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The use of high-dimensional biology (genomics, transcriptomics, proteomics, and metabolomics) to understand the preterm parturition syndrome

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Abstract

High-dimensional biology (HDB) refers to the simultaneous study of the genetic variants (DNA variation), transcription (messenger RNA [mRNA]), peptides and proteins, and metabolites of an organ, tissue, or an organism in health and disease. The fundamental premise is that the evolutionary complexity of biological systems renders them difficult to comprehensively understand using only a reductionist approach. Such complexity can become tractable with the use of 'omics' research. This term refers to the study of entities in aggregate. The current nomenclature of 'omics' sciences includes genomics for DNA variants, transcriptomics for mRNA, proteomics for proteins, and metabolomics for intermediate products of metabolism. Another discipline relevant to medicine is pharmacogenomics. The two major advances that have made HDB possible are technological breakthroughs that allow simultaneous examination of thousands of genes, transcripts, and proteins, etc., with high-throughput techniques and analytical tools to extract information. What is conventionally considered hypothesis-driven research and discovery-driven research (through 'omic' methodologies) are complementary and synergistic. Here we review data which have been derived from: 1) genomics to examine predisposing factors for preterm birth; 2) transcriptomics to determine changes in mRNA in reproductive tissues associated with preterm labor and preterm prelabor rupture of membranes; 3) proteomics to identify differentially expressed proteins in amniotic fluid of women with preterm labor; and 4) metabolomics to identify the metabolic footprints of women with preterm labor likely to deliver preterm and those who will deliver at term. The complementary nature of discovery science and HDB is emphasized.

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INTRODUCTION

The traditional scientific approach to solving problems has been based upon reductionism, for which there are many definitions, but in essence, it can be described as “divide and conquer.”¹ In other words, a complex system can be understood by studying smaller, simpler and therefore more tractable units of the whole. The success of this approach in physics, biology, and medicine is unquestionable. Indeed, reductionism will continue to be an important part of biomedical research, but Strange² has proposed that “naive reductionism” defined as the belief that reductionism alone will lead to a complete understanding of living organisms is not tenable. High-dimensional biology (HDB) and systems biology have emerged as an alternative/complementary scientific paradigm. The fundamental tenet of these disciplines is that a complex system can be understood more completely by considering it in its entirety, including dimensions such as time, space, and context.

High dimensional biology, the “ome”, omics sciences, and systems biology

The term “HDB” refers to the use of high-throughput techniques which allow simultaneous examination of changes in the genome (DNA), transcriptome (messenger RNA [mRNA]), proteome (proteins), or metabolome (metabolites) in a biological sample, with the goal of understanding the physiology or mechanisms of disease.^{3–5} Insights derived from these are expected to assist with the development of new diagnostic, prognostic, and therapeutic tools in medicine. The term “ome” refers to an abstract entity, group, or mass.⁶ “Omics” sciences refer to the study of entities in aggregate,⁷ and hence the term “genomics” to study the genome, “proteomics” for the proteome, etc. HDB encompasses the omics techniques.^{8,9}

The integration of “omic” techniques is called “systems biology.” This discipline aims to define the inter-relationships of several or, ideally, all the elements in a system, rather than study each element independently. Thus, systems biology will capture information from genomics, transcriptomics, proteomics, metabolomics, etc. (experimentally derived data) and combine it with theoretical models to predict the behavior of a cell or organism.^{10–14}

An important feature of HDB is its application as a “discovery tool” because it allows for a global description of changes in biological samples. Hence, it does not require a specific hypothesis, and it is presumably unbiased in nature, although there are constraints in existing technologies. In contrast, the traditional approach to study biological processes is considered to be reductionist^{1,2,15} because conclusions are drawn based on one or few hypotheses. For example, the early diagnosis of pregnancy is based on the detection of a single hormone, human chorionic gonadotropin. Reductionist approaches have been successful in identifying diagnostic and prognostic markers of disease. However, these approaches cannot provide a comprehensive description of the biological processes involved in complex disorders such as the preterm parturition syndrome.

Genomics

Genomics is the systematic study of an organism's genome. The elucidation of the role of human biology and environmental factors in health and disease requires the understanding of the genotype-phenotype relationship. The role of genomics in the study of preterm labor is aimed at determining if there is a genetic predisposition to spontaneous preterm labor and delivery. The clinical importance of this is predicated on the paradigm known as "personalized medicine," which proposes that it may be possible to ascertain the genetic predisposition of a disease or syndrome and then to implement behavioral and/or pharmacological interventions to prevent adverse outcomes or to use genetic information to tailor individualized treatment that maximizes the benefits and minimizes the risk of adverse reactions.^{16–20}

Table 1 describes the duration of pregnancy of some mammalian species. Duration of gestation ranges from 19–20 days in mice to 630 days in elephants. The mean duration of the human gestation is 280 days (post-menstrual age). Is duration of gestation determined genetically? Allen et al.²⁶ have addressed this question in equine breeds. Artificial reproduction technologies were used to study the effects of genetic versus environmental factors in the thoroughbred and pony. *In vitro* fertilization followed by embryo transfer was conducted in the following circumstances: 1) a thoroughbred fetus implanted in a thoroughbred mare; 2) a pony fetus implanted in a pony mare; 3) a thoroughbred fetus implanted in a pony mare (deprived *in utero* environment); and 4) a pony fetus implanted in a thoroughbred mare (luxurious *in utero* environment). The mean gestational age at birth and birthweight of a thoroughbred offspring of a thoroughbred mare were significantly greater than those of a pony offspring of a pony mare. Although both interbred combinations lead to intermediate mean gestational ages at birth and birthweights (Table 2), thoroughbred-in-pony offspring demonstrated classical signs of intrauterine growth restriction, including muscle wastage of the limbs, overextended joints, and ill-formed hooves, while pony-in-thoroughbred offspring had superior muscle tone and well-formed, hardened hooves.²⁶ The results indicate that both genetic and environmental factors influence the duration of pregnancy and fetal weight in equine breeds.

The arguments to consider preterm parturition as a syndrome have been developed elsewhere in this issue of the journal.²⁷ For each mechanism of disease responsible for the syndrome, there could be an environmental and/or a genetic component. The contribution of each varies according to the specific mechanism of disease. For example, a woman who has preterm labor caused by placental abruption following a motor vehicle accident has primarily environmentally induced preterm parturition. In contrast, a woman with Ehlers-Danlos syndrome and an affected fetus has a substantial genetic predisposition to preterm delivery usually caused by spontaneous rupture of the membranes.²⁸ Indeed, the risk of preterm delivery is 12.5% if the mother is affected but has a healthy fetus. In contrast, the risk is 40% if a healthy mother has an affected fetus.²⁸

Epidemiological evidence suggests that genetic factors play a role in the pathogenesis of preterm birth.^{29–31} In the United States, African-American women have a significantly higher rate of preterm birth.²⁹ This difference remained significant after adjusting for potential confounding factors including medical insurance type (a surrogate indicator of

socioeconomic status).³² Ethnicity was an independent risk factor for spontaneous preterm delivery at <32 and <35 weeks of gestation after controlling for confounding variables, including cervical length, history of preterm birth, and others.³³ Collectively, these data support a role for ethnicity as a risk factor for preterm birth.

A genetic predisposition for a particular disorder can be suspected if the following criteria are met: 1) demonstration of familial aggregation; 2) substantiation with segregation studies; and, finally, 3) identification of disease-susceptibility genes.³⁴ Familial aggregation, defined as the occurrence of a trait in members of a family that cannot be readily accounted for by chance, has been shown for preterm birth.^{35–39} However, all studies have focused thus far on preterm birth, rather than spontaneous preterm labor. Similarly, phenotypic differentiation between spontaneous preterm labor with intact membranes and preterm premature rupture of membranes (PPROM) has not been considered in these studies.

Women with a sister who gave birth to a preterm neonate have an 80% higher risk of delivering preterm.³⁶ Moreover, Porter et al.³⁵ reported that women born preterm had a significantly higher risk of delivering preterm (odds ratio [OR] 1.18; 95% confidence interval [CI] 1.02–4.16). Interestingly, these risks of preterm birth was inversely proportional to the gestational age of the mother at birth.³⁵

Another study supporting a genetic predisposition for preterm birth is based on an extensive genealogy database in the state of Utah (USA). Twenty-eight families were identified in which a woman who delivered a preterm singleton neonate had at least five first- or second-degree relatives who had had a preterm delivery. The coefficient of kinship for familial preterm delivery grandparents was more than 50 standard deviations higher than the control group, supporting the familial nature of preterm birth ($P < 0.001$).³⁹ Finally, twin studies in Sweden and Australia have suggested heritability estimates of 25–40% and 17%, respectively.^{37,38}

Segregation analysis is the main statistical tool for analyzing the inheritance of non-Mendelian traits or diseases. It can provide evidence for a susceptibility locus and suggest whether a complex disease is monogenic, oligogenic, or multifactorial.⁴⁰ This type of analysis requires large datasets of people affected by a familial but non-Mendelian disease and is sensitive to biases in data collection. Segregation analysis has not been reported for spontaneous preterm parturition.

The final and most persuasive evidence of a genetic predisposition is the identification of disease-susceptibility genes. This evidence is generally derived from genetic association studies. The design, execution, analysis, and interpretation of this type of study is a specialized subject, and the reader is referred to the recent reviews for details.^{34,41} Genetic association studies in obstetrics have the added complexity of dealing with two genotypes: maternal and fetal. Either of these genotypes may alter the risk for obstetrical syndromes and, therefore, warrant investigation. Moreover, the interaction of the genotypes or conflicting genotypes between mother and fetus may also modify the risk for a specific syndrome.

Polymorphisms (variations in DNA at a specific site) in several genes have been studied in preterm birth^{42–51} (Table 3) and PPRM^{52–63} (Table 4). The results of these genetic association studies have been reviewed elsewhere;⁶⁴ however, some examples will be described for illustration. The fetal genotype, as well as the maternal genotype, have been found to contribute to obstetrical disease. Matrix metalloproteinases (MMPs) have been implicated in PPRM because: 1) this family of enzymes degrades components of the fetal membranes such as collagens;^{65,66} 2) they are produced within the fetal membranes;^{65–67} 3) their production is increased in women with PPRM, as evidenced by higher amniotic fluid concentrations of MMPs in these patients;^{68–70} and 4) pro-inflammatory cytokines induce the production of MMPs.⁷¹

MMP-8 degrades fibrillar collagens (types I and III), which confer tensile strength to the fetal membranes. Promoter fragments containing the minor alleles of three single-nucleotide polymorphisms (SNPs), –799 (T), –381 (G), and +17 (G), were found to have 3-fold greater activity in chorion-like trophoblast cells, compared with the major allele promoter construct. Fetal carriage of the three-allele minor haplotype of MMP-8 confers a significantly increased risk for PPRM (OR 4.63; 95% CI 2.01–11.94; $P < 0.0001$).⁵³ Conversely, homozygosity for the three-allele major haplotype of MMP-8 confers protection from PPRM (OR 0.52; 95% CI 0.36–0.75; $P < 0.0002$).

Another example of the identification of fetal disease-susceptibility genes for PPRM involves heat-shock protein 47, encoded by the *SERPINH1* gene.⁶³ This heat-shock protein plays an essential role in collagen metabolism by serving as a chaperone to stabilize the collagen triple helix in the endoplasmic reticulum. The minor allele of a functional SNP in the promoter of the *SERPINH1* gene, –656 (T), was found to be more frequent in individuals of African ancestry. Wang et al⁶³ investigated the potential contribution of this polymorphism to the risk of PPRM in an African-American population. The authors reported that the minor allele was significantly more frequent in African-American neonates of pregnancies that were complicated by PPRM (OR 3.22; 95% CI 1.5–7.2; $P < 0.0009$). Statistical significance remained after adjustment for admixture in the population and was confirmed in a second case-control study.⁶³

The maternal and fetal genotypes interact with one another through their complement of gene products at the maternal-fetal interface, as well as during disruptions of this interface. This interaction may lead to either reproductive success or diseases unique to the pregnancy state.^{72,73} One example of a detrimental interaction of maternal and fetal genotypes is alloimmune hemolytic disease of the fetus such as rhesus (Rh) incompatibility.⁷⁴ This disorder results from antigenic exposure of a Rh-negative mother to antigens that are expressed on fetal red blood cells [Rh(D) positive]. This adverse outcome is mediated through the humoral response and is dependent upon a precondition of “conflicting genotypes” between mother and fetus, as well as environmental exposure.^{74–76}

This well-known example of “conflicting genotypes” which can lead to severe disease was the first of its kind to be described. However, conflicting genotypes may generate disease through a variety of other mechanisms. Conflicting maternal and fetal genotypes may be detrimental because they are, in fact, too similar. The cheetah, *Acinonyx jubatus*, is

considered a paradigm for disease vulnerability because of a loss of genetic diversity caused by a population bottleneck about 12,000 years ago.⁷⁷ The loss of genetic diversity predisposes the cheetah to neurodegenerative and multisystemic diseases in both juveniles and adults.⁷⁸

A large candidate gene association study of mothers and their offspring has been performed to examine genes that may predispose to PPRM.⁷⁹ Candidate genes were selected for biological plausibility for a role in the pathogenesis of PPRM and included genes involved in the immune response, extracellular matrix regulation, angiogenesis, and coagulation, among others. Separate, but distinct, maternal and fetal genes were associated with PPRM. Maternal genes associated with increased risk of PPRM included *interleukin (IL)-6*, *IL-6R*, and *COX-1*. Fetal genes associated with PPRM included the antimicrobial peptide beta-defensin 1 and *MMP-19*. A novel model for genetic predisposition of disease, “genotype conflict,” was also examined. “Conflicting” maternal-fetal genotypes for four genes were associated with PPRM. The study⁷⁹ highlights the importance of investigating both maternal and fetal genotypes, as well as their interaction and “genotype conflict,” when examining genetic factors for obstetrical diseases.

For each mechanism of disease responsible for the preterm parturition syndrome, there could be an environmental and/or a genetic component which could also interact. Moreover, there could be gene-to-gene interaction (epistasis). The first evidence of a gene-environment interaction was demonstrated with maternal carriage of an allele of the tumor necrosis factor (*TNF*) alpha gene and bacterial vaginosis.⁸⁰ A single nucleotide polymorphism (SNP) within the promoter of the *TNF* gene at position –308 has been correlated with differences in both constitutive and inducible expression. Carriage of the TNF-2 allele, in particular, yields increased gene expression. Results of the multivariable analysis showed that neither the carriage of the TNF-2 allele (OR 1.6; 95% CI 0.9–2.8) nor bacterial vaginosis (OR 1.3; 95% CI 0.5–2.9) were associated with an increased risk for spontaneous preterm birth. However, the combination of maternal carriage of the TNF-2 allele with bacterial vaginosis resulted in significantly increased risk for preterm birth (OR 6.0; 95% CI 1.6–22.7).⁸⁰ Similarly, Engel et al⁴³ found that maternal carriage of a polymorphism in the *IL-6* gene (C/G –237) did not result in increased risk of spontaneous preterm birth for Caucasian (OR 1.8; 95% CI 0.8–3.6) or African-American women (OR 1.6; 95% CI 0.7–3.8). However, African-American carriers of this allele with concomitant bacterial vaginosis had a 2-fold greater risk of spontaneous preterm birth compared with those who carried the variant but did not have bacterial vaginosis (OR 4.4; 95% CI 1.2–16.4). Together, these data provide evidence for a gene-environment interaction in spontaneous preterm birth. The study of gene-environment interactions may lead to the discovery of modifications of the environmental exposure which might lead to effective therapeutic interventions in the population at risk.

Recent technological advances and completion of the HapMap project (which identified a large number of DNA variants in some specific ethnic groups) have made possible the conduction of whole-genome association studies.^{81–86} Thus, genetics is evolving from a “candidate gene approach,” in which the DNA variants of biologically interesting genes are studied, to a true genomic approach that aims to examine the entire genome. High-density

arrays now allow simultaneous examination of 500,000 or more DNA variants in the same individual. It has been proposed that the sample size required for an initial whole-genome association study should include at least 1000 cases and 1000 controls. Accurate phenotype characterization, DNA quality, population stratification, and availability of a large number of samples for replication are crucial elements for this strategy to be successful in identification of genetic factors predisposing to complex disorders. For example, a whole-genome association study has been successful in identifying DNA variants in complement H which predispose to age-related macular degeneration.^{87,88}

Proteomics

The proteome is the entire set of proteins encoded by the genome, and proteomics is the discipline which studies the global set of proteins and their expression, function, and structure.^{89,90}

In early years of proteomics, protein composition studies relied on two-dimensional gel electrophoresis⁹¹ in which proteins were separated in one dimension by molecular weight and in the second dimension by isoelectric point. Spots in the polyacrylamide gel were then cut and protein identification performed by using trypsin digestion and mass spectrometry (MS). The MS tracing yields information about the mass/charge ratio (m/z ratio) of ions,⁸⁹ which is used to search protein databases such as SwissProt.

A major impetus for proteomics in medicine was the report that serum analysis with a combination of solid chromatography and MS could identify women with ovarian cancer.⁸⁹ The technique employed in this study was surface-enhanced laser desorption-ionization time-of-flight (SELDI-TOF). Since that report, many other studies have employed proteomic techniques to identify biomarkers for a wide range of disorders including preterm labor and PPRM.

Analysis of amniotic fluid and serum from women with either PPRM or term prelabor rupture of membranes (PROM) and control patients was undertaken to identify biomarkers characteristic of membrane rupture.⁹² Samples were analyzed using two-dimensional high-resolution electrophoresis followed by MS. Five amniotic fluid spots were considered possibly discriminatory. Of the five proteins, two were identified as new potential markers of PROM, meaning they were present only in amniotic fluid and absent in maternal plasma. These were agrin and perlecan.⁹² Clinical studies about the utility of these markers have not been published. Subsequently, SELDI-TOF MS was used for the identification of biomarkers of intra-amniotic inflammation in patients with preterm labor and intact membranes and PPRM. Four markers, including neutrophil defensins 1 and 2 and calgranulins A and C, identified patients with intra-amniotic inflammation (defined as a white blood cell count of 100 nucleated cells/mm³) with a sensitivity of 92.9 % and specificity of 91.8 %.⁹³

Studies in monkeys which had experimental intra-amniotic infection and in women with proven intra-amniotic infection showed that a protein profile of amniotic fluid could identify such patients. The informative peptides included calgranulin B and a fragment of insulin-like

growth factor-binding protein-1 (molecular weight of 11kDa). Moreover, some of the markers were also identified in maternal serum.⁹⁴

Subsequently, proteomic analysis of amniotic fluid with SELDI-TOF MS was reported in women with preterm labor and PPROM separately. The protein profiles of women with preterm labor and intra-amniotic infection were different between women with preterm labor and those with PPROM with intra-amniotic inflammation. Intra-amniotic inflammation was defined as a concentration of IL-6 of ≥ 1.5 ng/mL in amniotic fluid in women with preterm labor and ≥ 0.80 ng/mL in women with PPROM. The authors reported that 17 proteins were significantly overexpressed in amniotic fluid from intra-amniotic inflammation cases, including human neutrophil protein 1–3, calgranulins A and B.⁹⁵ This study included examination of cervical fluid; however, no differences could be demonstrated among the groups.

Other studies have attempted to identify biomarkers in maternal blood or vaginal fluid⁹⁶ using different proteomic techniques such as matrix-assisted laser desorption ionization, SELDI, and electrospray ionization time-of-flight MS. One study used two-dimensional chromatography (the first dimension separated proteins by isoelectric points and the second by protein hydrophobicity) followed by MS. Two-dimensional protein maps allowed the identification of bands differentially expressed in women with preterm labor with intact membranes who delivered preterm (with and without intra-amniotic inflammation) from women with preterm labor who delivered at term. MS was used to identify the proteins in the bands (Figure 1).⁹⁷

Collectively, these studies suggest that proteomic techniques can be used to identify biomarkers in women with preterm labor with intra-amniotic inflammation. A crucial question is whether these observations could be translated into a clinically applicable test. Clearly, the approaches used for discovery may not be optimal for a point-of-care test. Specifically, the expense of the equipment, expertise required to interpret the results, and issues of cost and time should be considered.

If the purpose is to identify intra-amniotic inflammation, this can be accomplished with a simple amniotic fluid white blood cell count determination, which requires a hemocytometer chamber and a microscope, available in every hospital around the world. There is no evidence that an MS-based test should be used to obtain the same information as that available rapidly and inexpensively worldwide with the use of a hemocytometer chamber. Moreover, if the desire is to render the diagnosis of intra-amniotic inflammation more objective, this can be accomplished with an enzyme-linked immunosorbent assay for IL-6.^{98–100} There is evidence that an elevated concentration of IL-6 in amniotic fluid is associated with intra-amniotic inflammation, and also short- and long-term neonatal morbidity.^{101–104}

A relevant challenge is to develop a point-of-care test for the detection of intra-amniotic inflammation. Such a test has been developed and consists of a rapid method for detecting an elevated concentration of MMP-8 in amniotic fluid.¹⁰⁵ Extensive studies^{70,106–110} support an association between an elevation in MMP8 in amniotic fluid and intra-amniotic

infection, histologic chorioamnionitis, impending preterm delivery, and adverse neonatal outcome.

Yoon et al developed a test which is configured as a rapid pregnancy test (Figure 2).¹⁰⁵ It requires 20 μ L of amniotic fluid; laboratory equipment is not required, and results are available within 15 minutes. Initial studies indicated that the test had a 95% sensitivity and a 93% specificity in the detection of intra-amniotic inflammation in women in preterm labor with intact membranes.¹¹¹ A subsequent study by Nien et al¹⁰⁵ showed that the test has a high sensitivity and specificity as well as a likelihood ratio for a positive result in the identification of intra-amniotic inflammation (Table 5) and high positive predictive value for delivery within 14 days (Table 6). This test fulfills most of the criteria for a point-of-care test, namely: 1) simple testing method; 2) rapid availability of the results; 3) easy interpretation of the results; 4) low maintenance because the kit can be stored at room temperature; 5) strong correlation with standard laboratory procedures; and 6) low cost because there is no need for capital equipment and because the market price can be driven by need.¹¹²

The availability of this rapid test opens avenues to evaluate interventions in women with intra-amniotic inflammation, such as antibiotic administration and modulators of the inflammatory response. This is pertinent because of beneficial effects of the administration of anti-inflammatory agents such as IL-10,^{113,114} steroids,¹¹⁴ and antioxidants.¹¹⁵

The end result of the use of a discovery technique or an “omic” methodology is the development of such point-of-care tests. An important challenge of proteomics in premature labor is whether analysis of vaginal fluid, maternal serum, or amniotic fluid can identify women destined to deliver preterm in the absence of intra-amniotic inflammation and also of women who will deliver at term without requiring tocolysis.

Transcriptomics

The transcriptome is the full complement of mRNA in a cell or tissue at any given moment.¹¹⁶ A transcriptome forms the template for protein synthesis, resulting in a corresponding protein complement or proteome.¹¹⁶ Transcriptomics has been used to describe the global mRNA expression of a particular tissue, yielding information about the transcriptional differences between two or more states.

Microarrays for analysis of the mRNA expression profile were first reported in 1995^{117,118} and have since been applied to a wide range of processes, notably, cancer. Microarrays have been used to: 1) classify tumors; 2) assess cancer prognosis; 3) predict tumors' response to therapy; and 4) identify new sub-types of cancer that could not be discerned with conventional techniques such as histopathologic examination.^{119–121} The case of breast cancer has been particularly noteworthy. Conventional methods, including staging of the disease, histologic grade, etc., have not been adequate to predict which woman will respond to treatment (chemotherapy or hormonal therapy). A recent advance was made by a group of investigators in the Netherlands who used microarrays in women with breast cancer who only had surgical treatment and found that the expression levels of 70 genes identified

women who subsequently developed metastases and that this profile was better than histology and conventional clinical staging.^{119,120}

In the case of parturition, gene expression has been studied using targeted and non-targeted approaches such as Northern blot analysis and quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), arrays of complementary DNAs (cDNA) spotted on membranes (macroarrays), and microarrays.^{122–136} It is possible to identify differentially expressed mRNA species in the myometrium, cervix, and chorioamniotic membranes from women not in labor and those in labor. These studies have shown that genes encoding proteins involved in prostaglandin synthesis and in the control of the inflammatory response are differentially expressed in labor. The reader is referred to a review of the subject which describes previous studies, techniques, and findings.¹³⁷

Studies of the transcriptome can be used to develop a molecular taxonomy of preterm labor and improve the understanding of the physiology and pathology of term and preterm parturition. The analysis of microarray experiments requires considerable expertise, and the reader is referred to recent reviews of the subject for details.^{138–142} Gene expression profiling can be used for three main purposes: 1) class comparison, 2) class prediction, and 3) class discovery. Class comparison studies are undertaken in order to characterize the gene expression profiles of two or more groups of women. For example, it is possible to compare the transcriptome of myometrium in labor versus those not in labor. In class prediction applications, the classes are predefined (e.g. women with and without preterm labor), and the goal is to build a “classifier” able to distinguish between these classes based on the gene expression profiles of the samples. In class discovery studies, a given set of gene expression profiles is analyzed with the goal of discovering subgroups that share common features. The biological interest of this approach is to understand the mechanisms of disease underlying the syndrome of preterm parturition. This can be realized by examining the functional classifications and pathways of genes differentially expressed.^{140,143}

Aguan et al¹⁴⁴ were the first to use functional genomics for the study of parturition. Using arrays for 588 genes and RNA isolated from the myometrium of three women at term in spontaneous labor and from three women not in labor, they noted up- and down-regulation (defined as a change of 2-fold or greater) of 21 genes involved in a wide range of physiological processes, including smooth muscle contraction and relaxation, regulation of DNA metabolism, as well as transcriptional and cell cycle regulation. Chan et al¹⁴⁵ used an unrestricted approach to identify differentially regulated genes during spontaneous labor at term. Suppression subtractive hybridization (SSH) was used with cDNA libraries constructed from the myometrium of one woman not in labor and a woman who underwent a cesarean section because of failure to progress in labor. Dot-blot screening of 400 positive clones indicated that 14 genes were up-regulated and 16 were down-regulated. Up-regulated genes included those encoding proteins implicated in the mechanism of parturition in the pregenomic era (oxytocin receptor, MMP-9, fibronectin, and IL-8), those not previously implicated, and four genes with no matching sequences in available databases. Northern blot analysis was performed for six genes and qRT-PCR for three (IL-8, Mn superoxidase dismutase [MnSOD], and cyclophilin) in a set of samples from a larger cohort. The major findings were that: 1) these three genes were up-regulated during spontaneous labor; 2) there

were topographic differences in the expression of MnSOD in the lower uterine segment and fundal myometrium, but not for IL-8; and 3) IL-8 expression was higher in spontaneous labor than in induced labor.

Transcriptomics has been used to examine the differences in gene expression profiles in the fetal membranes in women with PPROM and preterm labor with intact membranes with and without histologic chorioamnionitis. The gene with the most differential expression between preterm labor and PPROM was proteinase inhibitor 3 (PI3), also known as elastase-specific inhibitor. This serine proteinase inhibitor is capable of inhibiting neutrophil elastase and proteinase-3.¹⁴⁶ The former had been implicated in the mechanisms of membrane rupture both at term and preterm gestation.¹⁴⁷ Decreased expression of PI3 in PPROM was found by microarray experiments and was confirmed by qRT-PCR. Immunohistochemistry showed decreased PI3 protein expression. This study shows that a genome-wide approach can identify deficient expression of PI3 in PPROM, a gene that was not suspected previously to play a role in parturition. Moreover, the authors proposed that patients who are not capable of producing adequate amounts of PI3 in the fetal membranes may be predisposed to PPROM.¹⁴⁶ For this pregnancy complication, Tashima et al¹⁴⁸ was the first to use SSH to investigate differentially expressed genes.

Haddad et al¹⁴⁹ performed a prospective cohort study to examine differential gene expression of the chorioamniotic membranes of women not in labor and those in spontaneous labor at term (in the absence of histologic chorioamnionitis). The authors reported that multiple transcripts controlling each of the defined steps of acute inflammation increased during labor and that an “acute inflammation gene expression signature” appeared to be coordinately expressed and was not associated with the duration of labor. For example IL-8, IL-6, PBEF, TLR2, and SOD2 were overexpressed in samples of women in labor compared with those not in labor (Figure 3).¹⁴⁹ These observations are consistent with the analysis reported by Bisits et al,¹⁵⁰ indicating that genes involved in the control of inflammation participate in the activation of a tissue central to parturition: myometrium.

Hassan et al¹⁵¹ characterized the transcriptome of cervical tissue in women at term not in labor (n=7) and in those after spontaneous labor (n=9), and reported that the cervical transcriptome of women without labor was dramatically different from those who underwent labor. Indeed, unique genes (n = 1192) were differentially expressed in the cervical tissue from women after spontaneous labor, compared to women in term without labor (false discovery rate less than 0.05, absolute fold change greater than 2). Gene ontology analysis indicated that multiple “biological process” categories were enriched, including “response to biotic stimulus,” “apoptosis,” “epidermis development,” and “steroid metabolism.” Moreover, genes involved in neutrophil chemotaxis were dramatically up-regulated in specimens from women after spontaneous labor. The authors confirmed the increased expression of IL-8, IL-6, and vascular endothelial growth factor in women after spontaneous labor using real-time qRT-PCR. Of interest, toll-like receptor-3 and toll-like receptor-5 showed decreased gene expression in women after spontaneous labor.

One of the most comprehensive studies reported to date is that of Bukowski et al,¹⁵² who compared the transcriptome of the uterine fundus, lower uterine segment, and cervix before

and during labor. The authors concluded that labor results in a change in the transcriptome in each component of the uterus. Moreover, they have provided a list of differentially regulated genes and performed confirmatory studies with real-time qRT-PCR for two genes: repressor of estrogen receptor activity and retinoid X receptor alpha, both of which were down-regulated in the uterine fundus in women in labor. Genes with similar expression profiles were identified, and networks of co-regulated and co-expressed genes during parturition were discovered.^{152,153}

Transcriptomics has been used to study preterm labor in mice. Muhle et al¹⁵⁴ used an experimental paradigm to identify genes differentially expressed in pregnant mice subjected to inoculation with heat-killed bacteria (a model for infection-induced preterm labor)¹⁵⁵ and ovariectomy (a model of progesterone-withdrawal-induced preterm delivery). Each model of preterm labor was associated with a different set of differentially expressed genes. We have confirmed these findings using a similar experimental approach.¹⁵⁶ Specifically, bacteria-induced preterm labor substantially increased the expression of genes involved in prostaglandin synthesis. In contrast, ovariectomy-induced preterm labor increased the expression of genes involved in lipoxin, leukotriene, and hydroxyecosatetraenoic acid synthesis. Thus, bacteria-induced and ovariectomy-induced preterm labor express a different profile of genes involved in the synthesis of prostaglandins, lipoxins, leukotrienes, and hydroxyecosatetraenoic acids.¹⁵⁶ These observations suggest that transcriptomics may provide novel insights into the mechanisms involved in different forms of preterm parturition.

The results of the studies reviewed here shows that transcriptome analysis is feasible to study spontaneous preterm and term parturition and that the results can yield novel insights. Future challenges include: 1) the development of a taxonomy of the preterm parturition syndrome; 2) definition of the molecular pathways involved in each taxon; and 3) determination of whether or not the study of the peripheral blood transcriptome can be applied to identify women at risk for preterm delivery and those with intra-amniotic inflammation/intra-amniotic infection.¹⁵⁷

Metabolomics

Metabolomics is a discipline that aims to identify and quantify the global composition of “metabolites” of a biological fluid, tissue, or organism.^{158–160} The “metabolome” (analogous to the genome or transcriptome) would refer to the comprehensive catalogue of metabolites in a specific organ or compartment under a set of conditions (e.g. the plasma metabolome or the amniotic fluid metabolome).¹⁶¹ A working definition of a “metabolite” is a native small molecule (non-polymeric compound) that participates in general metabolic reactions and is required for the maintenance, growth, and normal function of cells.¹⁶¹ Metabolomics has theoretical advantages over genomics, transcriptomics, and proteomics because the metabolic network is downstream from gene expression and protein synthesis, and, thus, may reflect more closely the cell activity at a functional level.^{158,162} In addition, the concentration of a given metabolite is the result of the activity of all enzymes involved in the synthesis and catabolism of that compound and, thus, metabolic profiling has the potential to provide integrative¹⁶³ information. Moreover, the coupling of reactions in the

metabolic network allows that even small perturbations in the proteome (concentrations of a set of enzymes) could cause major changes in the concentrations of several metabolites. Importantly, gene deletion may not result in a visible phenotype change, suggesting that the organism can compensate for the absence of a specific gene, mRNA and protein. This is presumably accomplished by using alternative pathways.¹⁵⁹ Metabolic profiling has been used to identify such silent phenotypes.^{164,165}

The experimental approach for global metabolic analysis has evolved over time. Nuclear magnetic resonance spectroscopy (NMR) has been used for the study of the chemical composition of biofluids for decades.¹⁶⁶ The current approach consists of using a combination of separation techniques and MS. The typical separation techniques are gas chromatography (GC) (for non-polar compounds) or liquid chromatography (LC) (for polar compounds), followed by MS to identify the specific compound. However, other techniques have been proposed, including pyrolysis MS,¹⁶⁷ Fourier transform infrared spectroscopy,¹⁶⁷ Raman spectroscopy,¹⁶⁸ and direct infusion electrospray-MS.¹⁶⁹ NMR has the disadvantage of lower sensitivity than LC-MS or GC/MS but is non-destructive, covers a wide range of metabolites, and requires minimal sample preparation. The reader should be aware that there is no current method capable to detect and quantitate the entire¹⁵⁹ metabolome on its own. Indeed, subdivisions of metabolomics are now emerging, such as glycomics, lipidomics, etc.

Metabolic profiling of amniotic fluid has been used to identify women at risk for preterm delivery and also women with intra-amniotic infection/intra-amniotic inflammation.¹¹¹ Amniotic fluid obtained by amniocentesis from women with premature labor and intact membranes was subjected to separation with GC and LC, followed by MS. Identification of the compounds was performed by using authentic standards. Metabolic profiling was able to identify women as belonging to the correct clinical group with a 96.3% precision (53/55). Indeed, 15 out of 16 women with premature contractions who delivered at term were correctly classified, and all patients with preterm labor without infection and inflammation who delivered preterm neonates were correctly clustered (19/19). Moreover, among women with infection/inflammation, 19 out of 20 were correctly classified. Thus, metabolic profiling can be of value to assess the risk of preterm delivery in the presence or absence of infection/inflammation. Metabolic profiling has also been used in pre-eclampsia.^{160,170,171}

Epigenetics and Epigenomics

“Epigenetics” (etymologically “outside conventional genetics”) refers to heritable changes in gene expression that occur without modification of DNA sequence.¹⁷² These changes have been implicated in the integration of environmental and intrinsic signals into the genome. Specifically, epigenetics is concerned with how changes in gene function led to different phenotypes without a change in genotype.¹⁷³ Epigenomics is the global study of the epigenetic marks of chromatin, such as DNA methylation sites and post-translational modifications.¹⁷⁴

Epigenetic processes are essential for development and differentiation. However, they can also arise or be deleted after the embryonic period. Epigenetic changes provide a molecular link between the environment and the expression of the genome, and they have been implicated in the genesis of diseases such as cancer and the process of aging.

The mechanisms of epigenetic regulation are chromatin remodeling and modification (e.g. DNA methylation and histone modifications, such as acetylation, phosphorylation, methylation, ubiquitination, etc.) and RNA interference.^{175,176} There are hundreds of potentially methylated cytosines in a gene and dozens of known post-translational modifications of chromatin. Thus, there is a need for high-throughput technology to map the sites susceptible to such processes. Several epigenome projects have already been launched to characterize such sites on a genome-wide scale (epigenomics).

DNA methylation is the most widely studied mechanism for underlying epigenetic changes. Methylation can result in gene “repression” by attaching a methyl group to the cytosine nucleotide in CpG islands,¹⁷⁷ which are short sequence domains that generally remain unmethylated at all times, regardless of gene expression.¹⁷⁸ In most cases, increased DNA methylation is associated with gene “silencing” and decreased methylation is related to gene activation.¹⁷⁹

DNA methylation is dependent on folate, vitamin B12, and vitamin B6, which are co-factors in the enzymatic reaction.¹⁸⁰ Thus, nutrition can induce epigenetic changes.¹⁸¹ Indeed, maternal supplementation of methyl donors and co-factors (folic acid, vitamin B12, choline, and betaine) in a specific strain of mice has been shown to permanently alter the fur color of the offspring. The mechanism by which this occurs is CpG methylation at the *A^{vy}* locus of agouti mice.¹⁸²

Could there be a link between epigenetic changes and spontaneous preterm delivery? Preterm birth has been associated with folate deficiency.¹⁸³ Furthermore, mutations in genes involved in DNA methylation (such as *MTHFR* C677T and polymorphic deletion of 19 base pairs within intron I of *DHFR*) have been associated with preterm birth (the relative rate of spontaneous preterm birth and indicated preterm birth has not been reported in this cohort).^{184,185} Thus, both environmental and genetic susceptibility to impaired methylation were associated with preterm birth. Interestingly, preterm birth has been associated with low concentrations of zinc, another co-factor in the methylation pathway.^{186,187} Taken together, these findings suggest that epigenetic mechanisms may play a role in the pathogenesis of preterm birth. Indeed, epigenetics may provide a molecular mechanism for the impact of maternal nutrition, environmental factors, and genetic susceptibility on preterm labor.

Infection is a major environmental factor, which has been causally linked to spontaneous preterm labor and delivery. The role for pro-inflammatory cytokines, which act through the transcription factor NF- κ B, is well established in the molecular mechanisms responsible for infection-associated preterm parturition.^{188–195}

IL-6 plays a central role in the fetal host response to microbial invasion of the amniotic cavity. Fetuses with systemic inflammation (defined as an elevated fetal plasma IL-6) have impending onset of labor and multi-systemic organ involvement: the fetal inflammatory response syndrome.^{196,197} The possibility that IL-6 can induce re-programming of several fetal physiologic responses, including the immune response through an epigenetic mechanism, must now be considered in light of recent findings that IL-6 upregulates the expression of DNA methyltransferases^{198–200} and histone methyltransferases.²⁰¹

Furthermore, impaired DNA methylation can increase IL-6 expression.²⁰² Hence, DNA-methylation-related events may affect the magnitude of the inflammatory response in the mother and fetus and predispose to preterm labor and fetal injury.

Experimental evidence that epigenetics may be important in the onset of labor was reported in 2003.²⁰³ The administration of a histone deacetylase inhibitor (trichostatin A) to pregnant mice on a daily basis from day 15 to 19 was associated with delayed onset of parturition (24 to 48 hours).²⁰³ The authors postulated that the mechanism of action was impairment of the function of the progesterone-progesterone receptor complex. Of interest is that women in labor had lower ratio of acetylated histone to total H3 in the nuclei of human myometrial cells obtained from uterine fundus than women not in labor.

An epigenetic mechanism has been implicated in the control of prostaglandin production by human amnion. Mitchell²⁰⁴ has proposed that the production of prostaglandins during pregnancy is downregulated by enhanced DNA methylation and increased histone deacetylation of the gene for cyclooxygenase-2 (also known as prostaglandin H synthetase-2). The author demonstrated that prostaglandin E₂ production by human amnion in response to IL-1 beta was reduced by inhibition of DNA methylation and histone deacetylation (with 5-aza-2' deoxycytidine and trichostatin A, respectively).

Recently, our group has demonstrated changes in the expression of specific microRNAs in the myometrium and chorioamniotic membranes of women with spontaneous labor at term, as well as in preterm labor associated with inflammation. MicroRNAs operate through RNA interference, which is an important mechanism for epigenetic change. These findings suggest that several mechanisms may operate to accomplish epigenetic regulation of parturition (R Romero, personal communication).

In summary, studies regarding the role of epigenetic regulation in spontaneous term and preterm labor have begun, and the evidence suggests that these mechanisms may be involved in the control of the duration of pregnancy. The possibility that exposure to environmental factors may re-program the fetal and maternal immune systems must be considered. A systematic study of the role of epigenetic mechanisms in term and preterm parturition is needed.

Conclusion

The beginning of the 21st century is likely to be considered a pivotal period in the comprehension of biology, as dramatic advances allow freedom from the constraints of reductionism and the birth of HDB and systems biology. A new theoretical framework assisted by computational biology may render tractable the study of many of the problems presented by the major obstetrical syndromes. It is hoped that these disciplines will improve the understanding of the taxonomy and pathobiology of obstetrical disorders and lead to the improved identification of the woman at risk, diagnosis, treatment, and prevention of these conditions.

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Figure 1.

Mass spectrometry profiles of amniotic fluid from patients with preterm labor who delivered at term (A) and preterm labor with infection/inflammation (B). Reproduced with permission from Romero R,⁹⁷ Bujold E, Andrews P. Michigan Proteome Center SU/Perinatology Research Branch.

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Figure 2.
MMP-8 rapid test. Reproduced with permission from Nien JK, et al. Am J Obstet Gynecol.
2006;195:1025–30.¹⁰⁵

Figure 3.

Hierarchical clustering of probe sets that discriminate the chorioamniotic membrane samples of term in labor (TIL) patients from their term no labor (TNL) counterparts. The top 224 probe sets (P% .02) with a minimum average expression difference of 1.4-fold are shown. Reproduced with permission from Haddad et al. *Am J Obstet Gynecol* 2006;195:394. e1–24.¹⁴⁹

Table 1.

Mean Duration of Gestation in Mammals

Species	Days
Mouse ²¹	19–20
Rabbit ²²	31
Sheep ²³	148
Human	280
Killer whale ²⁴	510
Elephant ²⁵	630

Table 2.

Mean gestational age at birth and mean foal birthweight in equines in pregnancies after artificial reproductive technologies.

	Fetus in mother			
	Thoroughbred in thoroughbred	Pony in pony	Pony in thoroughbred (luxurious <i>in utero</i> environment)	Thoroughbred in pony (deprived <i>in utero</i> environment)
Mean gestational age at birth (days)	339 $\pm$ 3	325 $\pm$ 3	331 $\pm$ 3	332 $\pm$ 3
Mean foal birthweight (kg)	53 $\pm$ 3	24 $\pm$ 1	38 $\pm$ 2	33 $\pm$ 2

Modified from Allen W.R. et al. *Reproduction* 2002;123:445–453.²⁶

Table 3.

Genetic polymorphisms that confer the greatest increased/decreased risk to preterm birth.

Gene	Polymorphism	P-value	Odds ratio	First author
Genes that increase the risk for preterm delivery				
<i>TNFA1</i>	G/A –308 (N) (NR)	0.002	7.3(2.85, 18.9) RR	Aidoo et al ⁴²
<i>IL6</i>	C/G –237 (M) (with bacterial vaginosis) (S)		4.4 (1.2, 16.4)	Engel et al ⁴³
<i>PON2</i>	S311C (N) Homozygosity (NR)		4.6 (1.5, 14) RR	Chen et al ⁴⁴
<i>PON1</i>	Q192R (N) Homozygosity (NR)		3.6 (1.3, 11) RR	Chen et al ⁴⁴
<i>TNF/IL6/IL6R</i>	–3448/–7227/33314 (M) (S)	0.001	3.5 (2.52, 4.87)	Menon et al ⁴⁵
<i>IL4</i>	–509 C/C genotype (M) (S)	0.02 with multi-variate analysis	3.4 (1.2, 9.6)	Annells et al ⁴⁶
<i>IL4</i>	–509 C/C genotype (M) (S)	0.02 with univariate analysis	3.0 (1.1, 10.3)	Annells et al ⁴⁶
<i>IL6</i>	G/C –174 (M) (S)	< 0.01	2.32 (1.23, 4.30)	Orsi et al ⁴⁷
<i>IL10</i>	A/G –1082 (M) (S)	< 0.05	1.95 (1.04, 3.64)	Orsi et al ⁴⁸
<i>IL1B</i>	C/T –511 (M) (NR)	< 0.015		Greenfield et al ⁴⁹
Genes that decrease the risk for preterm delivery				
<i>TGFB1</i>	G/C –800, G/C –509 haplotype (M) (S)	0.01	0.7 (0.5, 0.9)	Annells et al ⁴⁶
<i>MBL2</i>	LYA (N) (NR)	0.02	0.61 (0.40, 0.93)	Bodamer et al ⁵⁰
<i>IL10</i>	A/G –1082 (M) (S)	0.01	0.6 (0.4, 0.9)	Annells et al ⁴⁶
<i>IL4</i>	–509 C/T genotype (M) (S)	0.01 with univariate analysis	0.3 (0.1, 0.8)	Annells et al ⁴⁶
<i>TNFRSF6</i>	AG/GA –1377, –670 haplotype (M) (S)	0.02	0.1 (0.0, 0.8)	Annells et al ⁴⁶
<i>ADRB2</i>	Arg16Gly (M) (S)	0.01	0.08 (0.01, 0.58)	Landau et al ⁵¹

M, maternal; N, neonatal; NR, mode of preterm birth not reported; RR, relative risk; S, spontaneous preterm birth.

Table 4.

Genetic polymorphisms that confer an increased risk to PPRM.

Gene	Polymorphism	OR (95% CI)	P-value	Reference
<i>MMP1</i>	G/GG –1607 (N)	2.29 (1.1–4.8)	0.028	Fujimoto et al ⁵²
<i>MMP8</i>	C/T –799, A/G –381, C/G +17 (N)	4.63 (2.0–11.9)	<0.0001	Wang et al ⁵³
<i>MMP9</i>	14 CA repeat (N)	3.06 (1.8–5.3)	<0.0001	Ferrand et al ⁵⁴
Caspase-recruitment-domain-containing protein15	2936insC (N)	—	0.017	Ferrand et al ⁵⁵
<i>TNFα</i>	G/A –308 (M)	3.18 (1.3–7.8)	0.008	Roberts et al ⁵⁶
	G/A –308 (N1)	5.98 (1.7–22.1)	0.002	Kalish et al ⁵⁷
	G +488, G –238, G –308 (M)	0.7 (0.5–1.0)	0.03	Annelis et al ⁴⁶
IL1 receptor antagonist	240-bp tandem repeat (N1 + 2)	8.0 (1.7–50.3)	0.005	Kalish et al ⁵⁸
	86-bp tandem repeat (N)	6.5 (1.3–37.7)	0.021	Genc et al ⁵⁹
	240-bp tandem repeat & CD14 T/T –159 genotype (M)	4.9 (1.4–15.9)	0.009	Kalish et al ⁶⁰
Heat-shock protein 70	A/G +1267 (N1)	—	<0.05	Kalish et al ⁵⁷
<i>IL10</i>	G –1082, C –819, C –592 (M)	1.9 (1.1–3.3)	0.01	Annelis et al ⁴⁶
<i>FAS</i>	A/G –670 (N)	3.05 (1.25–7.43)	0.01	Kalish et al ⁶¹
	A/G –670 (N1)	—	0.003	Fuks et al ⁶²
<i>CD14</i>	–159 T/T genotype (M)	2.94 (1.12–7.73)	0.036	Kalish et al ⁶⁰
Heat-shock protein 47	C/T –656 (N)	3.22 (1.5–7.22)	0.001	Wang et al ⁶³

M, maternal; N, neonatal; N1, neonate 1 in a multi-fetal pregnancy.

Table 5.

Diagnostic indices, predictive values, and likelihood ratios of MMP-8 PTD Check™ (In2Gen Co., Ltd., Seoul, Korea) for the detection of intra-amniotic infection and intra-amniotic inflammation.

	Prevalence % (n)	Sensitivity % (n)	Specificity % (n)	PPV % (n)	NPV % (n)	LR (+) (95% CI)	LR (-) (95% CI)
Intra-amniotic Infection	7.3% (24/331)	83% (20/24)	95% (291/307)	56% (20/36)	99% (291/295)	15.9 (9.6–26.6)	0.2 (0.1–0.3)
Intra-amniotic inflammation	11.5% (38/331)	84% (32/38)	99% (289/293)	89% (32/36)	98% (289/295)	61.7 (23.1–164.8)	0.2 (0.1–0.4)

PPV: positive predictive value, NPV: negative predictive value, LR: likelihood ratio.

Accuracy for intra-amniotic infection: 94%

Accuracy for intra-amniotic inflammation: 93%

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Table 6.

Diagnostic indices, predictive values and likelihood ratios of MMP-8 PTD Check™ (In2Gen Co., Ltd., Seoul, Korea) for the identification of women with spontaneous preterm delivery within 48 hrs, 7 days, 14 days and <32 and <34 weeks.

	Prevalence	Sensitivity	Specificity	PPV	NPV	LR (+) (95% CI)	LR (-) (95% CI)
Delivery within 48 hours	11.6% (38/327)	61% (23/38)	97% (279/290)	70% (23/33)	95% (279/294)	17.5 (9–33.9)	0.4 (0.2–0.8)
Delivery within 7 days	20.2 (66/327)	47% (31/66)	99% (259/261)	94% (31/33)	88% (259/295)	61.3 (15.1–250)	0.5 (0.1–2.2)
Delivery within 14 days	24.5% (80/327)	39% (31/80)	99% (245/247)	94% (34/36)	83% (245/295)	50 (12–196)	0.6 (0.2–2.5)

PPV: positive predictive value, NPV: negative predictive value, LR: likelihood ratio.

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