiredoxin 2	0.0012	0.024	−1.043
SIRT2	Sirtuin 2	0.0015	0.024	−1.020
CAT	Catalase	0.0033	0.031	−1.066
PNKP	Polynucleotide kinase 3′-Phosphatase	0.0059	0.031	−1.064
CCS	Copper chaperone for superoxide dismutaase	0.0065	0.031	−1.003
NUDT1	Nudix hydrolase 1	0.0067	0.031	−1.002
GPX1	Glutathione peroxidase 1	0.0069	0.031	−1.391
SEPP1	Selenoprotein P, plasma 1	0.010	0.039	−1.003
AKT1	AKT serine / threonine kinase 1	0.015	0.045	−1.198
EGFR	Epidermal growth factor receptor	0.018	0.045	−3.289
DYNLL1	Dynein light chain LC8-Type 1	0.018	0.045	−1.417
MTL5	Tesmin	0.019	0.045	−1.002
NOS3	Nitric oxide synthase 3	0.021	0.045	−1.138
RNF7	Ring finger protein 7	0.021	0.045	−1.115
PRDX5	Peroxiredoxin 5	0.022	0.045	−1.368
HSP90AB1	Heat shock protein 90 alpha family class B member 1	0.023	0.045	−1.418
GLRX2	Glutoaredoxin 2	0.024	0.045	−1.042
DUSP1	Dual specificity phosphatase 1	0.032	0.053	−1.628
GPX5	Glutathione peroxidase 5	0.034	0.053	−1.001
SGK2	Serine / Threonine kinase 2	0.035	0.053	−1.002
GPX4	Glutathione peroxidase 4	0.035	0.053	−1.193
GPX2	Glutathione peroxidase 2	0.039	0.056	−1.002
ALOX12	Arachidonate 12-lipoxygenase, 12S type	0.042	0.056	−1.003
MBL2	Mannose binding lectin 2	0.047	0.056	−1.001
KRT1	Keratin 1	0.047	0.056	−1.001
DUOX1	Dual oxidase 1	0.048	0.056	−1.010
OXR1	Oxidation resistance 1	0.048	0.056	−1.034

Table 3

Significant placenta nitric oxide pathway methylation results corresponding to top 27 differentially expressed nitric oxide pathway genes.

Gene Symbol	Number of differentially methylated CpG sites (progesterone responder vs. non-responder)		Mean fold change, progesterone responder vs. non-responder (range)	
	Down	Up	Down	Up
PRDX2	12	7	−1.47 (−5.69 to −1.002)	1.20 (1.003 to 1.51)
SIRT2	17	15	−1.16 (−2.12 to −1.0006)	1.16 (1.002 to 2.003)
CAT	15	17	−1.21 (−2.44 to −1.10)	1.28 (1.01 to 2.58)
PNKP	10	6	−1.14 (−1.48 to −1.01)	1.13 (1.02 to 1.46)
CCS	5	3	−1.23 (−1.79 to −1.01)	1.14 (1.014 to 1.300)
NUDT1	13	10	−1.59 (−6.69 to −1.0004)	1.82 (1.008 to 7.99)
GPX1	10	11	−1.05 (−1.10 to −1.002)	1.19 (1.004 to 1.89)
SEPP1	3	5	−1.23 (−1.47 to −1.04)	1.17 (1.00 to 1.50)
AKT1	26	34	−1.33 (−6.16 to −1.0001)	1.24 (1.008 to 2.28)
EGFR	56	53	−1.19 (−3.16 to −1.0009)	1.19 (1.002 to 3.08)
DYNLL1	13	21	−1.21 (−2.93 to −1.0003)	1.24 (1.008 to 2.40)
MTL5	15	16	−1.15 (−1.88 to −1.007)	1.63 (1.03 to 6.92)
NOS3	17	20	−1.12 (−1.58 to −1.004)	1.20 (1.004 to 1.60)
RNF7	10	8	−1.08 (−1.22 to −1.005)	1.07 (1.003 to 1.30)
PRDX5	13	9	−1.14 (−1.45 to −1.009)	1.23 (1.005 to 2.63)
HSP90AB1	15	12	−1.20 (−1.53 to −1.02)	1.19 (1.04 to 1.60)
GLRX2	10	11	−1.23 (−1.51 to −1.02)	1.12 (1.001 to 1.34)
DUSP1	18	15	−1.07 (−1.36 to −1.001)	1.23 (1.001 to 1.72)
GPX5	18	18	−1.13 (−1.66 to −1.002)	1.17 (1.002 to 1.98)
SGK2	18	18	−1.31 (−2.92 to −1.006)	1.16 (1.004 to 2.17)
GPX4	9	14	−1.20 (−1.47 to −1.03)	1.25 (1.0003 to 1.80)
GPX2	6	6	−1.32 (−2.54 to −1.03)	1.23 (1.01 to 1.63)
ALOX12	12	24	−1.35 (−3.00 to −1.02)	1.34 (1.003 to 2.82)
MBL2	5	5	−1.42 (−2.50 to −1.03)	1.14 (1.05 to 1.22)
KRT1	6	11	−1.19 (−1.55 to −1.02)	1.26 (1.01 to 1.86)
DUOX1	25	27	−1.21 (−2.057 to −1.010)	1.24 (1.005 to 4.44)
OXR1	35	52	−1.15 (−2.34 to −1.0003)	1.43 (1.001 to 4.94)

Table 4

Leading CpG methylation results (by Bonferroni corrected p-value) for each of the 27 genes significant in the gene expression analysis.

Gene Symbol	CpG marker	Fold change (progesterone responder vs. non-responder)	p-value (Bonferroni corrected)
PRDX2	cg18074016	−5.69	3.05×10^{-3}
SIRT2	cg16738915	1.05	2.48×10^{-4}
CAT	cg20234170	1.79	2.97×10^{-5}
PNKP	cg25136622	1.46	1.63×10^{-5}
CCS	cg15255291	1.10	2.42×10^{-4}
NUDT1	cg05061208	−1.85	4.64×10^{-4}
GPX1	cg11597332	−1.03	6.29×10^{-3}
SEPP1	cg00886598	−1.48	2.76×10^{-3}
AKT1	cg05726935	−1.21	8.4×10^{-5}
EGFR	cg10002850	−1.03	2.11×10^{-4}
DYNLL1	cg02699780	−2.93	8.22×10^{-4}
MTL5	cg06699275	1.62	9.07×10^{-4}
NOS3	cg24032393	1.58	1.11×10^{-4}
RNF7	cg27296459	−1.22	2.44×10^{-3}
PRDX5	cg23615572	−1.45	2.71×10^{-3}
HSP90AB1	cg16242498	−1.27	8.18×10^{-5}
GLRX2	cg11800710	−1.32	2.35×10^{-5}
DUSP1	cg26095194	1.72	1.69×10^{-4}
GPX5	cg02803996	1.20	2.02×10^{-4}
SGK2	cg03337502	−1.79	3.91×10^{-4}
GPX4	cg10732871	1.14	3.38×10^{-4}
GPX2	cg14947787	1.20	1.57×10^{-3}
ALOX12	cg13647527	−1.151	5.61×10^{-5}
MBL2	cg27418851	−2.50	3.88×10^{-4}
KRT1	cg03348792	1.76	1.37×10^{-4}
DUOX1	cg11230298	−2.06	2.65×10^{-4}
OXR1	cg06438976	−1.22	6.36×10^{-6}