Global alteration in gene expression profiles of deciduals from women with idiopathic recurrent pregnancy loss

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ABSTRACT: Recurrent pregnancy loss (RPL) occurs in ~5% of women. However, the etiology is still poorly understood. Defects in decidualization of the endometrium during early pregnancy contribute to several pregnancy complications, such as pre-eclampsia and intrauterine growth restriction (IUGR), and are believed to be important in the pathogenesis of idiopathic RPL. We performed microarray analysis to identify gene expression alterations in the deciduals of idiopathic RPL patients. Control patients had one antecedent term delivery, but were undergoing dilatation and curettage for current aneuploid miscarriage. Gene expression differences were evaluated using both pathway and gene ontology (GO) analysis. Selected genes were validated using quantitative reverse transcription–polymerase chain reaction (qRT–PCR). A total of 155 genes were found to be significantly dysregulated in the deciduals of RPL patients (>2-fold change, P < 0.05), with 22 genes up-regulated and 133 genes down-regulated. GO analysis linked a large percentage of genes to discrete biological functions, including immune response (23%), cell signaling (18%) and cell invasion (17.1%), and pathway analysis revealed consistent changes in both the interleukin 1 (IL-1) and IL-8 pathways. All genes in the IL-8 pathway were up-regulated while genes in the IL-1 pathway were down-regulated. Additionally, genes known to be critical for degradation of the extracellular matrix, including matrix metalloproteinase 26 and serine peptidase inhibitor Kazal-type 1, were also highly up-regulated. In this first microarray approach to decidual gene expression in RPL patients, our data suggest that dysregulation of genes associated with cell invasion and immunity may contribute significantly to idiopathic recurrent miscarriage.

Key words: recurrent miscarriage / decidual / interleukin-1 / interleukin-8 / matrix metalloproteinases

Introduction

Spontaneous pregnancy loss is the most common complication of pregnancy, occurring in ~15% of clinically recognized pregnancies. Approximately 1–5% of all women suffer from recurrent pregnancy loss (RPL), defined as two or more consecutive losses before 20 weeks of gestation. Although RPL has been attributed to various hematologic, anatomic, hormonal, immune and genetic defects, in most cases the etiology remains unknown after an extensive evaluation (Ford and Schust, 2009). Furthermore, RPL patients are at greater risk for multiple perinatal complications such as preterm delivery, neonatal demise and intrauterine growth restriction (IUGR; Jivraj et al., 2001). Thus, there is a critical need for the identification of factors involved in idiopathic RPL and a clearer understanding of its causes.

The establishment and maintenance of pregnancy occurs through the interaction of maternal endometrial (decidual) and trophoblastic tissue. The pathologies that lead to pregnancy loss must ultimately, either directly or indirectly, affect this interaction (Norwitz et al., 2010).
This interface is the conduit for communication between the maternal decidual cells and the invading trophoblasts enabling implantation and placental development. Furthermore, as most recognized losses occur after implantation, they arise during the process of decidualization, when endometrial and chorionic tissues are rapidly changing their architectures and regulatory environments (Norwitz et al., 2001; Lockwood et al., 2007). Among the requirements of successful pregnancy are the development of immune tolerance to the foreign, zygotic tissue, the suppression of inflammation, extensive invasion of the decidua by cytotrophoblasts and extensive remodeling of the decidual vasculature. Thus, the decidua is a locus of extensive regulatory control critical to normal pregnancy and a likely site of altered gene expression in many types of pregnancy complications, including RPL (Norwitz et al., 2001; Gellersen and Brosens, 2003; Hess et al., 2006; Lockwood et al., 2007).

A few previous studies have identified differences in gene expression between women with and without a history of RPL that have suggested potential involvement of immune, angiogenic and cell invasion regulators. In one of the larger studies Lee et al. (2007) found dysregulation of genes involved in retinoic acid metabolism (Lee et al., 2007). Baek and other groups have also identified abnormal endometrial expression of VEGF, C4BP, CRABP2, OLFM1, leukemia inhibitory factor (LIF) and matrix metalloproteinases (MMP), among others (Baek et al., 2002; Baek, 2004; Lee et al., 2007). Studies in the decidua, although far more limited, have identified B-cell activation factor, CD36, calpain and interleukin 10 (IL-10) as having altered expression in women with a history of RPL, suggesting a possible immune basis for such miscarriages (Jasper et al., 2007; Lee et al., 2007; Kumagai et al., 2008; Qu et al., 2008; Jin et al., 2009). However, these studies examined only a small subset of genes selected on the basis of existing hypotheses about the etiology of RPL and therefore may bias interpretation. Furthermore, some of these findings were based on analysis of chorionic villous tissue or secretory endometrium, and not decidual tissue where maternal effects on pregnancy loss are most likely to arise.

Despite its central role in the establishment and maintenance of pregnancy, the decidua has generally not been examined for potential gene or protein expression changes associated with RPL. This is largely due to limitations in access to tissues and the absence of associated clinical data. Existing gene and protein expression studies have looked primarily at the secretory phase (i.e. non-pregnant) endometrium. Furthermore, studies in the decidua have examined only genes based on investigator interest (Jasper et al., 2007; Lee et al., 2007; Kumagai et al., 2008; Qu et al., 2008; Jin et al., 2009). Many also lacked thorough controls, such as fetal karyotype at the time of loss, thereby making a diagnosis of idiopathic RPL uncertain. Previous investigations also compared decidual tissues isolated from women undergoing miscarriage to those from elective terminations, raising concern over the appropriateness of the control tissue due to the presence of a viable fetus.

To overcome the limitations of previous studies in the identification of genes and gene pathways specifically altered in RPL, we have undertaken a genome-wide microarray analysis of deciduas from RPL and those from pregnancy losses associated with aneuploidy. Significant gene expression differences were determined between the two groups. The aneuploid comparator group was experiencing their first miscarriage after known parity. Using this robust, global genomic approach we found dysregulation of immune- and cell invasion-related genes and pathways, among others.

## Materials and Methods

### Clinical samples and tissue processing

All patients provided informed consent for participation in this study under an IRB protocol approved by the Stanford University Committee on the Use of Human Subjects in Medical Research. Control patients had at least one normal term delivery and an euploid embryonic demise diagnosed at dilation and curettage (D&C). For the purpose of this study, idiopathic RPL was defined as three or more consecutive miscarriages with a normal evaluation. RPL patients must have had a complete screening per ACOG recommendations for recurrent miscarriage evaluation (American College of Obstetrics and Gynecology, 2001), including uterine cavity evaluation, prenatal karyotypes, thrombophilia testing, fasting glucose, thyroid-stimulating hormone and anti-phospholipid antibodies (anti-beta-2-glycoprotein antibodies, lupus anticoagulant and anticardiolipin antibodies; Table I). Embryonic demise was diagnosed during routine monitoring by transvaginal ultrasonography at which time an intrauterine pregnancy with a fetal pole and no cardiac activity was noted. At the time of D&C, deciduas were separated from any products of conception under a dissecting microscope. First, tissue samples were washed thoroughly with sterile normal saline to remove excess blood, mucous and fetal tissues. Then, fragments of decidua and deciduas with attached chorionic villi were transferred to a petri dish with sterile normal saline and examined under a dissecting microscope. The deciduas were carefully dissected out from the branching chorionic villi and flash frozen in liquid nitrogen for microarray studies. Chorionic villi were sent for cytogenetic analysis, and for those showing a 46XX karyotype, testing for maternal cell contamination was performed. Tissue blocks of decidua were also embedded in OCT for preparation of frozen tissue sections as previously described (Germeyer et al., 2005).

### Microarray studies

Eight specimens were analyzed—one each from the four control group patients and four RPL patients (patients 1 – 8, Table I). Total RNA was prepared from homogenized tissue using Trizol (Invitrogen, Carlsbad, CA, USA) and further purified using PureLink Micro- to Mid-totatal RNA purification kit (Invitrogen) per the manufacturer’s recommendations. RNA quality was confirmed by determining the A260/A280 ratio and examining the 28S and 18S RNA bands after denaturing formaldehyde gel electrophoresis. Using 250 ng of total RNA, each RNA sample was amplified using the Ambion Illumina TotalPrep RNA amplification kit with biotin-UTP labeling. The Ambion Illumina TotalPrep RNA Amplification Kit uses a T7 oligo(dt) primer to generate single-stranded cDNA, followed by a second strand synthesis to generate double-stranded cDNA, which is then column purified. In vivo transcription was performed to synthesize biotin-labeled cRNA using T7 RNA polymerase. In total, 750 ng of column-purified cRNA was hybridized to Illumina Human Ref 8 BeadChip (Illumina, Inc., San Diego, CA, USA) whole-genome expression arrays using standard Illumina protocols, and streptavidin-Cy was used for detection. Slides were scanned on an Illumina Beadstation and analyzed using BeadStudio (Illumina). Using this software, data underwent rank invariant normalization prior to data analysis. Initial analysis of normalized data used a two-tailed Student’s t-test to identify statistically significant changes in gene expression, and P-values < 0.05 were considered statistically significant. The data were also imported into GeneSpring GX Version 9 (Agilent Technologies Inc., Santa Clara, CA, USA) for pathway analysis using the Ingenuity Pathway Analysis package within Genespring.
Table I RPL subjects and controls.

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Age</th>
<th>Gravity</th>
<th>Fetal karyotype</th>
<th>Gestational age (weeks)</th>
<th>Medications</th>
<th>Study group</th>
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<td>2</td>
<td>47XX + 16</td>
<td>8</td>
<td>P4</td>
<td>Control</td>
</tr>
<tr>
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<td>2</td>
<td>47XY + 22</td>
<td>9</td>
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<td>Control</td>
</tr>
<tr>
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<td>39</td>
<td>2</td>
<td>47XY + 16</td>
<td>10</td>
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<td>2</td>
<td>48XY + 8 + 20</td>
<td>7</td>
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<td>Control</td>
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<tr>
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<td>P4</td>
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</tr>
<tr>
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<td>46XY</td>
<td>11</td>
<td>P4, Glucophage</td>
<td>RPL</td>
</tr>
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</table>

Gravity includes current miscarriage. Controls had one previous term live birth. RPL patients had only a history of first trimester miscarriages.

Average age RPL = 37; average gestational age RPL = 8.7 weeks; average age controls = 37.8; average gestational age controls = 8.6 weeks; P-value (age) = 0.70; P-value (gestational age) = 0.67.

MCC, maternal cell contamination; P4, progesterone.

*Donor egg.

Functional annotation was used to classify significant genes using the Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 to permit subgroup classification of genes based upon gene ontology (GO) code assignment (Dennis et al., 2003; Huang da et al., 2009).

Quantitative reverse transcription–polymerase chain reaction

Genes differentially expressed in RPL decidua were selected for validation by real-time PCR based upon pathway analysis findings and GO code clustering. The number of decidual samples utilized for validation was expanded from the original microarray samples (Table I) to include a total of 6 control samples and 10 RPL samples were used. cDNA was generated using moloney murine leukaemia virus (MMLV) reverse transcriptase (Invitrogen) according to the manufacturers’ specifications. Using random non-amers, 1 μg of DNase-treated RNA was reverse transcribed. All quantitative reverse transcription–polymerase chain reaction (qRT–PCR) primers were designed to span at least one intron (primer sequences provided in Supplementary data, Table SI). qRT–PCR was carried out in triplicate using an ABI 7900HT lightcycler (ABI biosystems, Foster City CA, USA). Quantitative PCR reactions utilized Power Sybr green master mix (ABI biosystems) according to the manufacturer’s directions. In all instances 18S cDNA was used for normalization of the data. Data were analyzed using the standard curve method to generate relative expression. Significance was ascertained using the Student’s t-test with Welch’s correction. Data are expressed as fold change where control RNA is the comparator as previously described (Krieg et al., 2010).

Immunohistochemistry

Frozen sections were prepared and processed for immunohistochemistry (IHC) as described previously (Tunuguntla et al., 2003; Fan et al., 2008). Briefly, 5 μm sections of decidua were thaw-mounted and lightly fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 min, washed in Tris-buffered saline (TBS), incubated with Peroxidase (Biocare Medical, Concord, CA, USA) for 10 min to inhibit endogenous peroxidase activity, washed in TBS, and incubated with the blocking serum for 20 min. The sections were then incubated with a rabbit polyclonal MMP-26 antibody (1:50 dilution, prepared by Dr Q. Sang, Florida State University; Park et al., 2000; Tunuguntla et al., 2003) and detected with Mach2 anti-rabbit horse-radish peroxidase (Biocare medical). The primary antibody was replaced with either PBS with 1.0% BSA or rabbit non-immune IgG as negative controls (Tunuguntla et al., 2003; Fan et al., 2008). Hematoxalin (blue) was used as a counter-stain. Visualization of slides was performed using a Nikon Eclipse 80i camera for brightfield imaging (Nikon Inc., Melville, NY, USA).

Results

Multiple genes are dysregulated in the decidua of RPL patients

Illumina Ref-8 gene chips allow the analysis of 24 500 gene products with high redundancy for each gene. Analysis of Illumina microarray data utilized rank invariant normalized data generated via Beadstudio software provided by Illumina. From the normalized data, we found 155 genes exhibiting a >2-fold (P < 0.05) change in expression, with 22 up-regulated and 133 down-regulated (Supplementary data, Table SI). Using GO code analysis, these genes were clustered into groups based upon cellular function (Fig. 1). A large proportion of these genes were found to be involved in immune function (23%). Other groups included those involved in proteolysis, enzyme inhibition, cell migration, cell signaling and transcriptional regulation.
Of the most up-regulated genes, MMP-26 and serine peptidase inhibitor Kazal-type 1 (SPINK 1) were increased 11.7- and 10.1-fold, respectively, by microarray. Both of these genes are important for degradation of the extracellular matrix, a necessary step for trophoblastic invasion (Li et al., 2002). Other up-regulated genes included those with immune function such as interleukin 17 receptor B and secretoglobin family 2A member 1 (SCGB2A1). Similarly, the down-regulated group included interleukins and zinc finger binding proteins, which are involved in immune function and transcriptional regulation (Supplementary data, Table SII).

Overall the largest proportion of dysregulated genes identified fall under the classification of immune function or proteolysis, enzyme inhibition and migration (Fig. 1). As these processes are central to implantation and placentation; dysregulation of these genes in RPL could adversely impact implantation by preventing appropriate trophoblast invasion into the decidua.

In addition to GO analysis, pathway analysis was undertaken using the Ingenuity Pathway Analysis Package within Genespring (Agilent, Santa Clara, CA, USA). This program allows identification of genes with similar or interrelated function while GO analysis groups genes based upon biological function. Both GO and pathway analysis revealed that most significant dysregulated genes belong primarily to the IL-1 and IL-8 pathways. The regulation of these genes was further examined using qRT–PCR in an expanded cohort of control (n = 6) and RPL (n = 10) samples (Table I), and these results were consistent with the microarray data.

### Genes impacting immune regulation and function

A large proportion of dysregulated genes were found to be associated with immune function, a finding further supported by pathway analysis (Figs 2–4A), which identified both the IL-1 and IL-8 pathways. Validation of the genes associated with these two pathways by qRT–PCR showed a 3-fold down-regulation of interferon induced protein 44 like (IFI44L), IFI44 and down-regulation of multiple genes involved in the IL-1 pathway (Figs 2 and 4A). Several other immune genes, including SCGB2A1, HLADR85, HLA29.1, IL17RB and those associated with IL-8 signaling were found to be up-regulated (Figs 3 and 4A). The genes associated with the IL-1 pathway include interleukin 1 receptor 2 (IL-1R2), caspase 1, IL-1α and IL-1β (Fig. 2). All of these IL-1-related genes were found to be down-regulated in RPL deciduas by both the microarray analysis and qRT–PCR. IL-1α and IL-1β were down-regulated 1.9-fold. Caspase 1, an enzyme necessary for IL-1β post-translational maturation was found to be down-regulated 1.5-fold in the RPL samples (Fig. 2). These proinflammatory gene products have been suggested by others to be necessary for implantation in murine models (Simon et al., 1994; Chen et al., 1995; von Wolff et al., 2000).

The abortigenic cytokine, IL-8, is up-regulated in RPL patients

Pathway analysis also identified the IL-8 pathway as being altered in RPL tissue. Genes in this pathway, including IL-8, neutrophil defensin (DEFA-1), proteoglycan 2 (PRG-2) and liposaccharide binding protein (LBP) were all found to be up-regulated in the microarray analysis (Fig. 3). Analysis of all the above genes in the IL8 pathway by qRT–PCR showed marked up-regulation identical to the pattern observed in the microarray analysis. IL8 expression, for example,
MMP-26 activity (Fig. 5). Dysregulation of these genes could impact biological inhibitor of MMP-26, potentially resulting in increased show no significant changes in the expression of TIMP-4, a major invasion (Fig. 5). IHC studies revealed that MMP-26 protein was 9-fold, which may represent a mechanism for abnormal trophoblast patients (Fig. 5). Additionally, both microarray and qRT–PCR data were found to be down-regulated, whereas MMP-10 was found to be up-regulated. However, there is little direct overlap in results between our screen and other comparative gene expression results for RPL, even though they have identified genes in the same functional areas (Baek et al., 2002; Choi et al., 2003; Kumagai et al., 2008; Qu et al., 2008). This likely reflects the fact that our study used decidual tissue, not chorionic villi or non-gravid endometrium between normal and RPL tissues have identified genes involved in immune regulation, angiogenesis, apoptosis, cell migration and enzyme inhibitors. Since most cellular events during tumor invasion and metastasis are analogous to the cellular processes that occur during blastocyst implantation (Norwitz et al., 2001), aberrant expression of these genes in the decidua could adversely impact trophoblast invasion and placenta. Other gene groups included cell signaling, cell cycle regulation, transcriptional regulation, apoptosis, enzyme inhibition and cation homeostasis. Previous studies examining gene expression differences either in chorionic villi or non-gravid endometrium between normal and RPL tissues have identified genes involved in immune regulation, angiogenesis, apoptosis, cell migration and cell attachment (Baek et al., 2002). However, there is little direct overlap in results between our screen and other comparative gene expression results for RPL, even though they have identified genes in the same functional areas (Baek et al., 2002; Choi et al., 2003; Kumagai et al., 2008; Qu et al., 2008). This likely reflects the fact that our study used decidual tissue, not chorionic villi or non-gravid endometrium, and used aneuploid loss not associated with RPL as the comparator group.

Although many of the functional groupings suggest plausible connections to the pathogenesis of RPL, the potential association with immune regulation and cell migration and invasion stand out. The
two most highly up-regulated genes in our microarray analysis (validated by qRT–PCR) were MMP-26 and serine peptidase inhibitor Kazal-type 1 (SPINK1), at 11.7- and 10-fold, respectively. Additionally, our IHC results demonstrated up-regulation of MMP-26 protein in the glandular epithelium. Consistent with our findings, MMP-26 is known to be expressed in the endometrial epithelium, degrades the extracellular matrix and to influence angiogenesis (Li et al., 2002; Tunuguntla et al., 2003; Bi et al., 2010). MMP-26 has been reported to be down-regulated in polycystic ovarian syndrome and up-regulated in endometrial hyperplasia (Li et al., 2002; Pilka et al., 2004; Huang et al., 2006; Qiao et al., 2008). Other MMP associated with RPL in our study include MMP-12 and MMP-10. MMP-12 was up-regulated in RPL tissue, and MMP-10 was found to be down-regulated (Fig. 4B). Other genes associated with proteolysis and metastasis such as KLK11, FGA and SPINK1 also show up-regulation (Fig. 4B; Gouyer et al., 2008). JMJD2B was up-regulated in RPL decidua. This histone demethylase is known to be hypoxia-inducible and promotes cancer cell proliferation and metastasis in a hypoxic environment (Krieg et al., 2010; Yang et al., 2010). The most plausible connection between the dysregulation of genes associated with cell invasion and RPL is through effects on trophoblast invasion of the endometrium and maintenance of decidual homeostasis. Inappropriate invasion may cause failure of placentation and early pregnancy loss. In ongoing pregnancies, inappropriate invasion is associated with several pregnancy complications such as bleeding, IUGR and pre-eclampsia (Jivraj et al., 2001; Lockwood et al., 2007; Tantbirojn et al., 2008).

The most dramatic result of our pathway analysis was the suggested involvement of both the IL-1 and IL-8 pathways in RPL (Figs 2 and 3). In the microarray analysis, all of the IL-1-related genes were all found to be down-regulated. All IL-8-related genes were found to be up-regulated. These changes were consistently validated by
In pregnancy, IFI44L is shown to be down-regulated in peripheral blood leukocytes of RPL patients taken early in pregnancy (Maas et al., 2010).

The IL-1-related genes identified by pathway analysis and validated by qRT–PCR included IL-1α, IL-1β, caspase 1, and IL-1R2 (Fig. 2). While IL-1 is a proinflammatory cytokine, it has been shown to be necessary for normal implantation and maintenance of pregnancy in mouse models. During the window of implantation, IL-1 plays a role in the expression of LIF and integrins and the production of prostaglandins (Simon et al., 1994; Chen et al., 1995; Sim on et al., 1997; von Wolff et al., 2000; Abad et al., 2011). All of these factors are necessary for appropriate blastocyst apposition and implantation. Furthermore, IL-1 deficiency has been found to be associated with miscarriage after blastocyst apposition in a murine model (Krussel et al., 2003).

The present study is the first report of aberrant IL-1 expression in RPL decidua, although reduced levels of IL-1 have been previously reported for the endometria of non-pregnant RPL patients (Choi and Kwak-Kim, 2008). At the genomic level, IL-1β polymorphisms are associated with RPL (Bi et al., 2010). Taken together, our findings and those reported previously by others suggest that RPL patients may have dysfunction within the IL-1 pathway, potentially resulting in abnormal implantation and failure to maintain early pregnancy.

In contrast to the IL-1 pathway, the IL-8 pathway is not known to play a role in normal pregnancy, and its up-regulation in RPL decidua suggests it may promote inflammatory responses in these tissues. IL-8 facilitates the extravasation of proinflammatory leukocytes from capillaries (Milne et al., 1999). Elevated levels of IL-8 in the serum, cervical mucus and in homogenates of intruterine contents have been found to be associated with miscarriage (Madhappan et al., 2003; Hattori et al., 2007; Morelli et al., 2008). Our results are consistent with these findings: we found a 2.4-fold increase in decidual IL-8 expression in RPL patients and up-regulation of several genes associated with IL-8 signaling in our qRT–PCR analysis (Fig. 3). While this is the first demonstration of elevated IL8 expression in idiopathic RPL decidua, Lockwood et al. (2004) found that IL-8 protein and mRNA levels were inhibited during decidualization in an in vitro system by progesterone and that hypoxia and thrombin induced expression of IL-8. Hess et al. (2007) found that, in an in vitro decidualized stromal cell system, IL-8 is highly up-regulated shortly after exposure to trophoblast-conditioned media, although its levels diminish with longer exposure. These in vitro reports may suggest a role for IL-8 in implantation. To our knowledge, ours is the first demonstration that IL-8 expression in the decidua is associated with idiopathic RPL.

Although our study has identified a number of intriguing gene expression differences between the decidua of idiopathic RPL and sporadic, aneuploid loss, it has several limitations. Without functional analysis of gene functions in the context of the decidua and the ability to test specific hypotheses about the roles of specific genes in RPL, the arguments that can be made are largely circumstantial. Pregnancy complications are notoriously hard to study in the laboratory because of the absence of appropriate models of human pregnancy. Furthermore, we found many changes in gene expression potentially affecting many different processes. This may suggest that underlying pathogenic events have multiple and complex effects on the regulatory environment of the decidua, very few of which may significantly contribute to pregnancy loss. Finally, what is now defined as idiopathic RPL may represent several different etiologies, yet our analysis used qRT–PCR analysis. Several other immune-related genes were also found to be altered in both the microarray and qRT–PCR analyses (Supplementary data, Table SII and Fig. S1). Of these other immune genes, IFI44L and IFI44, were significantly down-regulated (Supplementary data, Table SII and Fig. S4A). While little is known about these gene products they have been associated with rheumatologic disease and maintenance of the extracellular matrix (Woeckel et al., 2012).
a small sample size. There may be important differences in expression profiles among individuals that reflect distinct etiologies. These might be revealed in larger studies in the future.

Despite these considerations, the work presented here provides a comprehensive data set that was not previously available and may inform future studies. Furthermore, we believe these data are reliable indicators of differences between the idiopathic RPL and control groups. All genes analyzed by qRT–PCR were generally consistent with the initial microarray findings. The consistent effects on multiple components of the IL-1 and IL-8 pathways suggests that signaling by these pathways are affected in the RPL tissue as has been implicated in other species (Chen et al., 1995; Simon et al., 1997; Krusell et al., 2003; Lockwood et al., 2007). This is the first demonstration of dysregulation of these and other interrelated genes within both pathways in idiopathic RPL decidua and suggests that abnormal expression of these immune genes could provide a mechanism by which abnormal implantation occurs in some forms of RPL. Furthermore, abnormalities in gene expression of MMPs can impact the ability for the trophoblast to normally invade the decidua. Other groups of genes, such as those involved in transcriptional regulation and cell signaling, certainly warrant further study. Although preliminary in nature, these findings may ultimately lead to further investigation and alternate treatment options for patients experiencing repeated idiopathic miscarriages.

**Supplementary data**

Supplementary data are available at http://molehr.oxfordjournals.org/.

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**Authors’ roles**

S.A.K. was responsible for study design, patient recruitment, sample collection and preparation, QRT–PCR, data analysis and manuscript preparation. X.F. performed sample preparation. Y.H. performed QRT–PCR and IHC experiments. Q.-X.S. provided IHC assistance with the initial microarray findings. The consistent effects on multiple components of the IL-1 and IL-8 pathways suggests that signaling by these pathways are affected in the RPL tissue as has been implicated in other species (Chen et al., 1995; Simon et al., 1997; Krusell et al., 2003; Lockwood et al., 2007). This is the first demonstration of dysregulation of these and other interrelated genes within both pathways in idiopathic RPL decidua and suggests that abnormal expression of these immune genes could provide a mechanism by which abnormal implantation occurs in some forms of RPL. Furthermore, abnormalities in gene expression of MMPs can impact the ability for the trophoblast to normally invade the decidua. Other groups of genes, such as those involved in transcriptional regulation and cell signaling, certainly warrant further study. Although preliminary in nature, these findings may ultimately lead to further investigation and alternate treatment options for patients experiencing repeated idiopathic miscarriages.

**Conflict of interest**

None declared.

**References**


