Published in final edited form as:
Am J Obstet Gynecol. 2014 April ; 210(4): 321.e1–321.e21. doi:10.1016/j.ajog.2014.01.013.

OBSTETRICS:

Pharmacogenomics of 17-alpha hydroxyprogesterone caproate for recurrent preterm birth prevention

Tracy A. Manuck, MD, **M. Sean Esplin, MD**, **Michael W. Varner, MD**, and **G. Marc Jackson, MD**
Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, Intermountain Healthcare, University of Utah School of Medicine, Salt Lake City, UT

Department of Obstetrics and Gynecology, University of Utah School of Medicine, Salt Lake City, UT

W. Scott Watkins, MS, **Barry Moore, MS**, **Mark Yandell, PhD**, and **Lynn Jorde, PhD**
Human Genetics, University of Utah School of Medicine, Salt Lake City, UT

Abstract

OBJECTIVE—We hypothesized that genetic variation affects responsiveness to 17-alpha hydroxyprogesterone caproate (17P) for recurrent preterm birth prevention.

STUDY DESIGN—Women of European ancestry with ≥1 spontaneous singleton preterm birth at <34 weeks' gestation who received 17P were recruited prospectively and classified as a 17P responder or nonresponder by the difference in delivery gestational age between 17P-treated and -untreated pregnancies. Samples underwent whole exome sequencing. Coding variants were compared between responders and nonresponders with the use of the Variant Annotation, Analysis, and Search Tool (VAASl), which is a probabilistic search tool for the identification of disease-causing variants, and were compared with a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway candidate gene list. Genes with the highest VAAST scores were then classified by the online Protein ANalysis THrough Evolutionary Relationships (PANTHER) system into known gene ontology molecular functions and biologic processes. Gene distributions within these classifications were compared with an online reference population to identity over and under represented gene sets.

RESULTS—Fifty women (9 nonresponders) were included. Responders delivered 9.2 weeks longer with 17P vs 1.3 weeks' gestation for nonresponders ($P < .001$). A genome wide search for genetic differences implicated the NOS1 gene to be the most likely associated gene from among genes on the KEGG candidate gene list ($P < .00095$). PANTHER analysis revealed several over represented gene ontology categories that included cell adhesion, cell communication, signal

---

The authors report no conflict of interest.

Presented, in part, in oral format at the 34th annual meeting of the Society for Maternal-Fetal Medicine, New Orleans, LA, Feb. 3-8, 2014.

The racing flag logo above indicates that this article was rushed to press for the benefit of the scientific community.

transduction, nitric oxide signal transduction, and receptor activity (all with significant Bonferroni-corrected probability values).

CONCLUSION—We identified sets of over-represented genes in key processes among responders to 17P, which is the first step in the application of pharmacogenomics to preterm birth prevention.

Keywords

pharmacogenomics; progesterone; spontaneous preterm birth

More than 12% of babies are born preterm, which accounts for >70% of neonatal morbidity and deaths among nonanomalous infants in the United States.[1] Spontaneous preterm birth (SPTB) accounts for 50-80% of all preterm infants. Despite the magnitude of this clinical problem, few preventative or acute therapeutic interventions have proved effective; 17 alpha-hydroxyprogesterone caproate (l7P) is one notable exception. When administered weekly beginning in the mid trimester, intramuscular l7P injections are effective prophylaxis against recurrent SPTB.[2] Unfortunately, prophylactic l7P is not always effective, and two-thirds of high-risk women will have a recurrent preterm birth, despite l7P therapy.

There is strong evidence that genes contribute to SPTB susceptibility. SPTB recurs in 35-50% of women and tends to recur at similar gestational ages.[3] Likewise, the probability of SPTB increases with the number of previous SPTB that a woman has experienced, with the most recent birth being the most predictive.[4] Women who themselves were born prematurely at <30 weeks' gestation are more likely to deliver premature infants (odds ratio, 2.38; 95% confidence interval, 1.37–4.16).[5] Population studies have demonstrated ahigher rate of SPTB among African American women, even when adjustment is made for epidemiologic risk factors, which again suggests genetics as a contributing factor.[6,7] The heritable nature of this complication is further supported by the findings that the risk of SPTB is elevated in women whose sisters have experienced an SPTB (odds ratio, 1.94; 95% confidence interval, 1.26–2.99).[8]

Multiple candidate gene studies have demonstrated associations between several genes and SPTB, with genes involved in inflammation and coagulation pathways most commonly implicated.[9-15] Despite this knowledge, few studies have examined the role of genetic variation in the response to medications that are given for the prophylaxis or treatment of SPTB. An understanding of the reason that some women respond to 17P and others do not is crucial to understanding the cause of SPTB and optimizing prediction and therapeutic strategies.

We hypothesize that genetic variation influences this variability in response to 17P, particularly among women with more severe SPTB phenotypes (early <34 weeks' gestation and/or recurrent SPTB). Specifically, we sought to interrogate the coding regions of the genome and compare genotypes between women with recurrent SPTB despite 17P prophylaxis with those who have a favorable response to 17P. Additionally, we aimed to determine whether there are gene sets that are represented differentially when comparing those genes with the greatest variation between these 2 groups of women.

Materials and Methods

This was a case-control genetic association study. Women of non-Hispanic European ancestry with at least 1 previous documented singleton, nonanomalous SPTB who delivered at <34.0 weeks' gestation who received 17P in at least 1 subsequent pregnancy were recruited prospectively from a consultative Preterm Birth Prevention Clinic from 2008-2010 at Intermountain Medical Center (Salt Lake City, UT). The treatment of women in the prematurity prevention clinic has been described previously.[16] All women provided written informed consent, and this study was approved by the institutional review boards at Intermountain Healthcare and the University of Utah.

Women with a history of either idiopathic spontaneous onset of contractions and cervical dilation or preterm premature rupture of membranes (PPROM) in a previous pregnancy were considered to have a previous SPTB and were included. We excluded women with a known or suspected cause of SPTB, which included women who experienced SPTB after polyhydramnios, within 2 weeks of amniocentesis, because of hypertensive disorders that included preeclampsia or were related to abdominal trauma. Women with known uterine anomalies or a history of treatment for cervical dysplasia with cryotherapy, loop electrosurgical excision procedure, or cervical conization were also excluded.

Women were classified as a 17P responder or nonresponder based on response to 17P. Specifically, the difference between the earliest delivery gestational age because of SPTB without 17P and the delivery gestational age with 17P was calculated and was termed the "17P effect." If a woman had multiple pregnancies that had been treated with 17P after her initial SPTB, the 17P effects from each individual pregnancy were averaged to generate an overall 17P effect. Women with an overall 17P effect of ≥3 weeks (ie, the individual's pregnancy or pregnancies that had been treated with 17P delivered at least 3 weeks later compared with the gestational age of the earliest SPTB without 17P treatment) were considered 17P responders. Women with a negative overall 17P effect and those with an overall 17P effect of <3 weeks were classified as nonresponders. Demographic data were compared between responders and nonresponders with the Student *t* test and Fisher exact test, as appropriate (version 12.1; StataCorp LP, College Station, TX).

DNA was extracted from stored buffy coats. All extracted DNA underwent quality control with a spectrophotometer (NanoDrop Products, Wilmington, DE) reading and evaluation on a 1% agarose gel before genomic library construction. Genomic libraries were then constructed, and samples underwent additional quality control measures that included quantitative polymerase chain reaction quantitation of library concentration with primers (Illumina Inc, San Diego, CA) and evaluation of the library on an Agilent Bioanalyzer DNA 1000 chip (Agilent Technologies Inc, Santa Clara, CA). A PhiX control library (Illumina Inc) was spiked into each lane at a concentration that represented approximately 0.5% of the reads. This platform targeted approximately 180,000 protein-coding exons, in approximately 20,000 genes, for capture. Whole exome sequencing was then performed at The University of Utah Huntsman Cancer Institute's Microarray Core Facility with Illumina HiSeq2000 (Illumina Inc) technology. We indexed 4 samples per lane, with a goal of approximately ×40-50 average depth of coverage per sample.

Sequences were then called simultaneously on all samples with the University of Utah Department of Human Genetics variant-calling software pipeline. Paired-end 101 base pair fastq reads were aligned to the reference genome (b37) with the Burrows-Wheeler aligner software.[17] Additional processing that included sorting, mate-fixing, and duplicate read removal was performed with Samtools and Picard Tools.[18] Insertion and deletion realignment and base recalibration was performed with the Genome Analysis Tool Kit (Broad Institute, Cambridge, MA).[19,20] Processed call-ready BAM files were called jointly with the Unified Genotyper (Broad Institute). Raw genotypes were evaluated and filtered with the Variant Quality Score Recalibrator that is provided in the Genome Analysis Tool Kit package.

Individual exomes were analyzed for evidence of population stratification with Eigenstrat software (Harvard School of Public Health, Boston, MA).[21] Single nucleotide polymorphisms with a minor allele frequency of <0.05 and/or a strong deviation from Hardy-Weinberg equilibrium ($P < .00001$) were removed. Single nucleotide polymorphisms were also filtered to remove all single nucleotide polymorphisms with pairwise linkage disequilibrium of $r^2 > 0.2$. Population stratification was then analyzed among the remaining subjects, and individual outliers were excluded from further analysis.

The remaining exomes were compared between 17P responders and nonresponders using the Variant Annotation, Analysis, and Search Tool (VAAST). VAAST is a publicly available probabilistic search tool for the identification of disease-causing variants. VAAST scores coding variants that are based on the allele and amino acid substitution frequencies differences between case and control genomes have been demonstrated to be effective in the identification of causative disease alleles both in cases of rare variants in rare disease and combinations of rare and common variants in common disease.[22,23] The VAAST analysis produced a “raw” list of genes that was prioritized by the likelihood of allelic differences between 17P responders and nonresponders. We reported raw probability values (uncorrected for multiple comparisons) for our VAAST analysis gene list.

Next, the genes that were obtained from the VAAST analysis were compared with those potential candidate genes. Using the online Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database, we compiled a list of possible candidate genes that we suspected to be involved in either the pathogenesis of prematurity (ie, genes in pathways that affect inflammatory response, myometrial contraction/relaxation, oxidative stress, coagulation, and complement, calcium signaling) or the metabolism of 17P (ie, genes in pathways that involve steroid receptors, drug metabolism, steroid degradation). This list of candidate genes was compared with the raw list of VAAST genes.

In the next step of our pathway analysis, we again used the raw VAAST list of genes and selected the top 2.5% of genes with the highest VAAST scores from that list. Each gene on this raw list was classified by the online Protein Analysis THrough Evolutionary Relationships (PANTHER) system into known gene ontology molecular functions and biologic processes.[24,25] The percentage of genes within each molecular function/biologic process category was compared with an online referent population to search for areas of over- and under-representation with the use of the binomial test that is available through

PANTHER tools online.[26] A Bonferroni-corrected probability value < .05 for the binomial test was considered significant.

Results

Fifty-six women met initial inclusion criteria. All were of self-reported European ancestry. Of these, sequencing analysis failed in 2 women (average sequencing depth of coverage <1). On initial Eigenstrat analysis, population differentiation between cases and control subjects was not significant ($P > .09$). However, 4 samples deviated substantially from the main cluster of points. Removal of these 4 samples produced a much less stratified data set with little genome-wide differentiation between cases and control subjects ($P > .35$). Thus, the final cohort consisted of 50 women (41 responders and 9 nonresponders). All remaining samples met genotype quality filters. The average depth of exome coverage was 51 ± 18 base pairs (range, 13.4–102.6 base pairs).

Demographic and previous pregnancy characteristics were similar between responders and nonresponders with regard to parity, number of preterm births before the studied gestation, cervical insufficiency history, and PPROM history (Table 1). Four women (3 responders, 1 nonresponder) had a history of a cervical laceration that was related to delivery; this was not significantly different between groups ($P = .56$). This group of women was generally healthy. None of the women had a history of type I or II diabetes mellitus or chronic renal disease. Four women (8%) had a history of chronic hypertension; none of the women experienced preeclampsia or required preterm delivery because of worsening hypertension.

Responders delivered an average of +9.2 weeks later (range, +3.8 to +16.9 weeks) with 17P compared with +1.3 weeks (range, −1.9 to +2.9 weeks) for nonresponders ($P < .001$). Two women delivered earlier with 17P: 1 woman delivered 11 days earlier, and the other woman delivered 13 days earlier. There were no indicated preterm deliveries in the study population. Pregnancy management was similar between responders and nonresponders. Nonresponders delivered earlier and were more likely to be preterm (Table 2).

In our VAAST analysis, we allowed for recessive inheritance and locus heterogeneity and tested our genotypes using 1 million permutations. The genes with the greatest difference between responders and nonresponders are listed in Table 3 and represent the raw VAAST list of genes. The probability values that are displayed in Table 3 are unadjusted; none meet genome-wide significance ($P < 2.5 \times 10^{-6}$). Using the KEGG database as described earlier, we generated a list of 518 candidate genes in pathways that potentially are involved with SPTB and/or 17P metabolism (Supplementary Table 1). This list of 518 genes was compared with the raw list of genes from our VAAST analysis. The NOS1 gene was the eighth highest scoring gene on the overall raw VAAST list and was the highest scoring variant from genes on the KEGG candidate gene list (VAAST, $P < .00095$).

Next, the top 2.5% of raw genes (n = 457) that were generated from the initial VAAST analysis were selected for further analysis with the use of PANTHER (Supplementary Table 2). These top 457 genes were compared with the online referent population that was provided by PANTHER. This analysis revealed several differentially expressed biologic

processes (Table 4) from our gene list compared with the referent population. The frequencies of genes that were classified by pathway or function in our gene list compared with expected proportions in a general population are also given in Table 4. For example, based on gene distributions within the referent population, the expected frequency of genes in the nitric oxide pathway is 0.002*457 = approximately 1. However, from our list of 457 top genes that were identified by VAAST, the frequency of genes in the nitric oxide pathway was 0.02*457 = approximately 9 (Table 4). We identified 8 genes in the nitric oxide synthase pathway with different allele frequencies between responders and nonresponders; our list included SPTA2, GA2L2, DMD, SYNE1, NOS1, MICA2, SMTL2, and DESP. All statistically significant pathways that are listed in Table 4 were over-represented in our gene list. There were no under-represented biologic processes from among our top gene list.

Comment

Using a novel analytic approach, we have identified over-represented genes in key processes among responders to 17P, which is the first step in applying pharmacogenomics to preterm birth prevention. Our multistep approach identified both new individual candidate genes and general biologic processes (including functions such as cell adhesion and cell communication) that may influence an individual's response to prematurity prevention.

In both pathway analysis approaches, genes that were involved with nitric oxide were identified as potential mediators of 17P response. Nitric oxide synthase catalyzes the synthesis of nitric oxide from L-arginine. Animal model studies have shown that nitric oxide has a potent relaxant effect on uterine smooth muscle cells.[27,28] Nitric oxide metabolites have been found in higher concentrations among women in labor (both at term and preterm) compared with nonlaboring women.[29] Additionally, nitric oxide is thought to work synergistically with progesterone to inhibit uterine contractility. In humans, a functional withdrawal of progesterone (through changes in progesterone receptor expression) combined with decreased synthesis of nitric oxide is associated with the initiation of term and preterm parturition.[29-31] In 1 study, treatment of pregnant rats with a combination of an antiprogestin and a nitric oxide inhibitor induced preterm labor significantly faster than treatment with an antiprogestin alone.[32]

We have found that genotype nitric oxide pathway genes differ among women who do not respond to 17P for prematurity prevention, which provides additional insight into the possible mechanism of action of 17P. Transdermal nitroglycerin (a nitric oxide donor) has received some recent attention as a possible acute tocolytic, with mixed results. In one small randomized controlled trial of 158 women, it was found to reduce the risk of severe neonatal morbidity and death, although it was not associated with a prolongation of gestation.[33] In another study, it was not found to be effective.[34] To our knowledge, the role of nitroglycerin in preterm prevention or as an adjunct to 17P has not been investigated.

Our study has several strengths. This cohort included women with more severe SPTB phenotypes who were more likely to have a recurrent preterm birth compared with those with a single preterm birth or a preterm birth at a later gestational age. Additionally, our

cohort was ethnically homogeneous, because all women were of European ancestry, which was confirmed by our population stratification analysis. Because preterm birth is a complex phenotype and is likely the final common pathway that results from a variety of different initial causes or triggers and the relationship between genotype and clinical response is equally complex, it is unlikely that a single gene will be responsible for SPTB or for response to tocolysis. Therefore, our exhaustive interrogation of the coding regions of the genome was more comprehensive than previous candidate-gene studies and genome-wide association studies. Our pathway analysis also allowed for some refinement of results by considering genes that were involved in suspected SPTB pathways and 17P metabolic pathways.

Our classification of women into responder and nonresponder groups was objective and clinically relevant. Neonates who were delivered by women who gained at least 3 weeks gestation with 17P would be expected to have a clinically significant decrease in morbidity. One major limitation of our study was the small sample size. Unfortunately, because of sample size limitations, we were unable to conduct subgroup analyses of women with and without specific SPTB phenotypes, such as PPROM. Although the overall sample size was relatively small, our exome sequencing provided information regarding all coding regions of the genome. No single gene in our raw VAAST list results reached formal genome wide significance, but this was expected, given our relatively small sample size. However, the pathway analysis provides broad insight into this complex phenotype; our raw VAAST results and PANTHER pathway analysis did not rely on investigator assumptions regarding possible causative pathways. To date, few studies have undertaken a comprehensive, unbiased analysis of the genome to search for variants that are involved with obstetric pharmacogenomics.

Our study, like any sequencing study, was also limited by the genotyping depth of coverage, because samples with lower depths of coverage are more prone to genotyping errors. As genotyping technology continues to improve and the depth of coverage increases in the future, this will become less of a concern. Furthermore, functional data that are obtained through databases such as PANTHER depend on general genetic knowledge that is unrelated to pregnancy or preterm birth. The genes that are identified have functions that are cell and tissue specific, and the environment of pregnancy alone may alter function. It is beyond the scope of this investigation to study the pharmacokinetics and pharmacodynamics of 17P thoroughly. For example, it is possible that genotype alters response only in the presence of a certain critical threshold or serum level of 17P, but 17P concentration levels were not available for this cohort.

Physiologic changes during pregnancy result in the alteration of many processes, which include drug metabolism. Sharma et al[35] found that 17P metabolism is mediated by the cytochrome p450 enzyme system, specifically the CYP3A family. Genes that are involved in progesterone metabolism were not among the “top hits” from our VAAST analysis. Previous studies suggest that significant interindividual variability exists and that metabolic activity of these cytochromes varies throughout pregnancy.[35] Previous exploratory research in the area of preterm birth pharmacogenomics suggests that progesterone receptor polymorphisms may contribute to variable responsiveness to 17P but that known genetic

variation in this receptor accounts for only a small percentage of the clinical variation that was seen in response to this medication.[36] The results from this study suggest a role for both receptor genotypes and other biologic processes. However, quantification of the response to 17P based on metabolic enzyme polymorphisms, progesterone receptor polymorphisms, or variation in other genetic factors remains difficult and is an area for further research.

If the subset of women most likely to respond to 17P can be identified, women with SPTB could receive individualized treatment that would maximize benefit and limit side-effects. Additional advances in the prevention of SPTB with 17P can be made if women most likely to respond to 17P can be identified prospectively by genotype and if an appropriate individualized prevention regimen can be outlined. If this strategy is applied to nulliparous women with other risk factors for preterm birth (such as a personal history of Müllerian anomalies or a family history of preterm birth), those women with a high-risk genotype could be studied to determine whether treatment with 17P during their first pregnancy could reduce the risk of primary SPTB.

Future studies should confirm these findings in a larger cohort and further refine the role of genetic variation in response to 17P through identification of a specific set of genes that demonstrate the greatest relationship between genotype and response to medications. This has the potential to individualize preterm birth interventions and to lower the overall rate of both primary and recurrent SPTB and its corresponding neonatal morbidity and mortality rates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by grants from the University of Utah Program for Personalized Healthcare, Salt Lake City, UT; the Intermountain Research and Medical Foundation, project #556, Salt Lake City, UT; National Institutes of Health grant number 1K23HD067224; and grant number 8UL1TR000105 from the National Center for Advancing Translational Sciences/National Institutes of Health.

REFERENCES

1. Ananth CV, Vintzileos AM. Epidemiology of preterm birth and its clinical subtypes. J Matern Fetal Neonatal Med. 2006; 19:773–82. [PubMed: 17190687]
2. Meis PJ, Klebanoff M, Thom E, et al. Pre vention of recurrent preterm delivery by 17 alpha hydroxyprogesterone caproate. N Engl J Med. 2003; 348:2379–85. [PubMed: 12802023]
3. Adams MM, Elam Evans LD, Wilson HG, Gilbertz DA. Rates of and factors associated with recurrence of preterm delivery. JAMA. 2000; 283:1591–6. [PubMed: 10735396]
4. Esplin MS, O'Brien E, Fraser A, et al. Esti mating recurrence of spontaneous preterm de livery. Obstet Gynecol. 2008; 112:516–23. [PubMed: 18757647]
5. Porter TF, Fraser AM, Hunter CY, Ward RH, Varner MW. The risk of preterm birth across generations. Obstet Gynecol. 1997; 90:63–7. [PubMed: 9207815]
6. Manuck TA, Lai Y, Meis PJ, et al. Admixture mapping to identify spontaneous preterm birth susceptibility loci in African Americans. Obstet Gynecol. 2011; 117:1078–84. [PubMed: 21508746]

7. Fortunato SJ, Menon R, Velez DR, Thorsen P, Williams SM. Racial disparity in maternal fetal genetic epistasis in spontaneous preterm birth. Am J Obstet Gynecol. 2008; 198:666.e1–9. [PubMed: 18538149]
8. Winkvist A, Mogren I, Hogberg U. Familial patterns in birth characteristics: impact on individual and population risks. Int J Epidemiol. 1998; 27:248–54. [PubMed: 9602406]
9. Crider KS, Whitehead N, Buus RM. Genetic variation associated with preterm birth: a HuGE review. Genet Med. 2005; 7:593–604. [PubMed: 16301860]
10. Ehn NL, Cooper ME, Orr K, et al. Evaluation of fetal and maternal genetic variation in the progesterone receptor gene for contributions to preterm birth. Pediatr Res. 2007; 62:630–5. [PubMed: 17805208]
11. Gibson CS, MacLennan AH, Dekker GA, et al. Genetic polymorphisms and spontaneous preterm birth. Obstet Gynecol. 2007; 109:384–91. [PubMed: 17267840]
12. Velez DR, Fortunato SJ, Thorsen P, Lombardi SJ, Williams SM, Menon R. Preterm birth in Caucasians is associated with coagulation and inflammation pathway gene variants. PLoS One. 2008; 3:e3283. [PubMed: 18818748]
13. Velez DR, Menon R, Thorsen P, et al. Ethnic differences in interleukin 6 (IL 6) and IL6 receptor genes in spontaneous preterm birth and effects on amniotic fluid protein levels. Ann Hum Genet. 2007; 71:586–600. [PubMed: 17346257]
14. Wang H, Parry S, Macones G, et al. A functional SNP in the promoter of the SERPINH1 gene increases risk of preterm pre mature rupture of membranes in African Americans. Proc Natl Acad Sci U S A. 2006; 103:13463–7. [PubMed: 16938879]
15. Manuck TA, Major HD, Varner MW, Chettier R, Nelson L, Esplin MS. Progesterone receptor genotype, family history, and sponta neous preterm birth. Obstet Gynecol. 2010; 115:765–70. [PubMed: 20308837]
16. Manuck TA, Henry E, Gibson J, et al. Pregnancy outcomes in a recurrent preterm birth prevention clinic. Am J Obstet Gynecol. 2011; 204:320.e1–6. [PubMed: 21345407]
17. Li H, Durbin R. Fast and accurate short read alignment with Burrows Wheeler transform. Bioinformatics. 2009; 25:1754–60. [PubMed: 19451168]
18. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAM-tools. Bioinformatics. 2009; 25:2078–9. [PubMed: 19505943]
19. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and geno typing using next generation DNA sequencing data. Nat Genet. 2011; 43:491–8. [PubMed: 21478889]
20. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce frame work for analyzing next generation DNA sequencing data. Genome Res. 2010; 20:1297–303. [PubMed: 20644199]
21. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome wide association studies. Nat Genet. 2006; 38:904–9. [PubMed: 16862161]
22. Wang K, Li M, Hakonarson H. Analyzing biological pathways in genome wide association studies. Nat Rev Genet. 2010; 11:843–54. [PubMed: 21085203]
23. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009; 4:1073–81. [PubMed: 19561590]
24. Mi H, Muruganujan A, Thomas PD. PANTHER in 2013: modeling the evolution of gene function, and other gene attributes, in the context of phylogenetic trees. Nucleic Acids Res. 2013; 41:D377–86. [PubMed: 23193289]
25. Mi H, Muruganujan A, Casagrande JT, Thomas PD. Large scale gene function analysis with the PANTHER classification system. Nat Protoc. 2013; 8:1551–66. [PubMed: 23868073]
26. Cho RJ, Campbell MJ. Transcription, genomes, function. Trends Genet. 2000; 16:409–15. [PubMed: 10973070]
27. Izumi H, Garfield RE. Relaxant effects of nitric oxide and cyclic GMP on pregnant rat uterine longitudinal smooth muscle. Eur J Obstet Gynecol Reprod Biol. 1995; 60:171–80. [PubMed: 7641971]

28. Sladek SM, Regenstein AC, Lykins D, Roberts JM. Nitric oxide synthase activity in pregnant rabbit uterus decreases on the last day of pregnancy. Am J Obstet Gynecol. 1993; 169:1285–91. [PubMed: 7694467]
29. Marinoni E, Di Iorio R, Villaccio B, Alberini A, Rota F, Cosmi EV. Amniotic fluid nitric oxide metabolite levels and nitric oxide synthase localization in feto placental tissues are modified in association with human labor. Eur J Obstet Gynecol Reprod Biol. 2000; 89:47–54. [PubMed: 10733023]
30. Natuzzi ES, Ursell PC, Harrison M, Buscher C, Riemer RK. Nitric oxide synthase activity in the pregnant uterus decreases at parturition. Biochem Biophys Res Commun. 1993; 194:1–8. [PubMed: 7687424]
31. Madsen G, Zakar T, Ku CY, Sanborn BM, Smith R, Mesiano S. Prostaglandins differentially modulate progesterone receptor A and B expression in human myometrial cells: evidence for prostaglandin induced functional progester one withdrawal. J Clin Endocrinol Metab. 2004; 89:1010–3. [PubMed: 14764828]
32. Yallampalli C, Buhimschi I, Chwalisz K, Garfield RE, Dong YL. Preterm birth in rats pro duced by the synergistic action of a nitric oxide inhibitor (NG nitro L arginine methyl ester) and an antiprogestin (Onapristone). Am J Obstet Gynecol. 1996; 175:207–12. [PubMed: 8694053]
33. Smith GN, Walker MC, Ohlsson A, O'Brien K, Windrim R. Randomized double blind placebo controlled trial of transdermal nitroglycerin for preterm labor. Am J Obstet Gynecol. 2007; 196:37.e1–8. [PubMed: 17240225]
34. Bisits A, Madsen G, Knox M, et al. The Randomized Nitric Oxide Tocolysis Trial (RNOTT) for the treatment of preterm labor. Am J Obstet Gynecol. 2004; 191:683–90. [PubMed: 15467526]
35. Sharma S, Ou J, Strom S, Mattison D, Caritis S, Venkataramanan R. Identification of enzymes involved in the metabolism of 17alpha-hydroxyprogesterone caproate: an effective agent for prevention of preterm birth. Drug Metab Dispos. 2008; 36:1896–902. [PubMed: 18573861]
36. Manuck TA, Lai Y, Meis PJ, et al. Progesterone receptor polymorphisms and clinical response to 17 alpha hydroxyprogesterone caproate. Am J Obstet Gynecol. 2011; 205:135.e1–9. [PubMed: 21600550]

TABLE 1

Demographics and previous pregnancy characteristics

Variable	17P responders (n = 41)	17P nonresponders (n = 9)	*P* value
White, n (%)	41 (100)	9 (100)	—
Married, n (%)	36 (88)	6 (67)	.14
Tobacco use, n	0	0	—
Previous pregnancies, n[a]	2.9 ± 1.8	3.4 ± 1.7	.36
≥1 previous term delivery, n (%)	17 (41.5)	4 (44.4)	> .99
Total number of previous preterm (20.0-36.9 wks' gestation) deliveries, n[a]	1.7 ± 0.8	2.1 ± 0.9	.21
History of preterm premature rupture of membranes, n (%)	16 (39)	2 (25)	.69
Cervical cerclage placed in ≥1 pregnancies, n (%)	11 (27)	2 (22)	> .99
Delivery gestational age of earliest previous preterm birth, wk[a]	28.1 ± 4.0	31.5 ± 2.7	.02

17P, 17-alpha hydroxyprogesterone caproate.

Manuck. Pharmacogenomics of 17P for recurrent PTB. Am J Obstet Gynecol 2014.

[a]Data are given as mean ± SD.

TABLE 2

Pregnancy management and outcomes during the most recent pregnancy

Variable	17-alpha hydroxyprogesterone caproate responders (n = 41)	17-alpha hydroxyprogesterone caproate nonresponders (n = 9)	*P* value
Cervical cerclage placed, n (%)	10 (24)	2 (22)	> .99
Vaginal cervical length assessed at least once during pregnancy, n (%)	35 (92)	8 (89)	> .99
Delivery gestational age, wk[a]	37.3 ± 1.8	32.9 ± 3.7	< .001
Delivered <37 weeks' gestation, n (%)	16 (39)	8 (89)	.009
Delivered <32 weeks' gestation, n (%)	0	3 (33)	.004
Birthweight, g[a]	3001 ± 544	2094 ± 755	< .001

Manuck. Pharmacogenomics of 17P for recurrent PTB. Am J Obstet Gynecol 2014.

[a]Data are given as mean ± SD.

TABLE 3

Top VAAST genes with greatest difference between responders and nonresponders

Rank	Gene	Gene name	*P* value[a]	VAAST	Gene function (if known)[b]
1	CUBN	Cubilin	7.32e-5	23.3	Receptor for intrinsic factor-vitamin B12 complexes
2	TMTC1	Transmembrane and tetra-tricopeptide repeat containing 1	9.40e-5	17.1	
3	TMEM158	Transmembrane protein 158	.00035	23.8	Surface receptor proposed to function in neuronal survival pathway
4	ATMIN	ATM interactor	.00041	6.1	
5	MYOG	Myogenin	.00056	13.0	
6	NLRP10	NLR family pyrin domain containing 10	.00057	13.4	Multifunctional negative regulator of inflammation and apoptosis
7	ENP1	Essential nuclear protein 1	.00094	13.8	
8	NOS1	Nitric oxide synthase 1	.00094	11.2	Synthesizes nitric oxide from L-arginine
9	PRMT6	Protein arginine methyltransferase 6	.0011	8.5	Stimulates polymerase activity by enhancing DNA binding and processivity
10	ZFP28	Zinc finger protein	.0023	10.7	
11	CASZ1	Castor zinc finger 1	.00298	15.3	Tumor suppression, blood pressure variation
12	VPS13C	Vacuolar protein sorting 13 homolog C	.00301	11.4	
13	FCGR2A	Fc fragment of IgG, low affinity IIa receptor	.00305	14.6	Found on phagocytic cells and involved with clearing of immune complexes
14	OR56A5	Olfactory receptor family 56 subfamily A member 5	.00318	13.0	Smell perception
15	RPA4	Replication protein A4	.00337	5.5	DNA double-strand break repair, inhibition of viral replication
16	ZNF853	Zinc finger protein 853	.00352	5.3	
17	TSKS	Testis specific serine kinase substrate	.00373	8.0	Tumorigenesis pathways and progression
18	MICAL2	Microtubule associated monooxygenase, calponin and LIM domain containing 2	.00384	10.2	
19	CCDC50	Coiled-coil domain containing 50	.00401	11.1	Hearing loss, effector of epidermal growth factor–mediated cell signaling
20	ANP32D	Acidic nuclear phosphoprotein 32 family member D	.00408	7.5	Tumor suppressor

Rank	Gene	Gene name	*P* value[a]	VAAST	Gene function (if known)[b]
21	RALGAPA1	Ral GTPase activating protein alpha subunit 1	.00409	8.1	
22	C16orf46	Chromosome 16 open reading frame	.00409	5.9	
23	ATP6V0A2	ATPase lysosomal V0 subunit A2	.00425	7.4	Cutis laxa type II and wrinkly skin syndrome
24	SPRR1A	Small proline-rich protein 1A	.0457	4.9	
25	PIH1D1	PIH1domain containing 1	.00464	2.1	

VAAST, Variant Annotation, Analysis, and Search Tool.

Manuck. Pharmacogenomics of 17P for recurrent PTB. Am J Obstet Gynecol 2014.

[a]Unadjusted for multiple comparisons;

[b]Gene functions per National Center for Biotechnology Information gene database (ncbi.nlm.nih.gov).

TABLE 4

Pathway analysis results

Biologic process or molecular function	Frequency in referent group	Frequency among top VAAST genes	*P* value[a]
Cell adhesion	0.06	0.12	.0004
Cell communication	0.21	0.30	.0015
Signal transduction	0.20	0.28	.0021
Nitric oxide signal transduction	0.002	0.02	.0037
Receptor activity	0.09	0.15	.0110

Protein ANalysis THrough Evolutionary Relationships gene ontology pathway analysis results compared the distribution of the leading genes that were identified by the VAAST within known molecular functions with the biologic processes to evaluate for over- and under-representation.

VAAST, Variant Annotation, Analysis, and Search Tool.

Manuck. Pharmacogenomics of 17P for recurrent PTB. Am J Obstet Gynecol 2014.

[a] After Bonferroni correction for multiple testing.