Novel Expression and Regulation of Voltage-Dependent Potassium Channels in Placentas From Women With Preeclampsia

Hiten D. Mistry, Laura A. McCallum, Lesia O. Kurlak, Iain A. Greenwood, Fiona Broughton Pipkin, Rachel M. Tribe

Abstract—Preeclampsia is associated with structural/functional alterations in placental and maternal vasculature. Voltage-dependant potassium channels encoded by KCNQ1-5 genes have been detected in several types of blood vessels where they promote vascular relaxation. Voltage-dependant potassium channel function can be modulated by KCNE1-5–encoded accessory proteins. The aim of this study was to determine whether KCNQ and KCNE genes are differentially expressed in placentas from women with preeclampsia compared with normotensive controls and to examine any differences in those who delivered preterm (<37 weeks) or term. Placental biopsies (from midway between the cord and periphery) were obtained, with consent, from white European control (n=24; term) and preeclamptic (n=22; of whom 8 delivered before 37 weeks’ gestation) women. KCNQ/KCNE and GAPDH mRNA expressions were determined by quantitative RT-PCR. Protein expression/localization was assessed using immunohistochemistry. KCNQ3 and KCNE5 mRNA expressions were significantly upregulated in preeclampsia (median [interquartile range]: 1.942 [0.905 to 3.379]) versus controls (0.159 [0.088 to 0.288]; P=0.001) and exhibited a strong positive correlation with each other (P<0.001), suggesting a novel heterodimer. Enhanced protein expression of KCNQ3 and KCNE5 in preeclampsia was confirmed with localization mainly restricted to the syncytiotrophoblast. KCNQ4 and KCNE1 isoforms were suppressed in placentas from term preeclamptic women versus controls (P≤0.05). KCNQ1 mRNA expression was increased and KCNQ5 decreased in the preterm preeclamptic group versus controls (P<0.05). In summary, voltage-dependant potassium channels are expressed and markedly modulated in placentas from preeclamptic women. Differential expression of isoforms may lead to altered cell proliferation. The correlation between KCNQ3 and KCNE5 expression is indicative of a novel channel complex and warrants further investigation. (Hypertension. 2011; 58:497-504.)

Key Words: potassium channel ▪ placenta ▪ preeclampsia ▪ KCNQ ▪ KCNE

Preeclampsia is a pregnancy-specific condition affecting 2% to 7% of women and is associated with maternal multiorgan dysfunction. This disorder, which is responsible for ≈60,000 maternal deaths each year worldwide, increases perinatal mortality 5-fold and is commonly associated with preterm delivery and fetal growth restriction. Women who develop preeclampsia and their infants are at increased risk of hypertension, metabolic disorders, cardiovascular disease, and cardiovascular death in later life.

The pathophysiology underpinning the disorder is complex. Impaired placentation almost certainly plays a part. Placental and maternal oxidative stress and generalized systemic inflammatory activation are main components of the syndrome. Altered maternal and placental vascular reactivity and endothelial cell function have been implicated in the clinical manifestations of preeclampsia, for example, an increase in total peripheral vascular resistance, hypertension, and altered hemodynamics.

There is emerging evidence to suggest that potassium channels play important roles in the feto-placental vasculature. Specifically, it is proposed that suppression of voltage-dependant potassium channel function may increase vascular tone and, hence, be a mechanism affecting perfusion in placentas from women with preeclampsia.

The subfamilies of voltage-gated Kv7 channels (potassium channel complexes encoded by KCNQ1-5 and KCNE1-5 genes) are of particular interest, because preliminary data indicate the presence of functional Kv7 channels in human chorionic plate arteries. Kv7 channel activity is generally associated with outwardly rectifying, voltage-dependent K⁺
Table 1. Demographic, Pregnancy, Clinical, and Biochemical Data of Subject Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive (n=24)</th>
<th>Preeclampsia (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, y</td>
<td>30±7.0</td>
<td>32±6.0</td>
</tr>
<tr>
<td>Primipara, n (%)</td>
<td>14 (58)</td>
<td>15 (65)</td>
</tr>
<tr>
<td>Booking body mass index, kg/m²</td>
<td>26.2±5.3</td>
<td>26.8±4.7</td>
</tr>
<tr>
<td>Maximum systolic blood pressure outside labor, mm Hg</td>
<td>116±5.0</td>
<td>157±8.0†</td>
</tr>
<tr>
<td>Maximum diastolic blood pressure outside labor, mm Hg</td>
<td>76±3.0</td>
<td>98±4.0†</td>
</tr>
<tr>
<td>Proteinuria, g/L</td>
<td>...</td>
<td>1.0 (0.4, 1.7)</td>
</tr>
<tr>
<td>Gestation age at delivery, wk</td>
<td>40.1±1.2</td>
<td>36.8±3.6†</td>
</tr>
<tr>
<td>No. of deliveries &lt;37 wk, n (%)</td>
<td>0</td>
<td>8 (36)</td>
</tr>
<tr>
<td>Cesarean section, n (%)</td>
<td>4 (16.6)</td>
<td>8 (16.6)*</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.51 (3.25 to 3.76)</td>
<td>3.14 (2.14 to 3.52)†</td>
</tr>
<tr>
<td>Birth weight centile</td>
<td>45 (23 to 65)</td>
<td>35 (3 to 82)</td>
</tr>
</tbody>
</table>

Data are presented as mean±SD or median (interquartile range) as appropriate, except for proteinuria (median [minimum, maximum]) and parity, Cesarean sections, and No. of deliveries <37 wk (n [%]). These are a subgroup of our previous publication.28

*P<0.05 between normal and preeclamptic pregnancies.
†P<0.001 between normal and preeclamptic pregnancies.

Kv7 channels have been identified in numerous blood vessels, including human visceral and mesenteric arteries, and the cerebral, carotid, femoral, and mesenteric arteries of rodents, as well as the aorta and portal vein, where they appear to play a role in vascular reactivity. A potential role for these channels in pulmonary hypertension has also been reported.21 Kv7 channels are also important in nonexcitable cells, such as epithelia, and in regulation of cell volume and ion transport (Cl⁻ and Na⁺) across epithelia.22,23 In skeletal muscle myoblasts, KCNQ5 has been implicated in cell-cycle progression and cellular proliferation and differentiation;26 but this appears to have been less well studied in vascular smooth muscle. Voltage-dependent potassium channel dysfunction has been linked previously to abnormalities in proliferation and possibly also to remodeling.27 The presence of Kv7 channels in placenta and relative expression in blood vessels, endothelium, and syncytiotrophoblast is unknown, and the expression profiles in placentas from normotensive and preeclamptic women have not been determined.

The aim of this study, therefore, was to establish KCNQ/ KCNE mRNA expression profiles in placentas taken from women with preeclampsia both at term and preterm, as well as normotensive controls, and to confirm regulation and localization by immunohistochemistry.

Materials and Methods

Subjects

The study population consisted of white European women who had either a normotensive or preeclamptic pregnancy (Table 1).28 The investigations were approved by the Nottingham Hospital Ethics Committee, and written, informed consent was obtained from each participant. Cases were defined on admission with a clinical diagnosis of preeclampsia, using the International Society for the Study of Hypertension in Pregnancy definition of systolic blood pressure of ≥140 mm Hg and diastolic pressure (Korotkoff V) of ≥90 mm Hg on ≥2 occasions after 20 weeks’ gestation in a previously normotensive woman, together with proteinuria of ≥100 mg/L, ≥500 mg/d, or ≥2+ on dipstick analysis of midstream urine if ≥24-hour collection result was not available.29 Medical and obstetric histories, including delivery data, were obtained for each woman. The birthweight, gestational age, sex, maternal parity, and body mass index.30 Doppler velocimetry measurements are not routinely made with preeclampsia in our hospital.

Sample Collection

Placental tissue samples were collected from all of the subjects at a standardized location midway between cord insertion and placentaborder, avoiding placental infarcts. The placental samples were taken within 10 minutes of delivery; the membranes were removed and tissue washed in ice-cold 1X PBS to remove maternal blood contamination. One sample was snap frozen in liquid nitrogen and stored at −80°C for RNA analysis and another formalin fixed and wax embedded for immunohistochemical analysis.

RNA Extraction and cDNA Synthesis

Total RNA (n=22 preeclampsia and n=24 normotensive controls) was extracted from a known amount of placental tissue (between 50 and 100 mg) using QIAzol lysis reagent (Qiagen, Crawley, United Kingdom). RNA concentration and quality were verified by gel electrophoresis and spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies, Labtech, Ringmer, United Kingdom); all of the samples had an A260/A230 ratio >1.96 and were stored at −80°C. RNA (1 μg) was then reverse transcribed using the Quantitect Reverse Transcription kit containing a mix of random primers and oligo dT (Qiagen, Crawley, United Kingdom) in a Primus 96 advanced gradient thermocycler (Peqlab Ltd, Fareham, United Kingdom). The conditions used to generate first-strand cDNA were 42°C (15 minutes) and 95°C (3 minutes).

Quantitative RT-PCR

Real-time PCR was carried out with the use of SYBR Green chemistry (2×Sensimix; Bioline, London, United Kingdom) on a RotorGene 6000 (Corbett Research, Sydney, Australia) using the primers listed in Table 2. A pre-PCR cycle was run for 15 minutes at 95°C followed by 45 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 20 s. Melt-curve analysis was performed to confirm the presence of one single product and nontemplate controls run to assess contamination. Cycle threshold values were used for analysis, and abundance data were obtained by the use of quantified cDNA to generate a standard curve. Standards were quantified using densitometry and 10-fold serial dilutions (10⁶ to 10¹ copies) run in parallel with the samples. Abundance data for the genes of interest were expressed to GAPDH, a stably expressed housekeeping gene, suitable for human placental samples.31

Immunohistochemistry

Serial sections of placental tissue were cut (5 μm) in the same orientation from paraffin-embedded tissue blocks (Sledge Microtome, Anglia Scientific, Norwich, United Kingdom) and mounted onto Superfrost plus glass microscope slides (Menzel-Glaser, Braunschweig, Germany). Before use, sections were dewaxed by immersion in xylene followed by rehydration in descending concentrations of alcohol (3 minutes each).

Immunohistochemical staining was performed using the Vector Stain Elite ABC kit (Vector Laboratories). Two goat polyclonal antibodies (KCNQ3 and KCNE5, Santa Cruz Biotechnologies) were used for immunostaining of paraffin-embedded placental sections (n=6 preeclampsia and n=6 normotensive controls). The optimal dilution for each antibody was established by performing a dilution series, with final selection on the basis of maximal specific reactivity and minimal background staining. Heat-induced epitope retrieval was achieved by heating in a citrate buffer (pH 6.0) using a
Results

Subjects
Table 1 describes the demographic, obstetric, and pregnancy data of the 46 women who participated in the study; further clinical details are published.29 All of the women conceived naturally and carried singleton pregnancies. The normotensive group gave birth without developing hypertension or proteinuria, to infants weighing >2500 g, delivered at 37 weeks or later. The systolic and diastolic blood pressure levels were, by definition, significantly raised in preeclampsia compared with normal pregnancy (P<0.0001). Overall, the preeclamptic women all had moderate-to-severe disease and had lower gestational ages at delivery than the control group (P<0.05; Table 1). No preeclamptic woman had hemolysis elevated liver enzymes and low platelet count or required magnesium sulfate administration. All of the neonates from both pregnancy groups survived.

KCNQ/KCNE Expression in Placentas From Normotensive Control Versus All of the Preeclamptic Women
The expression of KCNQ and KCNE isoforms in placental tissue from normotensive controls (n=24) and women with preeclampsia (n=22), as determined by quantitative RT-PCR, is shown in Figure 1. The relative abundance of placental tissue KCNQ expression in both control and preeclamptic groups was KCNQ3>KCNQ5>KCNQ2>KCNQ1>KCNQ4. mRNA levels were, by definition, significantly raised in preeclampsia compared with controls (0.020 [0.006 to 0.060] versus 0.8 [0.3 to 0.96]; P<0.005).

All of the KCNE isoforms were detected in placental tissue in both control and preeclamptic groups (KCNES>KKCN1=KKCN3=KKCN4=KKCN2). However, KCNEN mRNA expression (Figure 1B) was significantly upregulated in preeclampsia compared with control samples (1.94 [0.91 to 3.38] versus 0.16 [0.09 to 0.29]; P<0.0001).

Association of Expression of KCNQ3 and KCN5E
The substantial fold increases in both KCNQ3 and KCNEN mRNA copy number in preeclamptic placental tissues were further examined, and a highly significant positive, curvilinear association between KCNQ3 and KCNEN mRNA expression was demonstrated (in preeclamptic placentas [Figure 2; r=0.96; R²=0.93; P<0.0001]). A significant association was also observed, at a much lower level of expression, in tissues from normotensive controls (r=0.73; R²=0.54; P<0.05; △).

Placental Expression of KCNQ/KCNE in Normotensive (Term), Preterm, and Term Preeclampsia
Because of the significant difference between the gestational ages in the control versus preeclamptic cohorts (Table 1), the preeclamptic group was further subdivided into those who had delivered preterm (<37 weeks; n=8) and those who

Table 2. Primers Used in Quantitative PCR

<table>
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<tr>
<th>Gene</th>
<th>Accession No.</th>
<th>Primers</th>
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<td>KCNQ1</td>
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<td>124</td>
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<td></td>
<td>5’-gctccgcacatgtagtga-3’</td>
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<td>123</td>
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<td></td>
<td></td>
<td>5’-ccacaggaagctaacctt-3’</td>
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<td>KCNQ3</td>
<td>NM_004519.2</td>
<td>5’-caggtcttcggaaggatc-3’</td>
<td>119</td>
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<td>5’-agccatcaaccaagagt-3’</td>
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<td>NM_004700.2</td>
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<td>102</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’-ttgactcaacagctacta-3’</td>
<td></td>
</tr>
</tbody>
</table>

microwave oven for 15 minutes, followed by incubation for 30 minutes in normal donkey serum (Vector Laboratories) to block nonspecific binding. Slides were then incubated with either anti-KCNQ3 (1:50) or anti-KCNE5 (1:50) overnight at 4°C. A positive control (human midbrain) was used to verify specificity. A negative control was performed for each test section by incubation with goat IgG. Sections were dehydrated and cleared in ascending concentrations of alcohol and xylene before mounting in DPX (BDH, Poole, United Kingdom).

All of the slides were assessed by the same observer, blinded to pregnancy outcome. For analysis of placental sections, digital images of 5 randomly selected, high-power (>400 magnification) fields were captured on NIS-Elements F2.20 microscope (Nikon United Kingdom Ltd. Surrey, United Kingdom). Quantifications of KCNQ3 and KCNEN5 were performed as described previously,32 using the Positive Pixel Algorithm of Aperio ImageScope software. This software is able to discriminate between positive- and negative-stained pixels and combines the number of positive pixels stained with the intensity of these same pixels to produce the value “positivity.” A visual check was also performed to ensure accurate discrimination of immunolabeled regions.

Statistical Analysis
We determined that a sample of 22 preeclamptic and 24 normal controls would provide 93% power to detect a difference of 1.25 SD in changes in gene expression with Bonferroni multiple testing. All of the tests were performed using SPSS for Windows version 16.0. The Kolmogorov-Smirnov test was used to test for normality of data distribution, and summary data are presented as mean±SD or median (interquartile range), as appropriate, for data distribution. Between-group comparisons were made using 2-tailed Student t tests or Mann-Whitney U tests, depending on the distribution. To assess association between mRNA expression of KCNQ and KCNE forms, data were normalized using log10. Visual inspection suggested a curvilinear association, which was tested by regression analysis. The null hypothesis was rejected where P<0.05.
delivered at term (n = 14). Placental KCNQ1 was significantly (P < 0.05) upregulated in preterm labor versus normotensive controls (Figure 3A), whereas KCNQ5 was significantly suppressed in placenta from preeclamptic women delivering preterm versus normotensive controls (Figure 3A; P < 0.001). KCNQ4 (Figure 3A) and KCNE1 (Figure 3B) were significantly down regulated in the preeclamptic group at term versus controls (P < 0.05). KCNQ5 was also significantly downregulated in the preterm but not the term preeclamptic group compared with controls (Figure 3A; P < 0.001). Interestingly, both KCNQ3 and KCNE5 were significantly increased in preeclamptic placentas delivered at both preterm and term compared with the normotensive controls (Figure 3A and 3B; P < 0.001). There was no significant difference in placental expression of any KCNQ or KCNE isoform when comparing a small subgroup (n = 5) of women with preeclampsia with fetal growth restriction to the remaining preeclamptic women (n = 17; P > 0.05).

KCNQ3 and KCNE5 Encoded Protein Localization and Expression in Control Versus Preeclamptic Placentas

Given the observed differences in mRNA expression for KCNQ3 and KCNE5, we performed immunohistochemistry
to determine protein localization and expression. Positive expression of both KCNQ3 and KCNE5 was seen in placental tissue from both groups in syncytiotrophoblast cells and in stromal areas, with little expression in the vasculature. Quantification of positive staining revealed that the expression was significantly increased in preeclampsia versus normotensive controls (Figure 4) for both KCNQ3 (P = 0.041) and KCNE5 (P = 0.015).

**Discussion**

This study has established Kv7 channel isoform expression profiles in human placental tissue. To our knowledge, this is the first description of altered K⁺ channel expression (involving both α and β subunits) in the placenta of women with preeclampsia. These novel data add to a nascent body of literature concerning Kv7 channels and their role in placental function and are important given the widespread impact that mutations of Kv7 channels have on human health (eg, cardiac arrhythmias, epilepsy, and deafness). The implications of our data are likely to be multifaceted because of the complex functions of the placenta and the specific Kv7 formation channels involved. Altered Kv7 channel expression could impact on many areas of placental function, including vascular tone, placental proliferation, ion transport, or even steroidogenesis.

Our data demonstrate specific upregulation of KCNQ3 and KCNE5 in placentas from women with preeclampsia and a highly statistically significant association between them (Figure 2), suggesting the presence of a novel heterodimer (see below). It has been suggested that a majority of KCNQ3 channels exist in a silent state. We speculate that a stimulus associated with preeclampsia may lead to activation of these channels, thus enhancing their function. This upregulation appears most relevant to preeclamptic women, because KCNQ3 and KCNE5 remain significantly upregulated in both term and preterm preeclampsia when compared with normotensive controls. A functional polymorphism (KCNQ3-A315T) has been reported markedly to increase trafficking of KCNQ3 subunits from the endoplasmic reticulum to the cell surface and to increase their stability in neuronal tissue. This polymorphism appears not to have been studied in the placenta or vascular tissue. Other isoforms, KCNQ1, KCNQ4, KCNQ5, and KCNE1, are also differentially modulated in term and preterm preeclampsia. One explanation for these gestation-related differences could be linked to severity of disease in those who deliver at earlier gestations.

The statistically significant association between KCNQ3 and KCNE5 expression in tissues from preeclamptic women (Figure 2) could suggest that they may form a novel heterodimeric channel complex. There are no published reports of this channel composition existing in other tissues, but, individually, each isoform has been shown to have a range of functions when coupled with other proteins. KCNQ3, along with KCNQ2 and/or KCNQ5, encodes for a channel that underlies the M current, which is important in determining the subthreshold excitability of neurons. KCNQ3, along with KCNQ5, is highly expressed in bladder smooth muscle, and KCNE5 is highly expressed in the femoral artery and can modulate KCNQ1-encoded channels with respect to the voltage and time-dependent functions of the channel. Such channel complex-specific activity makes it difficult to predict how different Kv7 channels may influence placental/vascular function. However, we hypothesize that the observed upregulation of KCNQ3 and KCNE5 would encode for a Kv7 channel subtype that promotes a greater outward potassium current. In this way, alteration in Kv7 channel isoforms could be viewed as a compensatory mechanism rather than a primary event responsible for reduced placental perfusion. The observation that KCNQ2/KCNQ3-encoded channels have also recently been shown to be inhibited by angiotensin II may also be relevant, because our group and others have reported that there is an increased vascular responsiveness to angiotensin II in preeclampsia. The importance of Kv7 channels to vascular reactivity has also been repeatedly demonstrated.

Immunohistochemistry data also provide some insight into the potential impact of altered Kv7 channel composition. The distribution of KCNQ3 and KCNE5 protein was not as localized to vascular regions as might be expected. The greatest protein density was detected in syncytiotrophoblast cells and in stromal areas, with little expression in the vasculature. This polymorphism appears not to have been studied in the placenta or vascular tissue. Other isoforms, KCNQ1, KCNQ4, KCNQ5, and KCNE1, are also differentially modulated in term and preterm preeclampsia. One explanation for these gestation-related differences could be linked to severity of disease in those who deliver at earlier gestations.

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Immunohistochemistry data also provide some insight into the potential impact of altered Kv7 channel composition. The distribution of KCNQ3 and KCNE5 protein was not as localized to vascular regions as might be expected. The greatest protein density was detected in syncytiotrophoblast cells, which may indicate that placental Kv7 channels are related to cell proliferation, angiogenesis, or solute transport. Indeed, Kv7 channels have been shown to mediate Na⁺ and Cl⁻ transport across other types of epithelia and other K⁺ channels; TASK1 and TASK2 expressed in placental trophoblast cells are proposed to play a role in regulating syncytiotrophoblast homeostasis and/or solute transport. It would be interesting to also determine the expression levels of Kv7 channels in preeclampsia.
channels in tissues from early pregnancy, particularly in relation to the extravillous trophoblast. Additional novel data obtained by Milan et al suggest that K^+ channels are involved in placental steroidogenesis via their contribution to mitochondrial K^+ concentrations. This opens up a further avenue of investigation in the role that KV7 channels may play in the placenta, which may also include cell proliferation, because K^+ channels have been linked previously to cell proliferation. Future experiments should aim to determine the channel complexes present and the functional role of KV7 channels in syncytiotrophoblast proliferation and migration and placental vascular reactivity.

 Perspectives 

The marked upregulation of KCNQ3 and KCNE5 KV7 channels in preeclamptic placentas, and their strong association, indicates a potential role for KV7 channels in the etiology of preeclampsia. This warrants further investigation into the roles that KV7 may play in syncytiotrophoblast and other placental cell function, such as trophoblast proliferation...
Figure 4. Expression of (A) KCNQ3 and (B) KCNE5 encoded proteins in placentas from women with preeclampsia (PE; n=6) vs normotensive controls (NC; n=6). KCNQ3 and KCNE5 expression was significantly upregulated in PE placentas (A2/B2 vs normotensive controls (A1/B1). Positive staining was localized mainly to stromal areas and syncytiotrophoblast (black arrow). Some staining was detected in blood vessels (white arrow). Data are expressed as median (interquartile range [IQR]), arbitrary units; ×400 magnification, positive controls (human midbrain) are shown in A3/B3 and negative controls in A4/B4 (*P<0.05, **P<0.001).

and migration, as well as, possibly, vascular reactivity. Their modulation and diversity may make them an interesting target for potential therapeutics to treat hypertension.

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Disclosures
None.

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