A polymorphism in the matrix metalloproteinase-9 promoter is associated with increased risk of preterm premature rupture of membranes in African Americans

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Fetal membrane rupture is associated with increased expression of matrix metalloproteinase-9 (MMP-9) and matrix degradation. We have determined the functional significance of a variable number tandem repeat and a single nucleotide polymorphism (SNP) in the MMP-9 gene on promoter activity and their association with preterm premature rupture of membranes (PPROM). The 14 CA-repeat allele was a stronger promoter than the 20 CA-repeat allele in amnion epithelial cells and WISH amnion-derived cells, but in THP-1 monocyte/macrophage cells the 14 and 20 CA-repeat alleles had similar activities. An SNP at –1562 did not significantly affect promoter activity. A case–control study of African American neonates revealed that the 14 CA-repeat allele was more common in newborns delivered of mothers who had PPROM than in those delivered at term. There was no association between the –1562 SNP and PPROM. We conclude that there are cell host-dependent differences in MMP-9 promoter activity related to CA-repeat number and that fetal carriage of the 14 CA-repeat allele is associated with PPROM in African Americans.

Key words: amnion epithelial cells/extracellular matrix/genetic association/PPROM/transcriptional control

Introduction

Preterm premature rupture of membranes (PPROM), defined as spontaneous fetal membrane rupture prior to the 37th week of gestation, occurs in 1% of pregnancies and accounts for 30–40% of all preterm deliveries (Parry and Strauss, 1998). Epidemiological studies suggest that preterm delivery is a condition that clusters in families (Hoffman and Ward, 1999). Moreover, preterm labour is a recurrent condition (Bloom et al., 2000) and the incidence of PPROM and preterm birth differs among ethnic groups (Hoffman and Ward, 1999). African Americans have a >2-fold greater risk of preterm birth than Caucasians, and PPROM is more likely to precede spontaneous preterm birth in African Americans (Ventura et al., 1999); these differences cannot be accounted for by socioeconomic status (Adams et al., 1993). These observations suggest that genetic factors contribute to the risk of preterm birth, in addition to environmental and medical risk factors. However, the genes conferring increased risk for PPROM and preterm birth remain largely unknown.

Matrix metalloproteinase-9 (MMP-9) is a member of the MMP gene family that encodes zinc-dependent enzymes that break down extracellular matrix (ECM) macromolecules (Nagase and Woessner, 1999). MMP-9 degrades type IV collagen, which is a major component of basement membranes, denatured fibrillar collagen, and other ECM molecules including laminin and fibronectin. In human and animal amnion, MMP-9 increases at the time of parturition (Vadillo-Ortega et al., 1995; Maymon et al., 2000; McLaren et al., 2000; Uchide et al., 2000) and high levels of MMP-9 are found in the amniotic fluid of women with spontaneous preterm labour (Athayde et al., 1998). Amnion epithelial cells produce MMP-9 and induction of MMP-9 in this cell layer promotes degradation of the underlying basement membrane leading to cell detachment and apoptotic death (Lei et al., 1999).

The expression of MMP-9 is primarily regulated at the level of transcription (Fini, 1998). Therefore, it is important to understand the factors that control the transcriptional response of this gene in relevant cells. Pro-inflammatory cytokines and bacterial endotoxin strongly induce MMP-9 expression in epithelial cells and monocyte/macrophages (Huhtala et al.,...
1991; Kondapaka et al., 1997). The magnitude of the response to cytokines and endotoxin may be influenced by the strength of the stimulus, the function of the cellular signal transduction system activated by the stimulus, and the structure of the MMP-9 gene (i.e. genetic variation in the MMP-9 locus). Several polymorphisms have been found in the MMP-9 gene, including polymorphisms in the proximal promoter (St Jean et al., 1995; Peters et al., 1999; Shimajiri et al., 1999; Zhang et al., 1999a,b; Yoon et al., 1999; Nelissen et al., 2000; Ye, 2000). Two of these polymorphisms have been reported to have functional significance with respect to promoter activity: a CA-repeat microsatellite polymorphism at position −55 to −13 (St Jean et al., 1995; Shimajiri et al., 1999; Yoon et al., 1999; Nelissen et al., 2000), and a single nucleotide polymorphism (SNP) at position −1562 (C to T substitution) (Peters et al., 1999; Zhang et al., 1999a,b).

We postulated that polymorphisms affecting MMP-9 expression are associated with PPROM, because increased transcription of the MMP-9 gene or untimely MMP-9 expression could lead to weakening of the fetal membranes and subsequent rupture. To test this hypothesis, we investigated the functional significance of different alleles with respect to MMP-9 promoter activity in human amnion epithelial cells, and conducted a case–control study to examine the association between the different MMP-9 promoter alleles and PPROM. The case–control study was conducted on African Americans because of the higher incidence of PPROM in this population.

Materials and methods

Subjects

Subjects in this study were African American women seeking obstetric care at the Hospital of the University of Pennsylvania, Philadelphia, PA and Hutzel Hospital, Detroit, MI, USA. The University of Pennsylvania, Wayne State University and National Institute of Child Health and Human Development Institutional Review Boards approved the study. Informed consent was obtained from mothers prior to collection of biological material including specimens for extraction of DNA. Cases were defined as neonates from pregnancies complicated by rupture of membranes prior to 37 weeks of completed gestation (n = 74). The diagnosis of membrane rupture was based on pooling of vaginal fluid and a positive nitrazine test. Controls (n = 215) were neonates from normal pregnancies delivered at term of mothers with no prior history of preterm birth or PPROM. Patients with multiple gestations, fetal anomalies and medical complications of pregnancy requiring induction of labour were excluded.

Isolation of amnion epithelial cells

Human amnion epithelial cells were isolated using a modification of a previously published procedure (Whittle et al., 2000). Term (37–40 weeks gestation) placentas with attached fetal membranes were collected immediately following spontaneous vaginal delivery. The amnion was peeled away from the chorion and washed in phosphate-buffered saline (Dulbecco’s PBS, pH 7.5; Gibco/BRL, Grand Island, NY, USA) and antibiotics (100 IU/ml penicillin G, 100 IU/ml streptomycin sulphate, 0.25 μg/ml amphotericin B; Gibco/BRL) for 5 min. Amnion pieces were transferred to a 250 ml flask containing 0.25% trypsin (Gibco/BRL) in Dulbecco’s modified Eagle’s medium (DMEM) (digestion media) and incubated for 30 min at 37°C with gentle shaking. The supernatant from the first digestion was discarded and the tissue was then subjected to two subsequent 60 min incubations in digestion media. Dispersed cells were collected by centrifugation at 2500 g for 10 min. The cell pellets were resuspended in DMEM supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 IU/ml penicillin G, 100 IU/ml streptomycin sulphate, 0.25 μg/ml amphotericin B; Gibco/BRL) and layered onto a discontinuous Percoll gradient (5, 20, 40 and 60; vol/vol). The gradient was centrifuged at 800 g for 20 min. Cells at the 20% Percoll layer were recovered and washed with DMEM containing 10% FBS and 0.8% Ultroser G (Biopool, Marlborough, MA, USA), and plated in 25 ml tissue culture flasks. The cells were maintained at 37°C in a water-saturated atmosphere under 5% CO2 in air. The medium was changed 24 h after plating and thereafter every 2–3 days. When the cultures were 90% confluent (~7 days of culture), the cells were harvested for use in experiments. Preparations in which >5% of cells had a morphology that resembled fibroblasts were discarded.

For experiments, cells were seeded into 24-well plates that had been pre-coated with fibronectin (Gibco/BRL) at a concentration of 2.5 μg/ml for 20 min; cells were also cultured on plates that had been pre-coated with laminin at a concentration of 5 μg/ml for 60 min (Gibco/BRL), or rat tail collagen (BD Biosciences, Bedford, MA, USA) at a concentration of 4 mg/ml for 10 min. The cells were cultured in DMEM supplemented with 10% FBS and 0.8% Ultroser G. After 80% confluence was achieved in about 2–3 days of culture, cells were stimulated with TNF-α (R&D Systems, Minneapolis, MN, USA) (50 ng/ml) and used in transfection experiments as described below.

WISH amnion-derived cells (American Type Culture Collection, Rockville, MD, USA) were cultured in 12-well plates in DMEM supplemented with 10% FBS and antibiotics (100 IU/ml penicillin G, 100 IU/ml streptomycin sulphate, 0.25 μg/ml amphotericin B), THP-1 monocyte/macrophage cells (American Type Culture Collection) were cultured in 6-well plates in Roswell Park Memorial Institute medium supplemented with 10% FBS and 50 μg/ml gentamicin.

Immunohistochemistry

Amnion cells were characterized using specific antibodies for two cytoskeletal proteins expressed in mesenchymal and epithelial cells, respectively. Cytokeratin, an epithelial cell lineage marker, was detected using a mouse monoclonal anti-human cytokeratin antibody (Sigma Chemical Co., St Louis, MO, USA) at a 1:400 dilution. Vimentin, a mesenchymal cell lineage marker, was detected using a mouse monoclonal anti-human vimentin antibody (Sigma Chemical Co.) at a 1:50 dilution. Antibody staining was visualized using the Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) with 3,3’-diaminobenzidine (FAST DAB tablets; Sigma Chemical Co.) as the chromagen.

Briefly, cells were washed with PBS, then fixed with 3% paraformaldehyde for 30 min, washed again with PBS, treated with 3% hydrogen peroxide and washed with PBS. The primary antibody was applied and incubated for 40 min at room temperature. Cells were washed and incubated with the secondary antibody for 30 min at room temperature, washed again and incubated with avidin–biotin–peroxidase complex for 30 min followed by incubation with diaminobenzidine for 5 min. The cells were counterstained with haematoxylin. Controls were treated in a similar fashion but without the addition of the primary antibody.

MMP-9 assay

We examined the secretion of MMP-9 by amnion epithelial cells after stimulation with tumour necrosis factor-α (TNF-α) using an MMP-9 enzyme-linked immunosorbent assay (Oncogene Research Products, Boston, MA, USA). MMP-9 concentrations in the conditioned media were standardized to total protein content of the cell lysates.
Analysis of MMP-9 promoter alleles

DNA was extracted from umbilical cords, cord blood, or neonate cheek swabs by conventional methods (Ausubel et al., 1987). The length of the CA-repeat in the MMP-9 promoter was determined using PCR products generated with a fluorescently-labelled forward primer 5'-6FAM-ACCTGGCAGTAGTTGACGCTC-3' (corresponding to base pairs −208 to −185) and reverse primer 5'-TGTGTCGGACTCACATAGTGGGCC-3' (corresponding to base pairs −33 to −13), based on the human MMP-9 gene sequence (Huhtala et al., 1991) (GenBank Accession No. D10051). PCR was carried out using Ready-To-Go PCR Beads (Amer sham Pharmacia Biotech Inc., Piscataway, NJ, USA). After initial denaturation at 94°C for 5 min, PCR was performed for 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension 1 min at 72°C. The PCR products were analysed with an ABI 3100 Genetic Analyzer using GeneScan software. The accuracy of the determination of CA-repeat number was verified by sequence analysis of PCR products derived from 15 different individuals. The results were completely concordant with the GeneScan analysis.

To analyse the −1562 polymorphism, we amplified a region of the MMP-9 promoter using a forward primer 5'-GCTGCGCATATGATAGGCGCC-3' (corresponding to base pairs −1871 to −1851) and a reverse primer 5'-CTTTCACTCGAGCCGCATC-3' (corresponding to base pairs −1339 to −1319). After initial denaturation at 94°C for 5 min, PCR was performed for 40 cycles of denaturation at 94°C for 1 min, annealing at 57°C for 1 min, and extension for 1 min at 72°C. The reaction products were digested with restriction endonuclease Sph I (New England Biolabs, Beverly, MA, USA) and fractioned on a 1.5% agarose gel. The −1562T allele is digested by SphI, yielding fragments of 247 and 188 base pairs; the −1562C allele is not cleaved by SphI.

Construction of promoter-reporter plasmids

To determine whether the variable length CA-repeats and the −1562 SNP influence transcription of the MMP-9 gene, we constructed fragments of the MMP-9 promoter containing either 14 or 20 CA-repeats and a −1562C allele. Two oligonucleotide primers were selected from sequences in the MMP-9 promoter (forward 5'-ACCTCGTGGAGCGCAAAGCAGA-3' and reverse 5'-AGAGGCTGCTGCTGAGCTGAG-3') for amplification of a region from base pairs −1871 to +12. Promoter constructs encompassing −1883 bp with either 14 or 20 CA-repeats and a −1562 C allele were cloned into the pGL3 vector (Promega, Madison, WI, USA), which contains the firefly luciferase gene as a reporter. We also designed two different promoter constructs with either C or T at −1562; these constructs contained 21 CA-repeats. The DNA sequences of the promoter constructs were verified prior to use.

Luciferase assay

WISH cells (10^5 cells/well) and THP-1 cells (80×10^5 cells/well) were seeded into 12-well plates. The amnion epithelial cells (10^5 cells/well) were cultured in 24 well plates. The MMP-9 promoter constructs and the pRLTK1 (Promega) reporter control were transfected into cells with 1 µg (THP-1 cells) or 2 µg (WISH cells and primary amnion epithelial cells) of promoter-reporter plasmid and 25 ng pRLTK1, a control plasmid expressing renilla that was used to correct for transfection efficiency, with FuGENE 6 (Roche, Indianapolis, IN, USA). The transfected cells were cultured for 48 h in DMEM supplemented 10% FBS and 0.8% Ultroser G. The cells were then lysed using passive lysis buffer (Promega). Aliquots of 20 µl of the supernatant were assayed for luciferase and renilla (Dual Luciferase-Renilla, Promega) using a luminometer (Lumat LB 9507, Berthold). Promoter activity was expressed as the ratio between luciferase and renilla activity.

Statistical analysis

The transfection experiments were evaluated using the Poisson distribution for each cell type separately. For the case–control study, demographic characteristics were compared using Students t-test. To evaluate the association between the different alleles and risk of PPROM, we calculated the odds ratio (OR) and confidence intervals (CI) comparing the specified allele to all others with the Cornfield method of variance estimation using Stata Statistical Software: Release 6.0 (Stata Corporation) (Feller, 1968; Selvin, 1995, 1996). To adjust for multiple testing, we adopted a conservative alpha (α) level utilizing a Bonferroni correction (Pocock et al., 1987).

Results

MMP-9 promoter allele frequencies

Because the frequency of MMP-9 promoter alleles has yet to be reported for African Americans, we characterized these in neonates derived from normal pregnancies in order to select alleles for functional analysis. The most prevalent MMP-9 promoter CA-repeat alleles in African Americans contain 14, 20, 21 or 22 repeats (Table I). These are also the most common MMP-9 promoter alleles reported in Caucasians (St Jean et al., 1995; Peters et al., 1999; Yoon et al., 1999; Nelissen et al., 2000), while in Japanese the most common allele contains 21 CA-repeats (Shimajiri et al., 1999). The frequencies of the −1562 C (87.1%) and −1562 T (12.9%) alleles in our African American population were similar to those previously reported for Caucasians (Zhang et al., 1999a).

Characterization of isolated human amnion epithelial cells

The functional significance of a dinucleotide (CA) repeat polymorphism and an SNP at −1562 in the MMP-9 gene on promoter activity has been evaluated in several different cell types. However, there has yet to be an examination of promoter function in amnion epithelial cells, a relevant cell type for analysis of MMP-9 expression related to fetal membrane rupture. We established a primary culture system for human amnion epithelial cells in order to study MMP-9 promoter function because the WISH amnion-derived cell line, which has frequently been employed for in-vitro studies, is probably contaminated with HeLa cells and may, therefore, not be a reliable model system to study the function of amnion epithelial cells (Kniss et al., 2001). The isolated amnion cells cultured on a fibronectin matrix multiplied and grew to confluent monolayers. In preliminary experiments, we examined the influence of different matrix coatings on cell growth and morphology. Cells cultured in the absence of matrix or cells grown on a laminin matrix had morphology similar to that of cells grown on fibronectin (Figure 1). However, their proliferation rate was slower and they reached confluence in 12-well plates in twice the time it took for cells growing on fibronectin. Cells grown on a rat-tail collagen matrix did not proliferate and remained isolated, never forming a confluent monolayer. The majority of the isolated amnion cells (>90%) grown on fibronectin stained for the epithelial marker cytokeratin (Figure 2A), and did not stain for the mesenchymal marker, vimentin (Figure 2B), after 14 days in culture. When the isolated amnion cells were stimulated with TNF-α, MMP-9 secretion into the culture medium was increased more than 2-
MMP-9 polymorphism and fetal membrane rupture

Table I. CA-repeat allele frequencies in the matrix metalloproteinase-9 promoter in African Americans

<table>
<thead>
<tr>
<th>Number of CA-repeats (%)</th>
<th>14</th>
<th>15</th>
<th>16</th>
<th>17</th>
<th>18</th>
<th>19</th>
<th>20</th>
<th>21</th>
<th>22</th>
<th>23</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of African Americans (215)</td>
<td>19</td>
<td>4.8</td>
<td>0.7</td>
<td>1.6</td>
<td>3</td>
<td>8.3</td>
<td>16.7</td>
<td>19.8</td>
<td>14.9</td>
<td>9.5</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Number of subjects analysed = 215.

Figure 1. Morphology of isolated amnion epithelial cells cultured on different matrices. (A) Cells cultured in absence of a matrix coating. (B) Cells cultured on a rat tail collagen. (C) Cells cultured on laminin. (D) Cells cultured on fibronectin. All the cultures were photographed 5 days after seeding.

fold (Figure 2D). The fold increase in TNF-α stimulated production of MMP-9 was greatest for cells grown on a fibronectin matrix and least for cells grown on rat-tail collagen.

Analysis of MMP-9 promoter activity

We elected to study the activity of the 14 CA-repeat and 20 CA-repeat alleles because they are among the most prevalent alleles in African Americans (Table I) and previous authors have reported that the promoter activity of the 14 CA-repeat allele differs from that of alleles with a larger number of repeats in non-amnion-derived cell hosts (Peters et al., 1999; Shimajiri et al., 1999). In primary cultures of amnion epithelial cells and WISH cells, the 14 CA-repeat MMP-9 promoter had 2–3-fold greater activity than 20 CA-repeat allele (Table II). However, in THP-1 cells the MMP-9 promoter with 14 CA-repeats had activity similar to that of the MMP-9 promoter construct with 20 CA-repeats. We could not detect significant differences in promoter activity between the –1562 C and –1562 T alleles when the constructs were transfected into primary amnion epithelial cell cultures, WISH or THP-1 cells (Table III).

Association of MMP-9 promoter alleles and PPROM

We carried out a case–control study to determine the association of MMP-9 promoter alleles with PPROM by genotyping 74 cases of PPROM and 215 controls. The demographic characteristics of our study population are shown in Table IV. The pregnant women were similar in age, gravidity and parity, but there were significant differences, as expected, in gestational age at birth and birthweight. We calculated the risk of PPROM for each of the fetal CA-repeat alleles from 14 to 24 repeats, compared with all the other alleles, and found that the 14 CA-repeat allele was associated with a significantly increased risk for PPROM (OR = 3.06; 95% CI 1.77–5.27) (Table V). The overrepresentation of the 14 CA-repeat allele in PPROM was significant.
Figure 2. Characterization of isolated amnion epithelial cells. (A) Cells stained for cytokeratin. (B) Cells stained for vimentin. (C) Control preparation processed in the absence of primary antibody. (D) Tumour necrosis factor-α (TNF-alpha) (50 ng/ml) induces matrix metalloproteinase-9 (MMP-9) production by amnion epithelial cells. Values are means ± SD from triplicate cultures.

<table>
<thead>
<tr>
<th>Table II. Allele-specific matrix metalloproteinase-9 (MMP-9) promoter activity in different cell lines</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell host</strong></td>
</tr>
<tr>
<td>Amnion epithelial</td>
</tr>
<tr>
<td>WISH</td>
</tr>
<tr>
<td>THP-1</td>
</tr>
</tbody>
</table>

The cells were transfected with pGL3 vector containing MMP-9 promoters with 14 or 20 CA-repeats and pRLTK-1. Values presented are the mean ± SE ratios of activities of the 14 CA- and 20 CA-repeat alleles (n = 3–4 separate experiments).

CI = confidence interval.

<table>
<thead>
<tr>
<th>Table III. Influence of the −1562 single nucleotide polymorphism on matrix metalloproteinase-9 (MMP-9) promoter activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cell host</strong></td>
</tr>
<tr>
<td>Amnion epithelial</td>
</tr>
<tr>
<td>WISH</td>
</tr>
<tr>
<td>THP-1</td>
</tr>
</tbody>
</table>

Cells were transfected with pGL3 containing C or T at −1562 and pRLTK-1. Values presented are the mean ± SE ratios of the activities of the C and T alleles (n = 6).

Table IV. Demographic characteristics of subjects in the case–control study

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 215)</th>
<th>PPROM (n = 74)</th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.2 ± 5.9</td>
<td>25.2 ± 6.5</td>
<td>1.00</td>
</tr>
<tr>
<td>Gravidity</td>
<td>3.1 ± 1.9</td>
<td>3.5 ± 2.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Parity</td>
<td>1.3 ± 1.4</td>
<td>1.6 ± 1.6</td>
<td>0.06</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)</td>
<td>39.2 ± 1.4</td>
<td>32.2 ± 3.1</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Birthweight (g)</td>
<td>3325 ± 439</td>
<td>1959 ± 607</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

PPROM = preterm premature rupture of membranes.

Discussion

MMP-9 expression in amnion increases at parturition, and the rise in this activity is postulated to promote ECM degradation after a Bonferroni adjustment for multiple comparisons. There was a nominally significant risk with the 17 CA-repeat, but the number of subjects carrying this allele was small and the P-value was not significant after adjusting for multiple comparisons. There was no association between fetal carriage of a −1562 C or −1562 T allele and PPROM (Table VI). In this analysis we could not obtain a genotype on seven of the controls, so the number of subjects in this group was lower than in the analysis of the CA-repeat alleles.
and apoptotic death of amnion epithelial cells, resulting in the weakening of the membranes and their rupture (Lei et al., 1999). Premature induction of MMP-9 is thought to contribute to the untimely rupture of the fetal membranes, leading to preterm birth (Vadillo-Ortega et al., 1995; Maymon et al., 2000; McLaren et al., 2000; Uchide et al., 2000). In the present study, we investigated whether polymorphisms in the MMP-9 promoter, which affect promoter function, are associated with risk of PPROM. We focused our attention on two previously described polymorphisms in the proximal promoter: a variable CA-repeat and an SNP at χC/C (Peter, 1999) and 21 CA- (Shimajiri et al., 1999) repeats, but the nature of the proteins binding to the CA-repeat allele in HeLa cell hosts (unpublished observations). The fact that similar results were obtained with primary amnion epithelial cell cultures and WISH cells does not rule out the possibility that WISH cells are contaminated with HeLa cells (Kniss et al., 2001). Indeed, we found that the 14 CA-repeat allele was also more active than the 20 CA-repeat allele in THP-1 cells. Because we did not examine promoter activities of the other CA-repeat alleles, we cannot rule out the possibility that alleles with different repeat numbers might have greater activity than the 14 CA-repeat allele in amnion epithelial cells. The CA-repeat may influence MMP-9 promoter function by serving as a binding site for regulatory proteins (Peters et al., 1999). Electrophoretic mobility shift assays have shown specific binding of nuclear proteins to oligonucleotides containing 23 CA- (Peter et al., 1999) and 21 CA- (Shimajiri et al., 1999) repeats, but the nature of the proteins binding to the CA-repeat alleles has not been determined.

Because the expression of MMP-9 is primarily controlled by the rate of gene transcription (Finne, 1998), differences in promoter activity should be reflected in levels of MMP-9. Unfortunately, it has not been possible for us to correlate MMP-9 protein levels in delivered fetal membranes with MMP-9 promoter genotype. This is due to the large variation

### Table V. Odds ratios (OR) for risk of preterm premature rupture of membranes (PPROM) for each matrix metalloproteinase-9 (MMP-9) CA-repeat allele and confidence intervals (CI)

<table>
<thead>
<tr>
<th>No. of CA-repeat</th>
<th>PPROM (%)</th>
<th>Controls (%)</th>
<th>OR *95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>47</td>
<td>78</td>
<td>3.06</td>
<td>1.77–5.27</td>
</tr>
<tr>
<td>15</td>
<td>4</td>
<td>19</td>
<td>0.59</td>
<td>0.20–1.70</td>
</tr>
<tr>
<td>16</td>
<td>1</td>
<td>3</td>
<td>0.97</td>
<td>0.14–6.68</td>
</tr>
<tr>
<td>17</td>
<td>8</td>
<td>7</td>
<td>3.60</td>
<td>1.30–9.99</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>11</td>
<td>0.52</td>
<td>0.13–2.08</td>
</tr>
<tr>
<td>19</td>
<td>4</td>
<td>30</td>
<td>0.35</td>
<td>0.13–0.98</td>
</tr>
<tr>
<td>20</td>
<td>12</td>
<td>59</td>
<td>0.51</td>
<td>0.26–1.01</td>
</tr>
<tr>
<td>21</td>
<td>28</td>
<td>78</td>
<td>1.07</td>
<td>0.62–1.84</td>
</tr>
<tr>
<td>22</td>
<td>14</td>
<td>62</td>
<td>0.58</td>
<td>0.30–1.10</td>
</tr>
<tr>
<td>23</td>
<td>10</td>
<td>41</td>
<td>0.66</td>
<td>0.32–1.38</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>6</td>
<td>0.97</td>
<td>0.22–4.26</td>
</tr>
</tbody>
</table>

*Statistically significant utilizing a Bonferroni adjustment for multiple comparisons with critical value α = 0.0042. The numbers in the PPROM and Controls columns refer to the number of individuals who have either one or two copies of the particular allele. OR = odds ratio for comparing the odds of CA-repeat for the specified allele compared to all others combined.

### Table VI. Matrix metalloproteinase-9 (MMP-9) promoter −1562 single nucleotide polymorphism genotype frequencies in African Americans and lack of association with preterm premature rupture of membranes (PPROM)

<table>
<thead>
<tr>
<th>MMP-9 promoter genotype</th>
<th>Controls (n = 207)</th>
<th>PPROM (n = 74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>C/C (n = 209)</td>
<td>154</td>
<td>55</td>
</tr>
<tr>
<td>C/T (n = 71) plus T/T (n = 1)</td>
<td>53</td>
<td>19</td>
</tr>
</tbody>
</table>

χ² = 0.8863, P = 0.88 (Yates’ correction).
in MMP-9 protein levels, which probably result from differences in the duration of labour and the potential impact of intrapartum infection on MMP-9 expression, in addition to genetic factors (P.E.Ferrand, E.Cho and J.F.Strauss III, unpublished observations).

A previous report suggested an association between the number of CA-repeats in the MMP-9 promoter and intracranial aneurysms in a Caucasian population (Peters et al., 1999). The authors postulated that MMP-9 activity influences the integrity of the blood vessel wall and that genetic variation in the MMP-9 promoter contributes to the risk of aneurysm. A similar study on intracranial aneurysm patients in the Finnish population did not, however, find an association (Yoon et al., 1999). In our study, the 14 CA-repeat allele was more represented in neonates derived from pregnancies complicated by PPROM than other alleles. Based on our observation that the 14 CA-repeat allele is a stronger promoter in amnion epithelial cells, we postulate that this allele in the fetal MMP-9 promoter could increase the likelihood of an exuberant response to stimuli that induce MMP-9 expression, increasing the risk of PPROM. In contrast, the −1562 SNP, which did not affect MMP-9 promoter activity in our hands, was not associated with risk of PPROM. Evidence has been presented that the −1562 SNP influences MMP-9 promoter function, with the T allele having greater activity than the C allele (Zhang et al., 1999b). However, the differences in promoter activity reported by these authors were modest. We found no significant differences in the activity of the −1562 T and C alleles in primary cultures of human amnion cells and WISH cells, in which we found differences in promoter function for the 14 CA and 20 CA-repeat alleles, and in THP-1 monocyte/macrophage cells. Zhang et al. (1999b) employed cultured macrophage cells in their analysis of MMP-9 promoter function with a similar-sized fragment of the promoter to that used in our experiments. The differences in results, while not readily apparent, could be explained by the different cell hosts employed.

It should be noted that the frequency of the 14 CA-repeat allele in African Americans is lower than in Caucasian populations (19 versus 51–67%) (St Jean et al., 1995; Peters et al., 1999; Yoon et al., 1999; Nelissen et al., 2000). This observation suggests that the higher incidence of PPROM in African Americans cannot be attributed to different frequencies of the 14 CA allele among the ethnic groups. Thus, other factors, possibly genetic (e.g. variation in expression of TIMPs, the endogenous tissue inhibitors of matrix metalloproteinases) or environmental, could modulate the influence of the MMP-9 promoter genotype on risk of PPROM in different ethnic groups. It is possible that fetal carriage of the 14 CA-repeat allele is associated with increased risk of PPROM in Caucasians. Future studies should determine whether this association holds true in large study groups of African Americans and in other ethnic groups.

Association studies must be interpreted with care because population stratification can influence results. This is a particular concern in populations that are heterogeneous such as African Americans. Moreover, an association between an allele and an outcome does not prove a causal relationship.

The association may be due to another gene in linkage disequilibrium with the allele under investigation. Therefore, the association between the 14 CA-repeat allele and risk of PPROM needs to be confirmed using methods that can establish linkage.

In conclusion, we have found that a variable CA-repeat polymorphism in the MMP-9 promoter has cell-specific effects on promoter function, and that the low number CA-repeat allele (14 CA-repeats) confers greater promoter activity in amnion epithelial cells. This allele is also significantly associated with risk of PPROM and could contribute to the increased occurrence of PPROM.

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References


References


MMP-9 polymorphism and fetal membrane rupture


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