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SPONTANEOUS PRETERM BIRTH: ADVANCES TOWARD THE DISCOVERY OF GENETIC PREDISPOSITION

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Abstract

Evidence from family and twin-based studies provide strong support for a significant contribution of maternal and fetal genetics to the timing of parturition and spontaneous preterm birth. However, there has only been modest success in the discovery of genes predisposing to preterm birth, despite increasing sophistication of genetic and genomic technology. In contrast, DNA variants associated with other traits/diseases have been identified. For example, there is overwhelming evidence suggests that the nature and intensity of an inflammatory response in adults and children is under genetic control. Since inflammation is often invoked as an etiologic factor in spontaneous preterm birth, the question of whether spontaneous preterm birth has a genetic predisposition in the case of pathologic inflammation has been of long-standing interest to investigators. Here, we review various genetic approaches employed for the discovery of preterm birth genetic variants in the context of inflammation-associated spontaneous preterm birth.

Candidate gene studies have sought genetic variants regulating inflammation in the mother and fetus; however, the promising findings have often not been replicated. Genome wide association studies (GWAS), an approach to identifying chromosomal loci responsible for complex traits, have also not yielded compelling evidence for DNA variants predisposing to preterm birth. A recent GWAS including a large number of Caucasian women (>40,000) revealed that maternal loci contribute to preterm birth. Although none of these loci harbored genes directly related to innate immunity, the results were replicated. Another approach to identify DNA variants predisposing to preterm birth is whole exome sequencing (WES), which examines the DNA sequence of protein coding regions of the genome. A recent WES study identified rare mutations in genes encoding for proteins involved in the negative regulation (dampening) of the innate immune response (e.g., *CARD6*, *CARD8*, *NLRP10*, *NLRP12*, *NOD2*, *TLR10*) and anti-microbial peptide/proteins (e.g., *DEFB1*, *MBL2*). These findings support the concept that preterm labor, at least in part, has an inflammatory etiology, which can be induced by pathogens (i.e. intra-amniotic infection) or “danger signals” (alarmins) released during cellular stress or necrosis (i.e. sterile intra-amniotic inflammation). These findings support the notion that preterm birth has a polygenic basis involving rare mutations or damaging variants in multiple genes involved in innate immunity and host defense mechanisms against microbes and their noxious products. An overlap among the WES-identified genes and other inflammatory conditions associated with preterm birth such as periodontal disease and inflammatory bowel disease was observed, suggesting a shared genetic substrate for these conditions.

We propose that WES, as well as whole genome sequencing, is the most promising approach for the identification of functionally significant genetic variants responsible for spontaneous preterm birth, at least in the context of pathologic inflammation. The identification of genes contributing to preterm birth by WES, or whole genome sequencing, promises to yield valuable population specific biomarkers to identify the risk for spontaneous preterm birth and potential strategies to mitigate such a risk.

Keywords

Defensins; DNA Variants; Exomes; Genetics; Inflammasome; Inflammation; Inflammatory bowel disease; Innate immunity; Interleukins; Periodontal disease; Preterm premature rupture of membranes (PPROM); Mutations; Nucleotide-binding Oligomerization Domain (NOD) proteins;

NOD-like receptors (NLR); Sequencing; Transcriptomics; Toll-like receptors (TLR); missing heritability; rare variants; high penetrance; genome wide association studies; single nucleotide polymorphisms

IS THERE EVIDENCE THAT SPONTANEOUS PRETERM LABOR/BIRTH HAS A GENETIC PREDISPOSITION?

Preterm labor and birth has a genetic predisposition^{1–5}. Such a concept is supported by the following evidence: 1) demonstration of familial aggregation^{6–12}, which is defined as the co-occurrence of a trait in members of a family that cannot readily be accounted by chance; 2) measures of heritability^{13–19}; 3) detection of disease-susceptibility genes²⁰; and 4) racial disparity in preterm birth rates^{21–28}, which could be associated with differences in frequency of risk-predisposing alleles^{29–33}. Studies in twins have demonstrated a heritability range from 17–40%^{8, 9, 14, 34}. Moreover, polymorphisms in several genes have been associated with spontaneous preterm labor with and without intact membranes^{5, 12, 24, 30, 33, 35–179}.

Studies combining twin and family based approaches have shown that both the maternal and the fetal genomes contribute to timing of delivery and that environmental factors, depending on the population, also play a key role¹⁸⁰. Maternal and fetal genetic factors, combined, are estimated to contribute 30% or more of the risk for early delivery¹⁸⁰. There has been considerable interest in identifying genetic variants that influence the inflammatory response in reproductive tissues that could lead to preterm birth, particularly those variants and their interacting environmental factors that could account for differences in the incidence of preterm birth among populations^{165, 170}.

CANDIDATE GENE APPROACH

One frequently employed technique to identify genetic risk factors for complex disorders is the candidate gene approach, which directly tests the effects of specific genetic variants of a selected gene in an association study¹⁸¹. Candidate gene studies may include the detection of genetic variants [including single nucleotide polymorphisms or SNPs, usually variants that have functional significance (e.g. alter protein function or expression) and relatively high minor allele frequencies] in a single (Figure 1) or multiple (Figure 2) genes. Such studies can be performed relatively quickly, and in an inexpensive manner¹⁸¹. The major problem with candidate gene studies is that investigators must have a deep understanding of the pathophysiology of the disease in order to select a candidate gene¹⁸¹. Therefore, a central limitation of this type of studies is that they do not identify genetic locations other than those suspected to influence disease¹⁸². Yet, candidate gene studies do not require large families with both affected and unaffected members and can be performed with unrelated cases and controls or with small families¹⁸¹. In addition, these types of studies are suitable for identifying genes implicated in common and complex diseases, in which the risk associated with any given candidate gene is minimal^{183, 184}. However, the case and control cohorts must be adequate based on a sample size calculation.

Candidate gene studies have shown that some polymorphisms in genes coding for matrix metalloproteinases^{20, 164}, cytokines/cytokine

receptors^{41, 48, 49, 51–53, 59–61, 63, 80, 82, 124, 125, 153, 185}, and other components of the innate immune system^{60, 118} are significantly associated with preterm birth. Yet, replication of the majority of the positive results has not been achieved^{165, 170, 186, 187}. The disparate results could be related to the polymorphism(s) investigated, inconsistent phenotype definitions, failure to account for multiple testing, the sample size, which has usually been quite small, and population differences, including different proportions of individuals of different race/ethnicity in cases and controls (population stratification).

GENOME-WIDE ASSOCIATION STUDIES

Genome-wide association studies (GWAS) emerged as the preeminent instrument for discovering genetic loci that contribute to disease risk and quantitative traits. GWAS includes the analysis of hundreds of thousands of SNPs across the entire genome (Figure 3) without bias imposed by pre-existing models and provide the opportunity of identifying the location of novel genes, regulatory loci, and pathways not previously considered^{188, 189}. These studies have been crucial to the development of our current understanding of the architecture of complex traits. When GWAS became popular, the dominant paradigm in the field was *the common disease-common variant hypothesis*^{190–193}. This paradigm posits that common diseases are attributable to DNA variants present in more than 1–5% of the population, that there are many of these DNA variants and each contributes a small amount to the total risk to a particular disease^{183, 191, 194}. Moreover, DNA variants may have an additive or a multiplicative effect in determining susceptibility to a particular trait or disease¹⁹⁵. GWAS scored important successes in identifying DNA variants predisposing to some diseases such as age-related macular degeneration (related to a gene encoding for complement factor H¹⁹⁶) and Crohn's disease (DNA variants in the *IL23R* gene¹⁹⁷). These successes have occurred because these specific DNA variants contribute a large amount to explain the heritability of these conditions. However, after many GWAS, it has become clear that most common variants identified so far confer only small increments in risk and therefore, would explain a small proportion of the familial clustering by genetic factors. This has raised the question of how to explain “the missing heritability”¹⁹⁸ of many complex traits.

The missing heritability problem has given rise to alternative paradigms, which attribute genetic predisposition to: 1) a large number of small-effect common variants across the entire allele frequency spectrum¹⁹⁹; 2) a large number of large-effect rare variants²⁰⁰; or 3) a combination of genotypic, environmental, and epigenetic interactions^{201, 202}. A full discussion of this subject is beyond the scope of this article and the interested reader is referred to publications that specifically address issues^{199, 203–206}. The practical issue is that with the advancements in sequencing techniques it is now possible to perform whole exome sequencing or whole genome sequencing, which allows the examination of the role of rare variants to the heritability of complex traits.

Several GWAS have been performed using panels of SNPs that cover the entire genome (Figure 3)²⁰³. The majority of these SNPs have not been within the coding sequences, and only serve to mark a chromosomal location (genetic locus) where a disease-related gene may reside¹⁹⁵. Further refined mapping and DNA sequence analysis are needed to follow-up any

GWAS finding to establish which DNA variants are responsible for the trait/phenotype¹⁹⁵. Moreover, a large number of cases and controls is necessary in order to identify loci to discover a statistically significant contribution to a trait²⁰⁶.

The GWAS of preterm birth reported to date have not been informative, nor confirmed the importance of the innate immunity candidate genes. They also have not yielded replicable loci. The largely negative results could reflect the complexity of the premature labor phenotype (a syndrome in which genetic predisposition could contribute to each mechanism of disease), the limitations in the characterization of the phenotype, limitations in sample size or a fundamental problem with the “common variant/common disease” paradigm. A very recent publication examining a large population, of more than 40,000 women, and replication cohorts of more than 8,000 women, identified several maternal loci (*EBF1*, *EEFSEC*, and *AGTR2*) that may contribute to preterm birth in Caucasian women²⁰⁷. *EBF1* encodes a protein implicated in B cell development, *EEFSEC* encodes a protein involved in the production of selenoproteins, and *AGTR2* encodes the type 2 angiotensin II receptor. Although none of these loci have a direct role in innate immunity or the inflammatory mechanisms implicated as important in spontaneous preterm birth, they may have a role in other mechanisms of disease for preterm labor and provide new lines of investigation. The relevance of the DNA variants to spontaneous preterm birth in populations other than those of European ancestry will undoubtedly be a subject of future research to determine how they predispose to spontaneous preterm birth and whether they confer risk in other ethnic groups such as African Americans or Hispanics.

WHOLE EXOME SEQUENCING

Whole exome sequencing (WES) entails the full characterization (nucleotide composition) of the protein-coding genes in a genome, known as the exome (Figure 4), which represents less than 2% of the genome but contains 85% of known disease-related variants²⁰⁸. The value of whole exome sequencing is based on the concept that rare as well as more common mutations in coding sequences of multiple genes make an important contribution to complex traits and common diseases. Based on their effect on the DNA sequence, mutations are clarified into: 1) point mutations, which are characterized by a change [insertion/deletion (indels) or substitution] of only one nucleotide base in a DNA sequence (Figure 5A); and 2) frame-shift mutations, which are caused by indels of a number of nucleotides in a DNA sequence that is not divisible by three, resulting in the change of the reading frame (the grouping of the codons), and therefore in a different translation from the original (different amino acid sequence) (Figure 5B). Based on their effect on the protein, mutations are also clarified into: 1) nonsense mutations, in which a sense codon that corresponds to one of the twenty amino acids is changed to a chain-terminating (Stop) codon (Figure 6A); and 2) missense mutations, which includes a single nucleotide change resulting in a codon that codes for a different amino acid (Figure 6B). Missense mutations can be further clarified into: i) silent mutations, which do not have an effect on the protein since the change in the codon does not result in a change in the amino acid (e.g. CCA, CCG, CCC, and CCT encode for the same amino acid, glycine); ii) conservative mutations, in which the new amino acid is the same type as the original (e.g. glutamic acid is replaced with aspartic acid, both are acidic amino acids); and iii) non-conservative, in which an amino acid is not the same type

as the original (e.g. serine, a small polar amino acid is replaced with L-phenylalanine a large nonpolar aromatic amino acid). Therefore, WES can identify damaging mutations that can disrupt protein amino acid sequence and truncate, inactivate, or diminish protein function, which contribute to complex traits such as preterm birth^{209, 210}.

There are limitations to WES; it can miss structural variation²¹¹ and cannot detect important intragenic variation, including regulatory elements controlling gene expression. In addition, WES, despite its name, can also miss some exons: if the causal variants lie within the missing exons, they will not be identified²¹¹. The currently used capture WES methods require sequencing of a large number of bases, which can make this technique expensive and comparable to low-coverage whole genome sequencing (WGS)²¹¹. Whole genome sequencing not only determines the exome but also provides information on variants in highly evolutionary conserved non-coding regions and other variants throughout the genome²¹¹ (Figure 7). However, low-coverage WGS may miss many of the variants and therefore high-coverage WES is the method of choice for complex traits as it is affordable and can be now performed in newly developed platforms²¹¹.

The first study using WES in idiopathic preterm birth was performed on 10 mothers from densely affected families, including two mother-daughter pairs¹². It was reported that maternal coding variants in complement receptor were implicated in the pathophysiology of preterm birth¹². Using WES, we discovered that genes that negatively regulate the innate immune response (e.g. TLRs and inflammasomes) or that encode proteins that defend the host against microbes such as defensins and lactoferrin (an iron-binding protein with antimicrobial properties which amniotic fluid concentrations increase in women with intra-amniotic infection who underwent preterm labor²¹²) harbor mutations or damaging variants that enhance inflammation in the fetal membranes, resulting in preterm PROM in neonatal DNA collected from pregnancies host by African American mothers²¹⁰. The PPRM case and term control neonates had similar % African ancestry, ruling out population stratification²¹⁰. Relevant to these findings, polymorphisms in the gene encoding TLR-10, a TLR that negatively regulates the inflammatory pathway, have been associated with preterm birth²¹³. Importantly, the *DEFB1* and *MBL2* nonsense mutations have allele frequencies that are more than 10-fold greater in African populations compared to European populations (*DEFB1* African: 0.00615; European: 0.00018; *MBL2*: African: 0.006537; European: 0.00004). These population differences in mutant alleles may help explain racial/ethnic differences in PPRM and preterm birth.

In addition, we showed that a number of these mutations/damaging missense variants would be associated with inflammatory conditions occurring in women who deliver preterm²¹⁰, including inflammatory bowel disease (IBD) and periodontal disease. A more detailed description of the results reported by Modi et al²¹⁰ is provided in the following section.

THE POWER OF WHOLE EXOME SEQUENCING FOR DETECTING PRETERM BIRTH GENES

Using a whole exome sequencing approach to analyze DNA from 76 neonates born preterm from African-American mothers subsequent to preterm PROM and 43 normal term

neonates, we interrogated 35 genes that negatively regulate the innate immune system or that encode anti-microbial proteins²¹⁰. We identified 14 nonsense and damaging frameshift mutations, as well as a number of predicted damaging missense variants, in genes that negatively regulate the innate immune system or that encode anti-microbial proteins (examples of which are presented in Supplementary Table 1²¹⁰). Noteworthy among the genes are those that protect the host against microbes (e.g., defensin β 1 (*DEFB1*), mannose binding lectin (*MBL2*)), the negative TLR regulator, TLR-10, and multiple **inflammasome** genes (Caspase Recruitment Domain Family Member 8 (*CARD8*), NLR Family Pyrin Domain Containing Domain 10 (*NLRP10*), *NLRP12*, and *NOD2*²¹⁰). A brief review on the role of inflammation, including the inflammasome and NOD proteins, in spontaneous preterm labor is shown below.

Of the 14 mutations identified, 10 had nominal allele frequencies that were higher in preterm PROM cases than controls in an expanded cohort of 188 preterm PROM cases and 175 term controls²¹⁰. A test of genetic burden (which assesses the potential impact of the aggregate mutational load) for the 14 mutations revealed a statistically significant association with preterm PROM risk ($P < 0.05$)²¹⁰.

The mutations and predicted damaging missense variants identified in cases of preterm PROM represent, for the most part, are very rare alleles and the majority were heterozygous mutations²¹⁰. However, cumulatively, they may have a population impact. This is exemplified by mutations and predicted damaging missense variants in the genes involved in the NOD2 pathway, which were found in 10 of 76 preterm PROM neonates and none of the 43 term controls²¹⁰.

With 76 preterm PROM cases (152 chromosomes) there was a greater than 75% chance to detect minor alleles with a frequency of 0.005. Thus, very rare mutations could have gone undetected²¹⁰.

The mutations reported by Modi et al²¹⁰ were found in genes encoding for innate immune mediators, which supports the concept that inflammation plays a central role in the mechanisms of disease for spontaneous preterm labor and birth.

SPONTANEOUS PRETERM LABOR/BIRTH: THE ROLE OF INFLAMMATION

Preterm labor is a syndrome caused by multiple pathological processes²¹⁴. Intra-amniotic inflammation has been causally linked to spontaneous preterm labor and delivery^{215–247}. This pathological inflammatory process can be caused by microorganisms invading the amniotic cavity (i.e. intra-amniotic infection) or danger signals/alarmins released during cellular stress or death (i.e. sterile intra-amniotic inflammation)^{214, 237, 248–255}. In both scenarios, cytokines, such as interleukin (IL)-1^{256–261}, IL-6^{250, 262–275}, IL-8^{276–279}, and tumor necrosis factor alpha (TNF- α)^{280–285} among others^{286–301}, play a central role in the pathophysiology of preterm labor and birth^{235–237}. Of all of these cytokines, IL-1 β is a central mediator in the pathological process of preterm labor since it can stimulate the expression and release of other labor mediators, such as prostaglandins^{229, 302–307}. Indeed, the administration of IL-1 β causes preterm birth in mice^{257, 260} and non-human

primates^{227, 308–314}, which can be abrogated by the administration of the IL-1 β receptor antagonist²⁵⁹, confirming a key role for this cytokine in the mechanisms responsible for preterm labor in the context of inflammation.

“The Inflammasome” in Spontaneous Preterm Labor

The inflammasome is not another “ome”, it is a multi-protein complex located in the cytoplasm of cells, which activation induces the transformation of pro-IL-1 β (immature form) into the mature/bioactive forms of IL-1 β ^{315–323} by the actions of active caspase-1 (CASP-1)^{324–329}. Figure 8 shows the inflammasome complex which contains: 1) a pattern recognition receptor or sensor molecule, 2) the adaptor protein ASC (an apoptosis-associated speck-like protein), and 3) pro-caspase-1 (pro-CASP-1)^{315–323}. The ASC component of the inflammasome protein is encoded by *PYCARD* and includes two death-fold domains: one pyrin domain and one caspase activation and recruitment domain (CARD)^{330, 331}. Upon activation, the inflammasome induces the release of active forms of CASP-1 which, in turn, participates in the processing of mature IL-1 β and IL-18^{324–328, 332} (Figure 8), inducing a pro-inflammatory programmed cell death termed pyroptosis^{333–335}.

Evidence in support of the participation of the inflammasome in the mechanisms of labor associated with inflammation³³⁶ is that the amniotic fluid concentrations of CASP-1³³⁷, IL-1 β ^{256, 258}, and IL-18²⁹¹ are greater in women who have spontaneous preterm or term labor with intra-amniotic infection/inflammation than in those without this clinical condition. In addition, the amounts of NLR family pyrin domain-containing protein 3 (NLRP3, an inflammasome-recruiting pattern recognition receptor), active forms of CASP-1 as well as mature forms of IL-1 β and IL-18 are increased in the chorioamniotic membranes from women who underwent spontaneous preterm³³⁸ or term³³⁹ labor with acute histologic chorioamnionitis compared to those without this placental lesion. Acute histologic chorioamnionitis is associated with intra-amniotic infection and/or inflammation^{340–350} and sterile intra-amniotic inflammation^{214, 250–252, 350, 351}. Therefore, the inflammasome is implicated in the physiological^{352, 353} and pathological^{338, 339, 354} inflammatory processes of labor (Figure 8).

Nucleotide Oligomerization Domain (NOD) Proteins in Spontaneous Preterm Labor

A second set of pattern recognition receptors implicated in the mechanisms leading to the inflammatory process associated with labor are the nucleotide oligomerization domain (NOD)1 and 2 proteins^{338, 339, 352, 354}. These intracellular molecules recognize bacterial peptidoglycan segments^{355–363} and alarmins³⁶⁴ without forming inflammasomes. Instead, the NOD proteins directly activate nuclear factor kappa B (NF- κ B) proinflammatory signaling, inducing the expression of pro-IL-1 β and pro-IL-18^{355, 365}. NOD proteins can also work synergistically with inflammasome components (e.g., NLRP3) to enhance immune responses in murine dendritic cells³⁶⁶. Consistently, the chorioamniotic membranes^{338, 339, 352} and myometrium³⁶⁷ express both NOD proteins during the physiological and pathological inflammatory processes of preterm and term labor.

A COMMON GENETIC SUBSTRATE FOR SPONTANEOUS PRETERM LABOR/BIRTH, PERIODONTAL DISEASE AND INFLAMMATORY BOWEL DISEASE

Based on the discovery of mutations and predicted damaging missense variants in preterm PROM cases in genes previously linked to inflammatory bowel disease (*CARD8*, *NOD2*, *TLR10*, *DEFB1*, *MBL2*) and periodontal disease (*DEFB1*, *MBL2*), we propose that there is a common genetic substrate that underlies the association of inflammatory bowel disease, periodontal disease, and spontaneous preterm labor/birth. This model would predict that there is overlap in their occurrence because of a genetic commonality, rather than a causal relationship as has been suggested for periodontal disease and preterm labor/birth (Figure 9).

Inflammatory bowel disease (Crohn's disease and ulcerative colitis) is associated with adverse pregnancy outcomes including preterm labor/birth, small for gestational age birth weight, and congenital anomalies^{368–370}. Pregnant women with inflammatory bowel disease, who have a high degree of disease activity, are at a much higher risk for preterm labor/birth than those who do not have disease activity during their pregnancy^{368–370}.

In contrast to preterm labor/birth, the genetics of inflammatory bowel disease has been well studied. GWAS has identified multiple loci that have been replicated and specific variants in genes that have been identified, as in the case of *NOD2*, in inflammatory bowel disease, 272, 371, 372. A genetic relationship between inflammatory bowel disease and preterm labor/birth has been suggested by others; however, it was heretofore not validated through mutation analysis³⁷³.

Periodontal disease has been reported to be related to both inflammatory bowel disease and preterm labor/birth. Evidence from multiple cross-sectional studies shows that inflammatory bowel disease is associated with increased risk of periodontal disease³⁷⁴. Inflammatory bowel disease and periodontal disease are similar in that they are both related to an inappropriate immune response to a microbiota dysbiosis. The interaction between the two diseases appears to be complex, with one disease possibly altering the microbiota and influencing the inflammatory response of the other³⁷⁵.

Most, but not all, studies have identified an association between preterm labor/birth and periodontal disease. Conflicting reports in the literature could be due to differences the populations studied (e.g., race/ethnicity), and the phenotypic characterization of cases and controls (e.g., the clinical definition of periodontal disease)³⁷⁶. Proposed links between preterm labor/birth and periodontal disease include translocation of bacteria to the placenta and amniotic fluid, as well as a systemic inflammatory response to periodontal disease, which is chronic in nature. A recent study showed no association between periodontal disease status and microbial invasion of the amniotic cavity (MAIC) and intra-amniotic inflammation (IAI). It is possible that the link between preterm PROM and periodontal disease could be through an exaggerated systemic inflammatory response; however, this requires further investigation³⁷⁷.

The genetic contribution to periodontal disease has been explored in candidate gene association studies and GWAS. Some of these genetic studies on candidate genes identified associations with genes that we found mutated in preterm PROM³⁷⁸.

There are also overlapping environmental risk factors for preterm labor/birth, inflammatory bowel disease, and periodontal disease. These environmental factors are thought to play key roles in risk for preterm labor/birth. We hypothesize that gene-environment (e.g., smoking) interactions produce the phenotypes in preterm labor/birth as well as in inflammatory bowel disease and periodontal disease. Smoking increases the risk of preterm labor/birth. One proposed mechanism is that smoking increases the risk of preterm PROM by reducing immunity and predisposing the mother to infection³⁷⁹. In addition, smoking promotes periodontal disease³⁸⁰ and influences inflammatory bowel disease, but affects Crohn's Disease and Ulcerative Colitis differently. In Crohn's disease, tobacco use increases disease activity, while in ulcerative colitis, tobacco is associated with a lower incidence of disease exacerbations, with smoking cessation even triggering flare ups³⁸¹.

Nutrition is another environmental factor influencing preterm birth, inflammatory bowel disease, and periodontal disease. Plasma vitamin C levels have also been found to be lower in women with preterm PROM, suggesting that relative deficiency of vitamin C may be a risk factor³⁸². This may relate to the co-factor role of ascorbic acid in collagen biosynthesis and the impact of diminished lysyl hydroxylase activity on the tensile strength of the fetal membranes. Vitamin C deficiency has long been known to predispose periodontal disease^{383, 384}. Ascorbic acid levels have also been shown to be decreased in patients with inflammatory bowel disease due to oxidative stress caused by inflammatory cells, thus reducing antioxidant buffering capacity and hindering the recovery of inflamed tissue³⁸⁵.

The microbiota is a third environmental factor that is critical to the inflammation associated with chorioamnionitis, preterm PROM, inflammatory bowel disease, and periodontal disease, since pathogenic bacteria or viruses trigger the innate immune response targeted in a recent whole exome association study²¹⁰. In some cases, specific bacteria are known to be critical to the disease (e.g., *Porphyromonas gingivalis* in periodontal disease), whereas in others it is thought to be a microbial community state that is associated with inflammation (bacterial vaginosis, microorganisms in the amniotic cavity in cases of intra-amniotic infection, and preterm PROM and preterm labor/birth).

Supplementary Table 1 presents information on the possible role of mutations or genetic variants shown in Crohn's disease, ulcerative colitis and periodontal disease. Note that there are conflicting data, which may relate to the population studied or the genetic variant examined (the majority of the reports are candidate gene-association studies examining polymorphisms in coding and non-coding regions, with the exception of the *NOD2* frameshift mutation (NC_000016.10:g.50729867_50729868insC)).

It is interesting that similar, but not necessarily identical genes may be involved in these conditions. For example, *NOD2* is strongly associated with inflammatory bowel disease, and apparently preterm PROM, as shown by our data, but not with periodontal disease.

Mutations or genetic variants in *MBL2*, however, appear to be associated with Crohn's disease, ulcerative colitis, periodontal disease and preterm PROM.

AN INTEGRATED VIEW OF THE GENETIC COMMONALITY MODEL

The genes harboring rare mutations and damaging missense variants identified in a recent study²¹⁰, as well as other genes that may have been missed, are candidates for other mechanisms of dysregulation that could result in the same consequences as inactivating mutations. One mechanism could be epigenetic control of gene expression. Methylation of DNA promoter elements that regulate gene transcription can silence gene expression by preventing access of transcription factors to control elements. Gene expression can also be silenced by chromatin modification or the action of microRNAs, small non-coding RNA molecules that bind to mRNA and promote RNA destruction or prevent translation. The identification of a suite of genes that are tied to preterm PROM and other inflammatory conditions places a spotlight on these genes as potential targets for epigenetic regulation including DNA methylation, chromatin modification, and microRNAs.

FUTURE CHALLENGES

Whole exome sequencing in preterm PROM did not determine the parent of origin of the mutant/damaging missense allele. However, we propose that maternal inheritance will be prominent, which would compromise innate immunity in both the maternal and the fetal compartments. This notion needs to be fully evaluated in future research.

The discovery that mutations in genes that encode host anti-microbial defense proteins raises interesting questions about the relationship of these mutations, or any mechanism that silences their expression, to the microbiota. It would be of interest to determine the impact of the mutations and damaging missense variants in host defense genes like *DEFB1* and *MBL2* on the reproductive tract, gut and oral microbiota. This information could help clarify whether an altered microbiota per se drives adverse outcomes, including preterm labor/birth, or whether it is the host genome, which shapes the microbiota composition and the host response.

Finally, we studied African-Americans, who have a higher incidence of preterm birth²¹⁰. It remains to be determined if the genetic commonality suggested by our findings in conjunction with previously published reports, that included diverse populations, are influenced by race/ethnicity or genetic admixture. Are their population-specific mutations or damaging missense variants that could be used to predict adverse pregnancy outcome?

CONCLUSIONS

Collectively, the observations discussed in this extended editorial suggest that rare mutations or damaging missense variants in innate immunity genes play an important role in preterm PROM risk, and that an overlapping genetic substrate could explain the occurrence of inflammatory conditions (inflammatory bowel disease and periodontal disease) in women who deliver preterm (Figure 9).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Whole exome sequencing

DNA sequence analysis of exons, the protein coding regions of genes

GWAS

Genome-wide association study, which utilizes thousands of single nucleotide polymorphisms covering the entire human genome to map chromosomal regions associated with a trait by comparing the differences in the variant frequency in a large number of cases and controls.

Nonsense mutation

A DNA variation that results in a stop codon that terminates translation of the messenger RNA. This truncates the protein at the site of the stop codon.

Frameshift mutation

Either insertion or deletion of DNA bases that alters the reading frame of the encoded messenger RNA such that it is either truncated as a result of a stop codon or the protein sequence is scrambled as a result of changes in the codon reading frame.

Missense variant

A DNA base change that alters a codon and results in an amino acid substitution in the translated protein.

Epigenetics

Regulation of gene expression by reversible chemical changes that do not involve alterations in DNA sequence such as the introduction of methyl groups into cytosine residues (DNA methylation) or acetylation of lysine molecules of histone proteins that comprise chromatin, proteins bound to DNA (chromatin modification).

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Figure 1. Candidate gene approach

This technique includes the detection of genetic variants (including single nucleotide polymorphisms or SNPs) in a single gene within the genome. SNPs may fall within coding sequences of genes (exons, blue color), non-coding regions of genes, or in the intergenic regions (introns, red color).

Figure 2. Multiple candidate gene approach

This technique includes the detection of genetic variants (including single nucleotide polymorphisms or SNPs) in multiple genes within the genome. SNPs may fall within coding sequences of genes (exons, blue color), non-coding regions of genes, or in the intergenic regions (introns, red color).

Figure 3. Genome-wide association approach

This technique includes the analysis of hundreds of thousands of single nucleotide polymorphisms (SNPs) across the entire genome without bias imposed by pre-existing models and provide the opportunity of identifying novel genes, regulatory loci, and pathways not previously considered. SNPs may fall within coding sequences of genes (exons, blue color), non-coding regions of genes, or in the intergenic regions (introns, red color). Data analysis results in candidate SNPs that require further validation.

Figure 4. Whole exome sequencing approach

This technique entails the full characterization (nucleotide composition) of the protein-coding genes in a genome, known as the exome, which represents less than 2% of the genome but contains 85% of known disease-related variants. Data analysis results in the identification of mutations in the exome.

Figure 5. Classification of mutations based on their effect on the DNA

A) A point mutation is characterized by a change [insertion/deletion (indels) or substitution] of only one nucleotide base in a DNA sequence. B) A frame-shift mutation is caused by insertion/deletion (indels) of a number of nucleotides in a DNA sequence that is not divisible by three, resulting in the change of the reading frame (the grouping of the codons), and therefore in a different translation from the original (different amino acid sequence).

Classification of mutations based on their effect on the protein

Cys = Cysteine
Trp = Tryptophan

Figure 6. Classification of mutations based on their effect on the protein

A) A nonsense mutation is when a sense codon that corresponds to one of the twenty amino acids is changed to a chain-terminating (Stop) codon. B) A missense mutation includes a single nucleotide change resulting in a codon that codes for a different amino acid.

Figure 7. Whole genome sequencing approach

This technique provides information of variants in the exome, highly evolutionary conserved non-coding regions, and throughout the entire genome. Data analysis results in the identification of mutations in the entire genome.

Figure 8. Basic mechanism of activation of the inflammasome in the chorioamniotic membranes Pathogen-associated molecular patterns (PAMPs) and/or danger signals (i.e. alarmins) in the amniotic cavity can induce the activation/assembly of the inflammasome complex [NLR family pyrin domain containing protein (e.g. NLRP3) + adaptor protein or ASC (Apoptosis-Associated Speck-Like Protein) + pro-caspase-1] in the chorioamniotic membranes. Upon activation, the inflammasome prompts the release of active forms of caspase-1 which, in turn, participates in the processing of mature interleukin (IL)-1 β and IL-18 in the amniotic cavity.

Figure 9.
Overlapping gene mutations, damaging missense variants and other polymorphisms in PPROM, Crohn's disease, ulcerative colitis, and periodontal disease.