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Evidence for the participation of CHCHD2/MNRR1, a mitochondrial protein, in spontaneous labor at term and in preterm labor with intra-amniotic infection

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Each author approved the final version of the manuscript prior to its submission to the Journal.

Declaration of Interest Statement

The authors report no potential conflicts of interest. The funders had no role in the design of the study, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results. Dr. Romero has contributed to this work as part of his official duties as an employee of the United States Federal Government.

Statement of Ethics

This research complies with the guidelines for human studies and was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. The study protocols (OH97-CH-N067, OH99-CH-N055, OH98-CH-N001, and OH99-CH-N056) were reviewed and approved by the Institutional Review Board of the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, National Institutes of Health, United States Department of Health and Human Services (NICHD/NIH/DHHS) and by the Human Investigation Committee of Wayne State University (IRB Nos. 110605MP2F, 082403MP2F(5R), 075299M1E(R) (RCR), and 103108MP2F RCR).

Consent to Participate Statement

Written informed consent was obtained from the study participants prior to the collection of maternal amniotic fluid and plasma samples.

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Abstract

Objective: Intra-amniotic inflammation (IAI), associated with either microbes (infection) or danger signals (sterile), plays a major role in the pathophysiology of preterm labor and delivery. Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 2 (CHCHD2) [also known as Mitochondrial Nuclear Retrograde Regulator 1 (MNRR1)], a mitochondrial protein involved in oxidative phosphorylation and cell survival, is capable of sensing tissue hypoxia and inflammatory signaling. The ability to maintain an appropriate energy balance at the cellular level while adapting to environmental stress is essential for the survival of an organism. Mitochondrial dysfunction was observed in acute systemic inflammatory conditions, such as sepsis, and proposed to be involved in sepsis-induced multi-organ failure. The purpose of this study was to determine the amniotic fluid concentrations of CHCHD2/MNRR1 in pregnant women, women at term in labor, and those in preterm labor (PTL) with and without IAI.

Methods: This cross-sectional study comprised patients allocated to the following groups: 1) mid-trimester (n=16); 2) term in labor (n= 37); 3) term not in labor (n=22); 4) PTL without IAI who delivered at term (n=25); 5) PTL without IAI who delivered preterm (n=47); and 6) PTL with IAI and delivered preterm (n=53). Diagnosis of IAI (amniotic fluid interleukin-6 concentration ≥2.6 ng/mL) included cases associated with microbial invasion of the amniotic cavity and those of sterile nature (absence of detectable bacteria, using culture and molecular microbiology techniques). Amniotic fluid and maternal plasma CHCHD2/MNRR1 concentrations were determined with a validated and sensitive immunoassay.

Results: 1) CHCHD2/MNRR1 was detectable in all amniotic fluid samples and women at term without labor had a higher amniotic fluid CHCHD2/MNRR1 concentration than those in the mid-trimester ($p=0.003$); 2) the amniotic fluid concentration of CHCHD2/MNRR1 in women at term in labor was higher than that in women at term without labor ($p=0.01$); 3) women with PTL and IAI had a higher amniotic fluid CHCHD2/MNRR1 concentration than those without IAI, either with preterm ($p<0.001$) or term delivery ($p=0.01$); 4) women with microbial-associated IAI had a higher amniotic fluid CHCHD2/MNRR1 concentration than those with sterile IAI ($p<0.001$);

5) among women with PTL with IAI, the amniotic fluid concentration of CHCHD2/MNRR1 correlated with that of interleukin-6 (Spearman Rho=0.7; p<0.001); and 6) no correlation was observed between amniotic fluid and maternal plasma CHCHD2/MNRR1 concentration among women with PTL.

Conclusion: CHCHD2/MNRR1 is a physiological constituent of human amniotic fluid in normal pregnancy, and the amniotic concentration of this mitochondrial protein increases during pregnancy, labor at term and preterm labor with intra-amniotic infection. Hence, CHCHD2/MNRR1 may be released into the amniotic cavity by dysfunctional mitochondria during microbial-associated intra-amniotic inflammation.

Keywords

amniotic fluid; bi-organellar; inflammation; parturition; premature

Introduction

Preterm birth, a leading cause of neonatal morbidity and mortality [1–5], represents a significant economic burden on society, as approximately $26.2 billion are spent in the United States annually on the care of premature infants [6]. Intra-amniotic inflammation (IAI) associated with microbial invasion of the amniotic cavity has been causally linked to spontaneous preterm delivery [7–12]. Furthermore, a subset of pregnant women diagnosed with IAI does not have identifiable microorganisms in the amniotic fluid [13–17], and this condition, also known as sterile IAI, is more common than the microbial-associated IAI among patients who delivered preterm [15,17]. Understanding the pathophysiology of these inflammatory conditions is key for improving preventive strategies and patient management.

Coiled-Coil-Helix-Coiled-Coil-Helix Domain-Containing Protein 2 (CHCHD2), also known as Mitochondrial Nuclear Retrograde Regulator 1 (MNRR1), is a recently characterized bi-organellar protein encoded by the homonymous gene [18,19], localized primarily to the mitochondria and, to a lesser extent, in the cell nucleus [20]. Within mitochondria, this protein is involved in oxidative phosphorylation and adenosine triphosphate (ATP) production [21–23], whereas, in the nucleus, it is a transcription factor for genes implicated in the cellular response to tissue hypoxia [20,22]. Moreover, CHCHD2/MNRR1 is required for optimal induction of cellular stress-responsive signaling pathways, such as the mitochondrial unfolded protein response [24], and it can inhibit apoptosis through Bax activation [25,26]. Therefore, this bi-organellar regulator of the mitochondria and the nucleus is important for cellular function, stress response, and survival.

The ability to maintain an appropriate energy balance at the cellular level while adapting to environmental stress is essential for the survival of an organism [27–29]. Mitochondrial dysfunction was observed in acute systemic inflammatory conditions, such as sepsis [30–33], and proposed to be involved in sepsis-induced multi-organ failure [31,34,35]. Reduction of the cellular CHCHD2/MNRR1 protein, as part of mitochondrial dysfunction, has recently been shown to play a role in the amplification of inflammatory cytokines in a murine model of lipopolysaccharide (LPS)-induced systemic inflammation leading to preterm birth [36].

However, whether this protein is involved in the physiological or pathological inflammatory processes of human pregnancy has never been examined.

The aim of this study was to determine whether CHCHD2/MNRR1 can be detected in the amniotic fluid of normal pregnant women and whether the concentration of CHCHD2/MNRR1 changes during pregnancy, parturition, or with IAI. The IAI group included cases associated with microbes and those of sterile nature. We also determined maternal plasma concentrations of CHCHD2/MNRR1 in women with preterm labor to evaluate whether there is a correlation between maternal plasma and amniotic fluid concentrations of this protein.

Materials and Methods

Study design and population

A cross-sectional study was conducted by searching our clinical database and bank of biological samples to identify 200 women allocated to the following groups: 1) women with an uncomplicated pregnancy who underwent amniocentesis in the mid-trimester for genetic indications and delivered at term (n=16); 2) women with an uncomplicated pregnancy at term with (n=37) and without spontaneous labor (n=22); 3) women with pregnancies complicated by an episode of spontaneous preterm labor (PTL) and intact membranes who were further classified as (*a*) PTL without IAI who delivered at term (n=25); (*b*) PTL without IAI who delivered preterm (n=47); and (*c*) PTL with IAI who delivered preterm (n=53) (Figure 1).

Amniocentesis was performed for the following indications: genetic indication, assessment of fetal lung maturity, or evaluation of microbial invasion of the amniotic cavity. The group of women with PTL and IAI included cases of either microbial-associated or sterile IAI. Patients with a multiple pregnancy, preeclampsia, maternal medical disease, fetal death, and fetal congenital or chromosomal abnormalities were excluded from the study. Each patient provided written informed consent upon enrollment and prior to the collection of samples. The Institutional Review Boards of Wayne State University and the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development (NICHD) approved the collection of samples and the use of biological specimens and clinical data for research purposes.

Clinical definitions

Spontaneous PTL was defined by the presence of at least two regular uterine contractions every 10 minutes with documented cervical changes between 20 and 36 6/7 weeks of gestation, and by the requirement of hospitalization [17,37]. Amniocentesis was performed to assess the presence of microbial invasion of the amniotic cavity. Preterm delivery was defined as birth occurring prior to the 37th week of gestation. The management of PTL in our hospital at the time of admission included corticosteroid (betamethasone or dexamethasone) administration between 24 and 34 weeks of gestation. Betamethasone was given intramuscularly in two doses (12 mg), 24 hours apart, and dexamethasone was administered intramuscularly in 4 doses (6 mg), 12 hours apart.

Women at term (≥37 weeks of gestation) without labor underwent amniocentesis to assess fetal lung maturity prior to a delivery by cesarean section. Women at term in labor were those who presented in labor with uncertain gestational age and who underwent amniocentesis for the assessment of fetal lung maturity and microbial invasion of the amniotic cavity. Retrospectively, these patients were considered to be at term given the following characteristics: (1) spontaneous labor; (2) delivery within 48 hours of amniocentesis; (3) analysis of amniotic fluid consistent with fetal lung maturity; (4) birthweight ≥2500 grams; (5) absence of respiratory distress syndrome or other complications of prematurity; and (6) physical examination by a pediatrician consistent with that of a term neonate. IAI was defined by an amniotic fluid interleukin (IL)-6 concentration ≥ 2.6 ng/ml [38]. Women with IAI were classified as having either microbial-associated or sterile IAI according to the presence or absence of microbial invasion of the amniotic cavity. The presence of micro-organisms was assessed by conventional cultures for bacteria and by the polymerase chain reaction technique coupled with electrospray ionization mass spectrometry ([PCR/ESI-MS]; Ibis® Technology, Athogen, Carlsbad, CA, USA) in amniotic fluid samples, as previously described [17,39]. Sterile IAI was defined by an IAI without evidence of microbial invasion of the amniotic cavity by both culture and PCR/ESI-MS. Amniotic fluid samples of women at term were assessed by conventional cultures for bacteria and without IL-6 results. Only women at term without evidence of microbial invasion of the amniotic cavity were included in this study.

Amniotic fluid and maternal peripheral blood collection

Amniotic fluid samples were obtained by transabdominal amniocentesis under ultrasonographic guidance and cultured for the presence of microorganisms (aerobic and anaerobic bacteria and genital Mycoplasmas). A white blood cell count [40], a glucose concentration [41,42], and a Gram stain [43] were also performed. The results of these tests were used to guide clinical management. Amniotic fluid samples not required for clinical assessment were centrifuged for 10 min at 4°C, and the supernatant was aliquoted and stored at −80°C until analysis.

Maternal blood samples, obtained by venipuncture in patients with an episode of preterm labor, were collected in tubes containing ethylenediaminetetraacetic acid. Samples were centrifuged shortly after collection and stored at −80°C until analysis. Only samples of maternal blood collected within 48 hours of amniocentesis were considered for analysis.

Determination of human CHCHD2/MNRR1 concentrations in amniotic fluid and maternal plasma

Concentrations of amniotic fluid CHCHD2/MNRR1 and maternal plasma CHCHD2/MNRR1 were determined by using a commercially available immunoassay (Human CHCHD2 ELISA Kit, Abbexa LTD, Cambridge, UK). Briefly, 100 μL of maternal plasma, amniotic fluid, or calibrator were dispensed into separate wells of the assay plates and incubated for 90 min at 37°C. After removal of the remaining sample and calibrator, the plates were washed twice with 1X wash buffer and 100 μL of detection reagent. A working solution was added into each well. Plates were then incubated for 60 min at 37°C. Next, the plates were washed three times with 1X wash buffer; 100 μL of detection reagent B

working solution were added to each well; and plates were then incubated for 30 min at 37°C. Subsequently, the plates were washed five times with 1X wash buffer, and 90 μL of TMB substrate were added to each well. Plates were then mixed thoroughly and incubated for 20 min at 37°C. Finally, 50 μL of stop solution were added into each well to halt the reaction. The SpectraMax iD5 (Molecular Devices, San Jose, CA, USA) was utilized to read the plates, and CHCHD2/MNRR1 concentrations were calculated with SoftMax Pro 7 (Molecular Devices). The inter- and intra-assay coefficients of variation were 8.3% and 7.1%, respectively, with an assay sensitivity of 12.3 pg/mL.

Statistical Analysis

The Kolmogorov-Smirnov test and visual plot inspection were used to assess the normality of data distribution. Demographic categorical data were summarized as proportions, whereas continuous variables were summarized as medians and interquartile ranges (IQR). Differences in proportions were assessed with a Chi-square or a Fisher's exact test, while continuous data were compared between two groups with the Mann-Whitney U-test or an independent t-test. Data were compared among groups by using the Kruskal-Wallis test with post-hoc Mann-Whitney U-tests or Analysis of Variance (ANOVA) with post-hoc t-tests, depending on data distribution. Generalized linear models were utilized to assess differences in biomarkers while adjusting for potential confounders (i.e., gestational age at amniocentesis). Data were log (base 2) transformed prior to analysis. A receiver operating characteristic (ROC) curve was generated among all patients with PTL to examine the diagnostic performance of CHCHD2/MNRR1 for the identification of patients with microbial-associated intra-amniotic inflammation. Statistical analyses were performed with R statistical language version 4.1.2 and IBM SPSS version 19.0 (IBM Corporation., Armonk, NY, USA).

Results

Concentration of CHCHD2/MNRR1 in the amniotic fluid of women with normal pregnancy

Clinical characteristics of women in the mid-trimester and term no labor groups are presented in Table 1. The median gestational age (IQR) at amniocentesis was 16.1 weeks (16.0–16.9) and 38.7 weeks (38.0–40.0), respectively. CHCHD2/MNRR1 was detectable in all samples. Women at term not in labor showed a higher amniotic fluid CHCHD2/MNRR1 concentration than women in the mid-trimester group [5055 pg/mL (3780–6101) vs 3473 pg/mL (2786–4124); p = 0.003, 1.4-fold change (FC)] as shown in Figure 2.

Concentration of CHCHD2/MNRR1 in the amniotic fluid of women at term with and without labor

There were no significant differences in maternal age, gestational age at amniocentesis and at delivery, duration of sample storage, birthweight, and frequency of nulliparity between the two groups (shown in Table 1). CHCHD2/MNRR1 concentration in the amniotic fluid of women at term in labor was significantly higher than that of women at term not in labor [5708 pg/mL (4822–6764) vs 5055 pg/mL (3780–6101); p=0.01, FC=1.23] as shown in Figure 2.

Concentration of CHCHD2/MNRR1 in the amniotic fluid of women with preterm labor, classified by gestational age at delivery and the presence of intra-amniotic inflammation

Table 2 displays the clinical characteristics of women with preterm labor according to the time of delivery and presence of IAI. Women with preterm labor and IAI presented the lowest gestational age at amniocentesis among the three preterm labor groups (shown in Table 2). After adjustment for gestational age at amniocentesis, women with IAI and preterm delivery showed a significantly higher amniotic fluid CHCHD2/MNRR1 concentration than those with preterm labor without IAI who delivered preterm or at term [5535 pg/mL (4703–8730) vs 5285 pg/mL (4402–5855), p<0.001, FC=1.31; and 5535 pg/mL (4703–8730) vs 5595 pg/mL (4893–6631), p=0.012, FC=1.23, respectively]. No significant difference in amniotic fluid CHCHD2/MNRR1 concentration was observed between women with preterm labor without IAI who delivered preterm and those who delivered at term (p=0.4) as shown in Figure 3. The clinical characteristics of women with sterile (n=37) and with microbial-associated (n=16) IAI are displayed in Table 3. Women with microbial-associated IAI had a higher amniotic fluid CHCHD2/MNRR1 concentration than those with sterile IAI [9829 pg/mL (7749–11818) vs 5296 pg/mL (4583–6236), p < 0.001] as shown in Figure 4. However, no significant difference was found in the amniotic fluid CHCHD2/MNRR1 concentrations between women with sterile IAI and those without IAI who delivered either preterm or term after adjustment for gestational age at amniocentesis (p >0.05). There was a significant correlation between IL-6 and CHCHD2/MNRR1 concentrations in the amniotic fluid of women with preterm labor (n=125, Spearman Rho =0.3; p<0.001). The strength of this correlation was higher among women with IAI (n=53, Spearman Rho = 0.7; p<0.001) as shown in Supplementary Figure 1. An elevated amniotic fluid CHCHD2/MNRR1 concentration ≥ 7632 pg/mL identified women with microbial-associated IAI with a sensitivity of 81% and a specificity of 90% (area under the ROC curve 0.90; 95% confidence interval, 0.80–0.98; p < 0.001) as shown in Supplementary Figure 2.

Concentration of CHCHD2/MNRR1 in the maternal plasma of women with preterm labor

Among women with preterm labor, CHCHD2/MNRR1 concentration was also evaluated in the maternal plasma of those whose blood sample was collected within 48 hours of amniocentesis (shown in Supplementary Table 1). There were no significant differences in maternal plasma CHCHD2/MNRR1 concentrations among the three subgroups (p=0.3) as shown in Supplementary Figure 3. Moreover, no correlation was observed between the concentrations of CHCHD2/MNRR1 in maternal plasma and in amniotic fluid (n=98, Spearman Rho =0.1; p=0.2).

Discussion

Principal findings of the study

The current study demonstrates that 1) CHCHD2/MNRR1 is a physiological constituent of human amniotic fluid in normal pregnancy; 2) amniotic fluid concentration of CHCHD2/MNRR1 increases as pregnancy progresses; 3) spontaneous labor at term is a physiological inflammatory state associated with an increase in amniotic fluid CHCHD2/MNRR1 concentration; 4) among women with PTL, those with IAI show a significantly higher

concentration of CHCHD2/MNRR1 than those without this condition, and this difference is mainly attributable to women with microbial invasion of the amniotic cavity; 5) a positive correlation exists between CHCHD2/MNRR1 and IL-6 concentrations in the amniotic fluid of women with PTL; and 4) there is no significant correlation between amniotic fluid and maternal plasma CHCHD2/MNRR1 concentrations in women with preterm labor.

What is CHCHD2/MNRR1?

CHCHD2/MNRR1, a recently characterized bi-organellar protein encoded by the homonymous gene [18,19], is a member of the coiled-coil-helix-coiled-coil-helix (CHCH) domain-containing protein family, a group of evolutionary conserved proteins [44,45] found in the mitochondrial intermembrane space and now recognized as cellular factors regulating respiration, redox equilibrium, lipid homeostasis, and membrane dynamics, among others. Furthermore, a growing body of evidence suggests the involvement of several of these protein family members in human disease [46].

The *CHCHD2/MNRR1* gene was first reported in a study designed to identify new genes that affect oxidative phosphorylation [18,19]. Suppression of CHCHD2/MNRR1 cell expression led to a 50% reduction in cellular oxygen consumption and a 2-fold increase in reactive oxygen species (ROS) levels [22]. As a transcription regulator in the nucleus, CHCHD2/MNRR1 acts by binding and activating a so-called oxygen-responsive element (ORE), a conserved 13-bp DNA sequence identified in the promoter regions of *COX4I2* and of *CHCHD2/MNRR1* itself. An *in vitro* study showed that ORE-stimulated transcription is maximally induced by CHCHD2/MNRR1 under moderate tissue hypoxia (4% oxygen), suggesting that, under moderate hypoxia, it promotes a transcriptional program that achieves its normal homeostatic state, similar to the function of hypoxia-inducible factors under more severe hypoxic conditions [22]. The ORE has recently been found in other genes apart from *COX4I2* and *CHCHD2/MNRR1,* some of which are implicated in functions such as cell migration and adhesion, e.g., Mucosal Vascular Addressin Cell Adhesion Molecule 1 (MADCAM1) and cadherin4 [23]. Furthermore, CHCHD2/MNRR1 has survival value, as this protein is capable of inhibiting apoptosis by binding to the anti-apoptotic protein Bcl-xL and by preventing the accumulation of the pro-apoptotic protein Bax in the mitochondria [25]. Lastly, CHCHD2/MNRR1 is required for optimal induction of cellular stress-responsive signaling pathways, such as the mitochondrial unfolded protein response [24]. The pleiotropic role of CHCHD2/MNRR1 in physiological and pathological inflammatory processes makes this protein a candidate for studies investigating normal pregnancy and its complications.

Concentration of CHCHD2/MNRR1 in the amniotic fluid increases as gestation progresses

CHCHD2/MNRR1 was detectable in all amniotic fluid samples obtained from normal pregnant women examined in the current study, indicating that this protein represents a physiological constituent of the amniotic cavity. In addition, amniotic fluid CHCHD2/MNRR1 concentration was higher in women at term than in mid-trimester, suggesting that its concentration increases as gestation progresses. Throughout pregnancy, amniotic fluid is in direct contact with fetal organs (including mucosal surfaces of the gastrointestinal and respiratory tracts) and the chorioamniotic membranes [47–49] where water and solute

exchanges take place [50–52]. Different cells could serve as potential sources for amniotic fluid components. Indeed, CHCHD2/MNRR1 is expressed in several cell types such as neurons [53,54], hepatocytes [55], and lung cells [56]. Additionally, previous analyses of the transcriptome of different reproductive tissues found *CHCHD2/MNRR1* gene expression in the chorioamniotic membranes [57], myometrium [58], and cervix of normal pregnant women [59] as well as in the umbilical cord blood of preterm neonates [60]. Changes in the protein and metabolite components of human amniotic fluid throughout gestation were reported in several studies [61–63]. A recent proteomic study of amniotic fluid from normal pregnant women showed that, among the 320 proteins significantly changed in abundance in term pregnancy compared to mid-gestation, about 48% increased in abundance with advancing gestational age and were implicated in antimicrobial, developmental, and inflammatory functions [63]. Furthermore, Gene Ontology analysis of such upregulated proteins identified terms related to immune effector processes involved in defense against invading microbes [63]. However, the significance of the increased amniotic fluid CHCHD2/MNRR1 concentration as pregnancy progresses as well as the source and mechanisms of CHCHD2/MNRR1 release in the amniotic fluid remains to be determined.

Women with intra-amniotic inflammation have a higher amniotic fluid CHCHD2/MNRR1 concentration than those without intra-amniotic inflammation

Among the processes implicated in the activation of the common pathway of preterm labor [64], inflammation of the amniotic cavity, or IAI, is the most well established [64–68]. Indeed, microbial-associated IAI [7,68–71] and sterile IAI [38] are associated with early preterm delivery, adverse neonatal outcome, and acute inflammatory lesions of the placenta [15,71–88]. Our findings 1) that the concentration of amniotic fluid CHCHD2/MNRR1 was significantly higher in women with preterm labor with IAI than in women without IAI, and 2) that amniotic fluid CHCHD2/MNRR1 concentration correlated with amniotic fluid IL-6 concentration, led us to hypothesize that CHCHD2/MNRR1 is a part of host response against intra-amniotic infection. Among women with IAI, those with microbial-associated IAI showed a higher amniotic fluid CHCHD2/MNRR1 concentration than those with sterile IAI, which is consistent with the previous observation of significantly higher IL-6 concentrations in the amniotic fluid of the former group compared to the latter [17,37,89]. However, only pregnant women with microbial-associated IAI, but not those with sterile IAI, had a significantly higher amniotic fluid CHCHD2/MNRR1 concentration than women without IAI who delivered term or preterm, after adjustment for gestational age at amniocentesis. Thus, the difference in amniotic fluid CHCHD2/MNRR1 concentrations between women in PTL with and without IAI is mainly attributable to the group of women with proven intra-amniotic infection.

Lastly, we found that an amniotic fluid CHCHD2 concentration ≥7632pg/ml can identify women with microbial-associated IAI among all women who presented with PTL, with a sensitivity of 81% and a specificity of 90%—this finding might be useful for targeted antimicrobial therapy.

The effect of microbial-associated IAI on CHCHD2/MNRR1 release during pregnancy was recently examined with the use of trophoblast cell cultures exposed to LPS and a murine

model of preterm birth induced by the intra-peritoneal administration of LPS. LPS exposure resulted in reduced levels of CHCHD2/MNRR1 in trophoblast cells due to the stabilization of YME1L1, a protease implicated in post-translational degradation of CHCHD2/MNRR1 [36]. Additionally, decreased levels of CHCHD2/MNRR1 were associated with increased mitochondrial ROS production and with transcription of TNF-α that was independent of the Toll-Like Receptor 4 signaling pathway [36]. Our finding that the amniotic fluid CHCHD2/MNRR1 concentration was increased in women with IAI seems to contradict the observed decrease of CHCHD2/MNRR1 in trophoblast cells. However, the reduced level of CHCHD2/MNRR1 in trophoblast cells could be due, in part, to the heightened extracellular release of this protein; indeed, murine macrophages treated with LPS release CHCHD2/MNRR1 in the supernatant (Purandare N, Grossman LI, Aras S, et al., unpublished data). Thus, invading bacteria may induce the extracellular release of CHCHD2/MNRR1 from either amniotic fluid leukocytes [90–94] or from the chorioamniotic membranes of women with IAI. Alternatively, this intracellular bi-organellar protein could be indirectly released into the amniotic cavity as a result of cell death caused by IAI, especially if infection is present. Future investigations of the chorioamniotic membranes, which are in direct contact with amniotic fluid, may help to examine these hypotheses.

Plasma concentration of CHCHD2/MNRR1 in maternal blood of women with preterm labor

We found no correlation between concentrations of amniotic fluid CHCHD2/MNRR1 and maternal plasma CHCHD2/MNRR1 in women with preterm labor. This observation is consistent with the asymptomatic or the subclinical nature of IAI in the majority of women [67,95–98], with only a small proportion developing systemic signs and symptoms of maternal infection (i.e., clinical chorioamnionitis) [99,100]. Thus, the increased concentration of CHCHD2/MNRR1 in the amniotic cavity may reflect the localized nature of the intra-amniotic inflammatory response. Amniotic fluid represents a unique source of information about processes localized to the uterus. However, techniques for amniotic fluid collection are invasive either if performed transabdominally or transcervically by needle amniotomy as recently described [101]. Devices have been designed for non-invasive transcervical collection of amniotic fluid in women with ruptured membranes [102]; however, non-invasive techniques are not available to retrieve amniotic fluid from women with intact membranes. We evaluated CHCHD2 in maternal plasma, but the results were disappointing. Future studies of this mitochondrial protein in the vaginal fluid as a non-invasive biomarker of intra-amniotic infection/inflammation may be possible.

Labor at term is associated with a higher concentration of CHCHD2/MNRR1 in amniotic fluid

The activation of the common pathway of spontaneous labor at term [65,103,104] is a coordinated physiological inflammatory process [79,105–111]. Multiple studies have shown that an increase in cellular and soluble inflammatory mediators occurs in the uterine tissues [112–120], decidua [107,114,115,121–125], chorioamniotic membranes [114,115,121,122,126], cervix [59,105,113,116,121,127], and amniotic fluid [128–134] during normal parturition at term. Furthermore, several cytokines, including TNF-α [132,133], are higher in the amniotic fluid of women at term in labor compared to those not in labor [128–133]. The localized inflammatory state may be responsible for the increased

release of mitochondrial protein CHCHD2/MNRR1 from the gestational tissues or immune cells that are in direct contact with the amniotic fluid of women at term in labor [135]. The changes in amniotic CHCHD2/MNRR1 concentration in women at term in labor may be due to enhanced protein release rather than to increased gene expression in the myometrium [58] and chorioamniotic membranes [136], as transcriptomic analysis did not show differences in *CHCHD2* expression in those tissues from women at term in labor compared to those not in labor. Elevated amniotic fluid CHCHD2/MNRR1 concentration may therefore reflect the physiological mild and localized inflammatory state that characterizes spontaneous labor at term. However, it is difficult to reconcile why the amniotic fluid CHCHD2/MNRR1 concentration was higher in women with spontaneous labor at term but not in those with PTL and sterile IAI, compared to controls without IAI.

Strengths and Limitations

This is the first study to report on the amniotic fluid concentration of CHCHD2/MNRR1 in pregnant women with an uncomplicated pregnancy, in women at term in labor, and in those with preterm labor with or without IAI. In women with PTL, the diagnosis of microbial invasion of the amniotic cavity was made by real-time PCR/ESI-MS. We also examined whether there was a correlation between CHCHD2/MNRR1 concentration in the amniotic fluid and in the maternal plasma of women with preterm labor. However, the cross-sectional nature of this study does not allow evaluation of the temporal relationship between the changes in CHCHD2/MNRR1 concentration and the clinical conditions examined.

Conclusions

We report herein for the first time on the presence of the mitochondrial protein, CHCHD2/MNRR1, in the amniotic fluid of human pregnancy. The amniotic fluid concentration of this protein increases as gestation progresses, in women at term in labor, and in the presence of intra-amniotic infection. It is possible that CHCHD2/MNRR1 represents a mitochondrial protein released by dysfunctional mitochondria during microbial-associated intra-amniotic inflammation. Further studies are needed to better understand the mechanisms of CHCHD2/MNRR1 release and the clinical significance of this protein in the context of obstetrical disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author at prbchiefstaff@med.wayne.edu (Dr. Romero).

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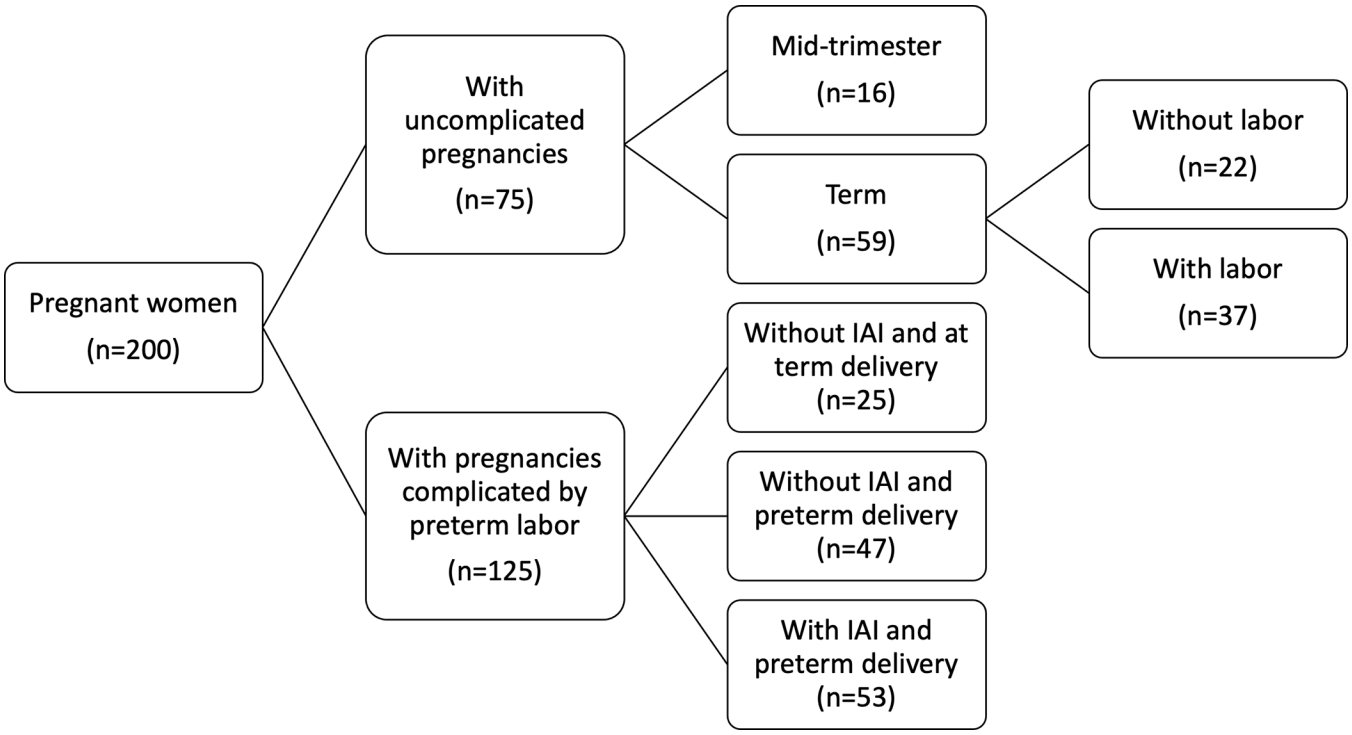

Figure 1.
Flow diagram of patients included in the study.

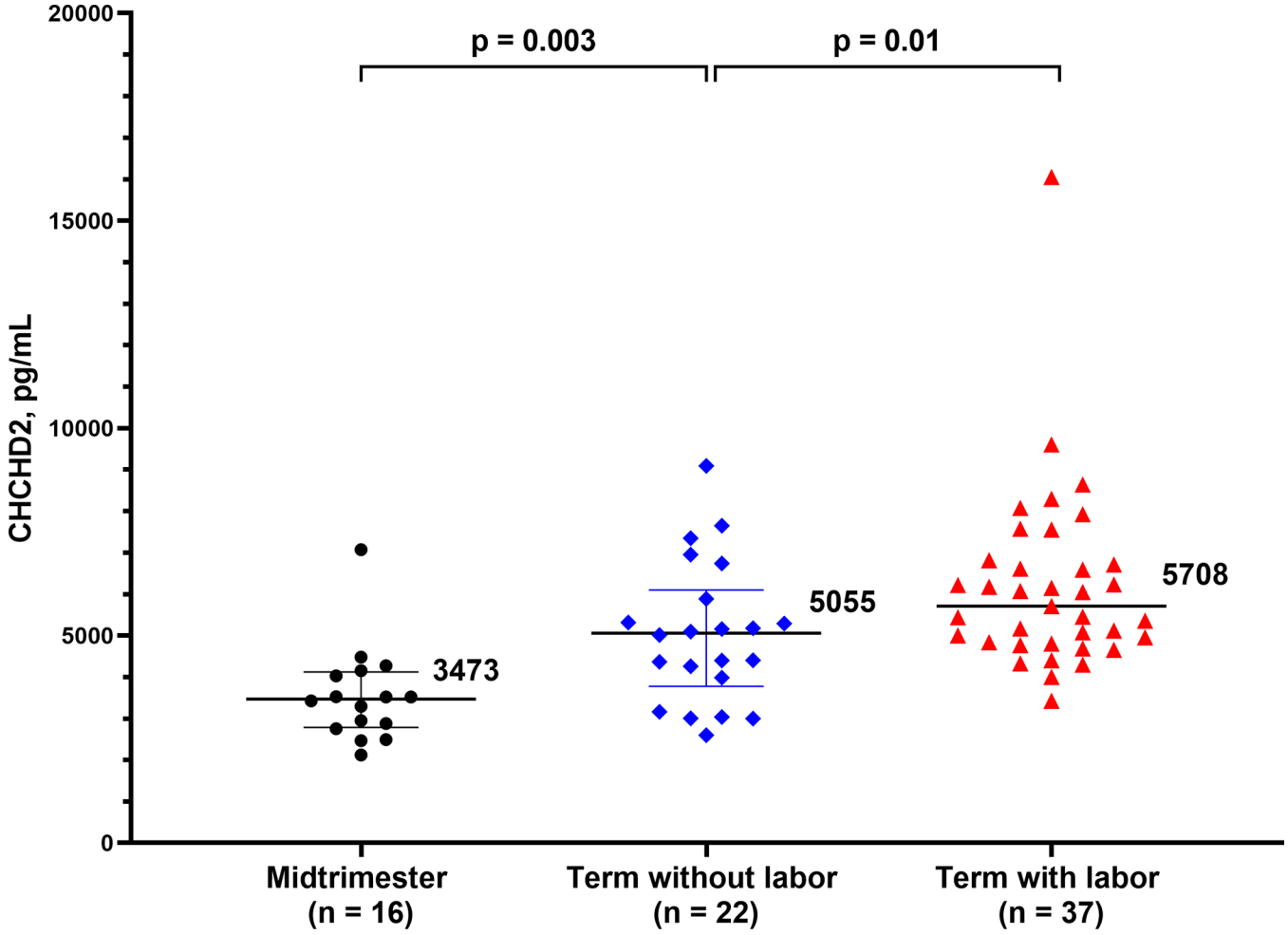

Figure 2.

Concentration of CHCHD2/MNRR1 (pg/mL) in the amniotic fluid of pregnant women in the mid-trimester, at term without labor and at term with labor. Data are reported as medians and interquartile ranges. The analysis was performed after $\log_2$ transformation of the data.

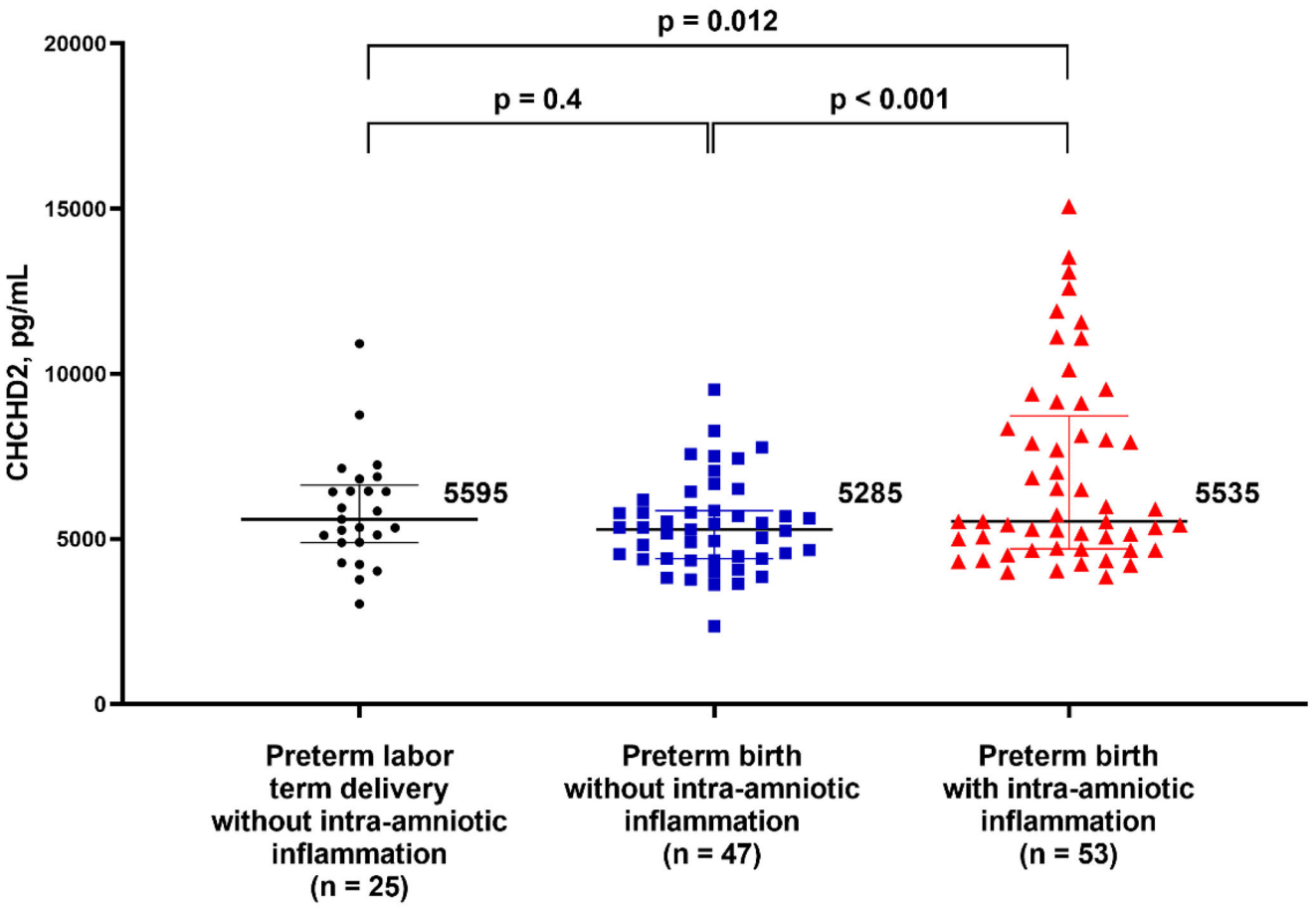

Figure 3.

Concentration of CHCHD2/MNRR1 (pg/mL) in the amniotic fluid of pregnant women in preterm labor classified by gestational age at delivery and the presence of intra-amniotic inflammation. Data are reported as medians and interquartile ranges. The analysis was performed after $\log_2$ transformation of the data.

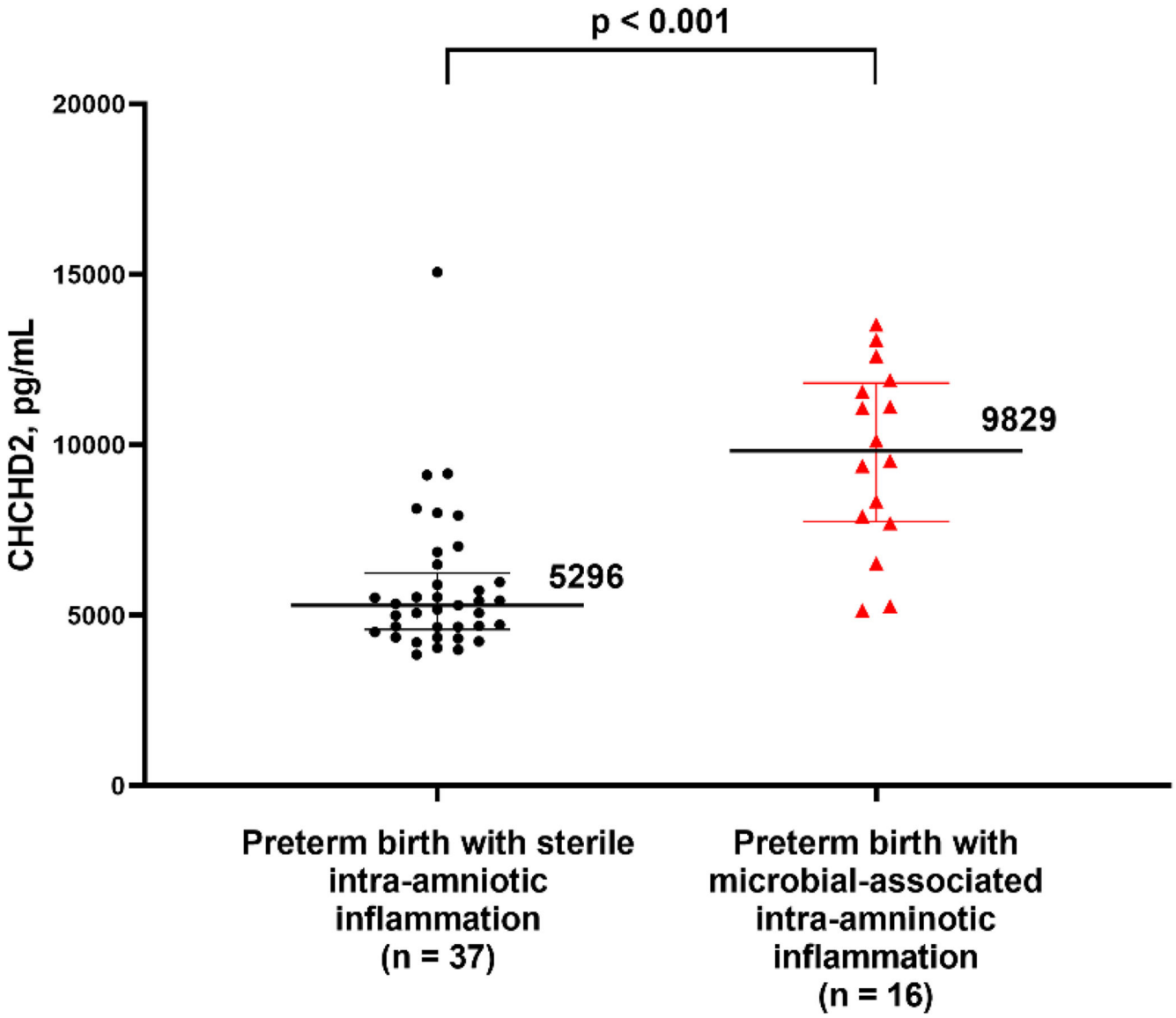

Figure 4.

Concentration of CHCHD2/MNRR1 (pg/mL) in the amniotic fluid of pregnant women in preterm labor with intra-amniotic inflammation classified by the presence or absence of microbial invasion of the amniotic cavity. Data are reported as medians and interquartile ranges. The analysis was performed after $\log_2$ transformation of the data.

Table 1.

Clinical characteristics of women in the mid-trimester of pregnancy and at term with and without labor.

	Mid-trimester (n=16)	Term No Labor (n=22)	p value*	Term in Labor (n=37)	P value**
Maternal age (years)	36.5 (33.5–39.7)	29.5 (19.7–35.0)	< 0.001	22.0 (19.5–24.5)	0.074
Nulliparity	31.3% (5)	25% (5/20)[a]	0.7	50% (18/36) [a]	0.1
GA at amniocentesis (weeks)	16.1 (16.0–16.9)	38.7 (38.0–40.0)	< 0.001	35.8 (37.7–39.2)	0.4
GA at delivery (weeks)	39.5 (39.0–40.0)	38.7 (38.0–40.0)	0.09	38.5 (37.7–39.2)	0.4
Duration of sample storage (years)	26.9 (25.8–27.4)	31.6 (31.6–31.7)	< 0.001	31.6 (31.3–31.7)	0.6
Birthweight (grams)	3460 (3261–3923)	3230 (3031–3686)	0.07	3390 (3040–3545)	0.9

GA: gestational age. Data are reported as the median (interquartile range) for continuous variables and as a percentage (number) for dichotomous variables;

[a] missing values.

* p value for mid-trimester vs term no labor;

** p value for term no labor vs term labor.

Table 2.

Clinical characteristics of women with preterm labor according to the gestational age at delivery (preterm and term) and the presence of intra-amniotic inflammation.

	Preterm labor and term delivery, without intra-amniotic inflammation (n=25)	Preterm labor and delivery, without intra-amniotic inflammation (n=47)	Preterm labor and delivery, with intra-amniotic inflammation (n=53)	p value
Maternal age (years)	23.0 (20.5–28.5)	23.5 (20.0–26.5)	23.0 (20.0–28.0)	0.9
BMI (kg/m2)	23.7 (20.8–30.7)	23.2 (19.6–28.9)	25.8 (20.5–32.6)	0.4
Tobacco use	16.0% (4)	21.7% (10/46) [a]	21.2% (11/52) [a]	0.8
Nulliparity	20.0% (5)	31.9% (15)	30.2% (16)	0.5
GA at amniocentesis (weeks)	31.6 (30.5–32.6)	31.6 (28.6–32.7)	25.6 (23.5–31.8)	<0.001
GA at delivery (weeks)	38.7 (37.8–39.6)	34.8 (33.4–36.0)	26.6 (24.4–32.3)	<0.001
Duration of sample storage (years)	16.2 (14.6–16.5)	15.4 (13.9–16.6)	16.2 (13.4–17.0)	0.6
Birthweight (grams)	3065 (2807–3340)	2330 (1935–2645)	910 (610–1892)	<0.001

BMI: body mass index; GA: gestational age; Data are reported as the median (interquartile range) for continuous variables and as a percentage (number) for dichotomous variables;

[a] missing values.

Table 3.

Clinical characteristics of women with preterm birth and intra-amniotic inflammation in the presence or absence of microbial invasion of the amniotic cavity.

	Preterm birth with sterile intra-amniotic inflammation (n=37)	Preterm birth with microbial-associated intra-amniotic inflammation (n=16)	p value
Maternal age (years)	23.0 (20.0–25.7)	24.0 (20.0–31.0)	0.2
BMI (kg/m2)	23.2 (19.6–32.4)	26.5 (23.5–35.1)	0.2
Tobacco use	22.2% (8/36) [a]	18.8% (3)	1.0
Nulliparity	27.0% (10)	37.5% (6)	0.5
GA at amniocentesis (weeks)	24.7 (23.3–30.5)	26.3 (23.8–32.3)	0.3
GA at delivery (weeks)	26.6 (24.2–32.1)	26.7 (24.8–32.6)	0.9
Duration of sample storage (years)	15.3 (13.4–17.0)	16.3 (13.7–17.4)	0.4
Birthweight (grams)	910 (558–1907)	913 (672–1877)	0.8

BMI: body mass index; GA: gestational age; Data are reported as the median (interquartile range) for continuous variables and as a percentage (number) for dichotomous variables;

[a] missing values.