Mistry, Hiten D. and McCallum, Laura A. and Kurlak, Lesia O. and Greenwood, Iain A. and Broughton Pipkin, Fiona and Tribe, Rachel M. (2011) Novel expression and regulation of voltage-dependent potassium (KV7) channels in placentae from women with preeclampsia. Hypertension, 58 . pp. 497-504. ISSN 0194-911X

Novel Expression and Regulation of Voltage-dependent Potassium (K_V7) channels in Placentae from Women with Preeclampsia

Hiten D. Mistry[1,4,5], Laura A. McCallum[1,4], Lesia O. Kurlak[2], Iain A. Greenwood[3] & Fiona Broughton Pipkin[2] & Rachel M. Tribe[1]

[1]*Division of Women's Health, King's College London and King's Health Partners, UK;*
[2]*Department of Obstetrics & Gynaecology, University of Nottingham, Nottingham, UK;*
[3]*Basic Medical Sciences, St George's University of London, UK.*

[4]*Authors made equal contribution*

[5] **Correspondence/reprint request address:** Dr. Hiten D. Mistry

Maternal and Fetal Research Unit

Division of Women's Health

King's College London

St Thomas' Hospital

Westminster Bridge Road

London, UK

SE1 7EH

Tel: +44(0)20 7188 8151

Fax: +44(0)20 7620 1227

Email: hiten.mistry@kcl.ac.uk

Short title: Placental K_V7 Channel Expression in Preeclampsia

Word Count: total: 4,670; abstract: 250; number of figures: 4

Abstract

Preeclampsia is associated with structural/functional alterations in placental and maternal vasculature. K_V7 (voltage-dependant potassium channels encoded by KCNQ1-5 genes) have been detected in several types of blood vessels where they promote vascular relaxation. K_V7 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 placentae from women with preeclampsia compared to 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') women. KCNQ/KCNE and GAPDH mRNA expression was determined by qRT-PCR. Protein expression/localisation was assessed using immunohistochemistry. KCNQ3 and KCNE5 mRNA expression was significantly up-regulated in preeclampsia (median [IQR]: 1.942 [0.905, 3.379]) *versus* controls (0.159 [0.088, 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 localisation mainly restricted to the syncytiotrophoblast. KCNQ4 and KCNE1 isoforms were suppressed in placenta from term preeclamptic women *versus* controls ($p \leq 0.05$). KCNQ1 mRNA expression was increased and KCNQ5 decreased in the preterm preeclamptic group *versus* controls (p<0.05). In summary, K_V7 channels are expressed and markedly modulated in placenta 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.

Keywords: Potassium channel, placenta, preeclampsia, KCNQ, KCNE

Introduction

Preeclampsia is a pregnancy-specific condition affecting 2-7% of women and is associated with maternal multi-organ dysfunction[1]. This disorder, which is responsible for approximately 60,000 maternal deaths each year worldwide[2], increases perinatal mortality five-fold[3] and is commonly associated with preterm delivery and fetal growth restriction[4]. Women who develop preeclampsia and their babies are at increased risk of hypertension, metabolic disorders, cardiovascular disease and cardiovascular death in later life[5, 6].

The pathophysiology underpinning the disorder is complex. Impaired placentation almost certainly plays a part. Placental and maternal oxidative stress and generalised systemic inflammatory activation[7] are main components of the syndrome[8]. Altered maternal and placental vascular reactivity and endothelial cell function have been implicated in the clinical manifestations of preeclampsia, e.g. an increase in total peripheral vascular resistance (TPVR) [9, 10], hypertension and altered haemodynamics.

There is emerging evidence to suggest that potassium channels play important roles in the feto-placental vasculature [11-15]. Specifically, it is proposed that suppression of K_V channel function may increase vascular tone and hence be a mechanism affecting perfusion in placentae from women with preeclampsia.

The sub-family of voltage gated K_V7 channels (potassium channel complexes encoded by KCNQ1-5 and KCNE1-5 genes) are of particular interest, as preliminary data indicate the

presence of functional K_V7 channels in human chorionic plate arteries[15]. K_V7 channel activity is generally associated with outwardly rectifying, voltage dependent, K^+ currents[16]. K_V7 channels have been identified in numerous blood vessels including human visceral and mesenteric arteries[17], and the cerebral, carotid, femoral and mesenteric arteries of rodents as well as the aorta and portal vein[18-20] where they appear to play a role in vascular reactivity. A potential role for these channels in pulmonary hypertension has also been reported[21]. K_V7 channels are also important in non-excitable cells such as epithelia[22-24] and in regulation of cell volume[25] and ion transport (Cl^-, 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. K_V channel dysfunction has previously been linked to abnormalities in proliferation and possibly also to remodelling[27]. The presence of K_V7 channels in placenta and relative expression in blood vessels, endothelium and syncytiotrophoblast is unknown and the expression profiles in placenta from normotensive and preeclamptic women have not been determined.

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

Materials and Methods

Human Placental Tissue Collection

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 (ISSHP) definition of systolic blood pressure of 140 mm Hg or more and diastolic pressure (Korotkoff V) of 90 mm Hg or more on 2 occasions after 20 weeks gestation in a previously normotensive woman, together with proteinuria of ≥300 mg/L, ≥500 mg/day or ≥2+ on dipstick analysis of midstream urine (MSU) if 24-hour collection result was not available[29]. Medical and obstetric histories, including delivery data, were obtained for each woman. The birthweight centile for each baby was computed, correcting for gestation age, sex, maternal parity and body mass index (BMI) [30]. Doppler velocimetry measurements are not routinely made with preeclampsia in our hospital.

Sample collection

Placental tissue samples were collected from all subjects at a standardised location midway between cord insertion and placental border, avoiding placental infarcts. The placental samples were taken within 10 minutes of delivery; the membranes were removed and tissue washed in ice cold 1 x PBS to remove maternal blood contamination.

One sample was snap frozen in liquid nitrogen and stored at -80ºC for RNA analysis and another formalin fixed and wax-embedded for immunohistochemical analysis.

RNA extraction and cDNA synthesis

Total RNA (n=22 pre-eclamptics and n=24 normotensive controls) was extracted from a known amount of placental tissue (between 50 to 100 mg) using QIAzol lysis reagent (Qiagen, Crawley, UK). RNA concentration and quality were verified by gel electrophoresis and spectrophotometrically using the Nanodrop ND-1000 (Nanodrop Technologies, Labtech, Ringmer, UK); all samples had an A_{260}/A_{280} ratio greater than 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, UK) in a Primus 96 advanced gradient thermocycler (Peqlab Ltd, Fareham, UK). The conditions used to generate first strand cDNA were 42 °C (15 min) and 95 °C (3 min).

Quantitative RT-PCR

Real-time PCR was carried out with the use of SYBR Green chemistry (2 x Sensimix; Bioline, UK) on a RotorGene 6000 (Corbett Research, Australia) using the primers listed in Table 2. A pre-PCR cycle was run for 15 min 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 non-template controls (NTCs) run to assess contamination. Cycle threshold (CT) values were used for analysis and abundance data was obtained by the use of quantified cDNA to generate a standard curve. Standards were

quantified using densitometry and 10 fold serial dilutions (10^9 to 10^1 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, UK) 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 min each).

Immunohistochemical staining was performed using the Vector Stain Elite ABC kit (Vector Laboratories, US). Two goat polyclonal antibodies (KCNQ3 and KCNE5, SantaCruz Biotechnologies) were employed for immunostaining of paraffin-embedded placental sections (n=6 preeclamptic 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 microwave oven for 15 min followed by incubation for 30 min in normal donkey serum (Vector Laboratories, US) to block non-specific binding. Slides were then incubated with either anti-KCNQ3 (1:50) or anti-KCNE5 (1:50) overnight at 4^{o}C. A positive control (human mid-brain) 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, UK).

All slides were assessed by the same observer, blinded to pregnancy outcome. For analysis of placental sections, digital images of 5 randomly selected high-power (x400 magnification) fields were captured on NIS-Elements F2.20 microscope (Nikon UK Ltd, Surrey, UK). Quantification of KCNQ3 and KCNE5 were performed as previously described [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 immunolabelled 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 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 means ± SD or median [interquartile range (IQR)] as appropriate for data distribution. Between-group comparisons were made using 2-tailed Student's *t* tests/ or Mann-Whitney *U*-tests depending on the distribution. To assess association between mRNA expression of KCNQ and KCNE forms, data were

normalised using $\log_{10}$. Visual inspection suggested a curvilinear association which was tested by regression analysis. The null hypothesis was rejected where $P < 0.05$.

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[28]. All 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 to 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 HELLP (Hemolysis Elevated Liver enzymes, and Low Platelet count) or required magnesium sulphate administration. All neonates from both pregnancy groups survived.

KCNQ/KCNE expression in placentae from normotensive control versus all 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 qRT-PCR is shown in Figure 1. The relative abundance of placental tissue KCNQs expression in both control and preeclamptic groups was KCNQ3 > KCNQ5> KCNQ2> KCNQ1> KCNQ4. mRNA for KCNQ3 (Figure 1A) was significantly higher in preeclampsia (median [IQR]: 0.02 [0.01, 0.06] *vs.* 470 [236, 1148], $p<0.0001$) while that for KCNQ5 (Figure 1A) was down-regulated in tissues from preeclamptic women compared to controls (0.02 [0.006, 0.06] *vs*. 0.8 [0.3, 0.2], $p<0.005$).

All KCNE isoforms were detected in placental tissue in both control and preeclamptic groups (KCNE5> KCNE1= KCNE3> KCNE4> KCNE2). However, KCNE5 mRNA expression (Figure 1B) was significantly up-regulated in preeclampsia, compared to control samples (1.94 [0.91, 3.38] *vs.* 0.16 [0.09, 0.29], $p<0.0001$).

Association of expression of KCNQ3 and KCNE5

The substantial fold increases in both KCNQ3 and KCNE5 mRNA copy number in preeclamptic placental tissues, was further examined and a highly significant positive, curvilinear association between KCNQ3 and KCNE5 mRNA expression was demonstrated (in preeclamptic placentae (Figure 2; ●) $r=0.96$; $R^2=0.93$; $p<0.0001$). a significant association was also observed, at a much lower level of expression, in tissues from normotensive controls ($r=0.73$; $R^2=0.54$; $p<0.05$; Δ).

Placental expression of KCNQ/KCNE in normotensive (term), preterm and term preeclampsia

Due to 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 delivered at term (n = 14). Placental KCNQ1 was significantly ($p<0.05$) up-regulated in preterm labour *versus* normotensive controls (Figure 3A,) while 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 down-regulated in the preterm, but not term preeclamptic group compared to controls (Figure 3A; $p<0.001$). Interestingly, both KCNQ3 and KCNE5 were significantly increased in preeclamptic placentae delivered at both preterm and term compared to 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 localisation and expression in control versus preeclamptic placentae

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 K_V7 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 K^+ channels and their role in placental function[14], and is important given the widespread impact mutations of K_V7 channels have on human health (e.g. cardiac arrhythmias, epilepsy, and deafness)[16]. The implications of our data are likely to be multifaceted due to the complex functions of the placenta and the specific K_V7 formation channels involved. Altered K_V7 channel expression could impact on many areas of placental function including vascular tone, placental proliferation, ion transport or even steroidogenesis.

Our data demonstrates specific up-regulation of KCNQ3 and KCNE5 in placenta 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[33]. We speculate that a stimulus associated with pre-eclampsia may lead to activation of these channels, thus enhancing their function. This up-regulation appears most relevant to preeclamptic women as KCNQ3 and KCNE5 remain significantly up-regulated in both term and preterm preeclampsia when compared to 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[34]. 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 to other proteins. KCNQ3 along with KCNQ2 and/or KCNQ5 encodes for a channel that underlies the M current, which is important in determining the sub-threshold excitability of neurones[35, 36]. KCNQ3 along with KCNQ5 is highly expressed in bladder smooth muscle[37]. KCNE5 is highly expressed in femoral artery[20] and can modulate KCNQ1 encoded channels with respect to the voltage and time-dependent functions of the channel[38]. Such channel complex specific activity makes it difficult to predict how different K_V7 channels may influence placental/vascular function. However, we hypothesise that the observed up-regulation of KCNQ3 and KCNE5 would encode for a K_V7 channel subtype that promotes a greater outward potassium current. In this way, alteration in K_V7 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 as our group and others have reported that there is an increased vascular responsiveness to angiotensin II in

preeclampsia[39-41]. The importance of K_V7 channels to vascular reactivity has also been repeatedly demonstrated [18, 20].

Immunohisotchemistry data also provides some insight into the potential impact of altered K_V7 channel composition. The distribution of KCNQ3 and KCNE5 protein was not as localised to vascular regions as might be expected. The greatest protein density was detected in syncytiotrophoblast, which may indicate that placental K_V7 channels are related to cell proliferation, angiogenesis or solute transport. Indeed, K_V7 channels have been shown to mediate Na^+ and Cl^- transport across other types of epithelia[22, 23] and other K^+ channels; TASK1 and TASK-2 expressed in placental trophoblast cells are proposed to play a role in regulating syncytiotrophoblast homeostasis and/or solute transport[13]. It would be interesting to also determine the expression levels of Kv7 channels in tissues from early pregnancy, particularly in relation to the extravillous trophoblast. Additional novel data obtained by Milan *et al.* (2010) suggests 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 K_V7 channels may play in the placenta which may also include cell proliferation, since K^+ channels have previously been linked to cell proliferation[42]. Future experiments should aim to determine the channel complexes present and the functional role of K_V7 channels in syncytiotrophoblast proliferation and migration and placental vascular reactivity.

Perspectives

The marked upregulation of KCNQ3 and KCNE5 K_V7 channels in preeclamptic placentae, and their strong association, indicates a potential role for K_V7 channels in the aetiology of preeclampsia. This warrants further investigation into the roles K_V7 may play in syncytiotrophoblast and other placental cell function such as trophoblast proliferation and migration, as well as, possibly, vascular reactivity. Their modulation and diversity may make them an interesting target for potential therapeutics to treat hypertension.

Acknowledgements

We would like to thank all the women who took part in this study and also the midwives and doctors whose support in sample collection, aided the successful completion of this project.

Sources of Funding

This study was funded by Tommy's the Baby Charity (registered charity nº: 1060508), Action Medical Research (registered charity nº: 208701) and the Rosetrees Trust (registered charity no: 298582).

Conflict of interests/disclosure

None of the authors have any conflicting interests

References

1. Sibai B, Dekker G, Kupferminc M. Pre-eclampsia. *Lancet*. 2005;365:785-799.
2. Broughton Pipkin F. Risk factors for preeclampsia. *N Engl J Med*. 2001;344:925-926.
3. Roberts JM, Lain KY. Recent insights into the pathogenesis of pre-eclampsia. *Placenta*. 2002;23:359-372.
4. Ness RB, Sibai BM. Shared and disparate components of the pathophysiologies of fetal growth restriction and preeclampsia. *Am J Obstet Gynecol*. 2006;195:40-49.
5. Sattar N, Greer IA. Pregnancy complications and maternal cardiovascular risk: Opportunities for intervention and screening? *BMJ*. 2002;325:157-160.
6. Staff AC, Dechend R, Pijnenborg R. Learning from the placenta: Acute atherosis and vascular remodeling in preeclampsia-novel aspects for atherosclerosis and future cardiovascular health. *Hypertension*. 2010;56:1026-1034.
7. Redman CW, Sargent IL. Pre-eclampsia, the placenta and the maternal systemic inflammatory response--a review. *Placenta*. 2003;24 Suppl A:S21-27.
8. Poston L. The role of oxidative stress. In: Critchley H, MacLean A, Poston L, Walker J, eds. *Pre-eclampsia*. London: RCOG Press; 2004.
9. Roberts JM, Redman CW. Pre-eclampsia: More than pregnancy-induced hypertension. *Lancet*. 1993;341:1447-1451.
10. Cunningham FG, Lindheimer MD. Hypertension in pregnancy. *N Engl J Med*. 1992;326:927-932.

11. Kang D, Mariash E, Kim D. Functional expression of tresk-2, a new member of the tandem-pore k+ channel family. *J Biol Chem*. 2004;279:28063-28070.

12. Mylona P, Clarson H, Greenwood SL, Sibley CP. Expression of the kir2.1 (inwardly rectifying potassium channel) gene in the human placenta and in cultured cytotrophoblast cells at different stages of differentiation. *Mol Hum Reprod*. 1998;4:195-200.

13. Bai X, Greenwood SL, Glazier JD, Baker PN, Sibley CP, Taggart MJ, Fyfe GK. Localization of task and trek, two-pore domain k+ channels, in human cytotrophoblast cells. *J Soc Gynecol Investig*. 2005;12:77-83.

14. Wareing M, Greenwood SL. Review: Potassium channels in the human fetoplacental vasculature. *Placenta*. 2011; 32 Suppl 2:S203-6.

15. Wareing M, Bai X, Seghier F, Turner CM, Greenwood SL, Baker PN, Taggart MJ, Fyfe GK. Expression and function of potassium channels in the human placental vasculature. *Am J Physiol Regul Integr Comp Physiol*. 2006;291:R437-446.

16. Robbins J. Kcnq potassium channels: Physiology, pathophysiology, and pharmacology. *Pharmacology & therapeutics*. 2001;90:1-19.

17. Ng FL, Davis AJ, Jepps TA, Harhun MI, Yeung SY, Wan A, Reddy M, Melville D, Nardi A, Khong TK, Greenwood IA. Expression and function of the k+ channel kcnq genes in human arteries. *Br J Pharmacol*. 2010;162:42-53.

18. Zhong XZ, Harhun MI, Olesen SP, Ohya S, Moffatt JD, Cole WC, Greenwood IA. Participation of kcnq (kv7) potassium channels in myogenic control of cerebral arterial diameter. *J Physiol*. 2010;588:3277-3293.

19. Ohya S, Sergeant GP, Greenwood IA, Horowitz B. Molecular variants of kcnq channels expressed in murine portal vein myocytes: A role in delayed rectifier current. *Circ Res*. 2003;92:1016-1023.

20. Yeung SY, Pucovsky V, Moffatt JD, Saldanha L, Schwake M, Ohya S, Greenwood IA. Molecular expression and pharmacological identification of a role for k(v)7 channels in murine vascular reactivity. *British journal of pharmacology*. 2007;151:758-770.

21. Morecroft I, Murray A, Nilsen M, Gurney AM, MacLean MR. Treatment with the kv7 potassium channel activator flupirtine is beneficial in two independent mouse models of pulmonary hypertension. *Br J Pharmacol*. 2009;157:1241-1249.

22. MacVinish LJ, Guo Y, Dixon AK, Murrell-Lagnado RD, Cuthbert AW. Xe991 reveals differences in k(+) channels regulating chloride secretion in murine airway and colonic epithelium. *Mol Pharmacol*. 2001;60:753-760.

23. Greenwood IA, Yeung SY, Hettiarachi S, Andersson M, Baines DL. Kcnq-encoded channels regulate na+ transport across h441 lung epithelial cells. *Pflugers Arch*. 2009;457:785-794.

24. Schroeder BC, Waldegger S, Fehr S, Bleich M, Warth R, Greger R, Jentsch TJ. A constitutively open potassium channel formed by kcnq1 and kcne3. *Nature*. 2000;403:196-199.

25. Jensen HS, Callo K, Jespersen T, Jensen BS, Olesen SP. The kcnq5 potassium channel from mouse: A broadly expressed m-current like potassium channel modulated by zinc, ph, and volume changes. *Brain Res Mol Brain Res*. 2005;139:52-62.

26. Roura-Ferrer M, Sole L, Martinez-Marmol R, Villalonga N, Felipe A. Skeletal muscle kv7 (kcnq) channels in myoblast differentiation and proliferation. *Biochem Biophys Res Commun*. 2008;369:1094-1097.

27. Burg ED, Remillard CV, Yuan JX. Potassium channels in the regulation of pulmonary artery smooth muscle cell proliferation and apoptosis: Pharmacotherapeutic implications. *Br J Pharmacol*. 2008;153 Suppl 1:S99-S111.

28. Mistry HD, Wilson V, Ramsay MM, Symonds ME, Broughton Pipkin F. Reduced selenium concentrations and glutathione peroxidase activity in pre-eclamptic pregnancies. *Hypertension*. 2008;52:881-888.

29. Brown MA, Lindheimer MD, de Swiet M, Van Assche A, Moutquin JM. The classification and diagnosis of the hypertensive disorders of pregnancy: Statement from the international society for the study of hypertension in pregnancy (isshp). *Hypertens Pregnancy*. 2001;20:IX-XIV.

30. Gardosi J, Francis A. Customised centile calculator. 2006

31. Murthi P, Fitzpatrick E, Borg AJ, Donath S, Brennecke SP, Kalionis B. Gapdh, 18s rrna and ywhaz are suitable endogenous reference genes for relative gene expression studies in placental tissues from human idiopathic fetal growth restriction. *Placenta*. 2008;29:798-801.

32. Williams PJ, Mistry HD, Innes BA, Bulmer JN, Broughton Pipkin F. Expression of at1r, at2r and at4r and their roles in extravillous trophoblast invasion in the human. *Placenta*. 2010;31:448-455.

33. Zaika O, Hernandez CC, Bal M, Tolstykh GP, Shapiro MS. Determinants within the turret and pore-loop domains of kcnq3 k+ channels governing functional activity. *Biophys J*. 2008;95:5121-5137.

34. Gomez-Posada JC, Etxeberria A, Roura-Ferrer M, Areso P, Masin M, Murrell-Lagnado RD, Villarroel A. A pore residue of the kcnq3 potassium m-channel subunit controls surface expression. *J Neurosci*.30:9316-9323.

35. Wang HS, Pan Z, Shi W, Brown BS, Wymore RS, Cohen IS, Dixon JE, McKinnon D. Kcnq2 and kcnq3 potassium channel subunits: Molecular correlates of the m-channel. *Science*. 1998;282:1890-1893.

36. Lerche C, Scherer CR, Seebohm G, Derst C, Wei AD, Busch AE, Steinmeyer K. Molecular cloning and functional expression of kcnq5, a potassium channel subunit that may contribute to neuronal m-current diversity. *The Journal of biological chemistry*. 2000;275:22395-22400.

37. Porter RJ, Partiot A, Sachdeo R, Nohria V, Alves WM. Randomized, multicenter, dose-ranging trial of retigabine for partial-onset seizures. *Neurology*. 2007;68:1197-1204.

38. Angelo K, Jespersen T, Grunnet M, Nielsen MS, Klaerke DA, Olesen SP. Kcne5 induces time- and voltage-dependent modulation of the kcnq1 current. *Biophysical journal*. 2002;83:1997-2006.

39. Gant NF, Daley GL, Chand S, Whalley PJ, MacDonald PC. A study of angiotensin ii pressor response throughout primigravid pregnancy. *J Clin Invest*. 1973;52:2682-2689.

40. Broughton Pipkin F, Rubin PC. Pre-eclampsia--the 'disease of theories'. *Br Med Bull*. 1994;50:381-396.

41. Baker PN, Kilby MD, Broughton Pipkin F. The effect of angiotensin ii on platelet intracellular free calcium concentration in human pregnancy. *J Hypertens*. 1992;10:55-60.

42. Millership JE, Devor DC, Hamilton KL, Balut CM, Bruce JI, Fearon IM. Calcium activated k+ channels increase cell proliferation independent of k+ conductance. *Am J Physiol Cell Physiol*.

Figure Legends

Figure 1. A) KCNQ and B) KCNE mRNA expression in placentae from women with preeclampsia (PE, n=22) *versus* normotensive controls (NC, n=24). Data is expressed as median [IQR] normalised to GAPDH. Note the substantial change of scale for KCNQ3 and KCNE5.KCNQ3 and KCNE5 were significantly up-regulated in PE *versus* NC, whereas KCNQ5 expression was suppressed in PE. (* $p<0.05$, ** $p<0.001$).

Figure 2. Scatter plot demonstrating the association between KCNQ3 and KCNE5 mRNA expression in preeclamptic placentae. Signifcant curvilinear associations were seen between KCNQ3 and KCNE5 expression in tissues from women with preeclampsia (r=0.96; R^2=0.93; $p<0.0001$; ●), and normotensive controls, although at a much lower level of expression (r=0.73; R^2=0.54; $p<0.05$;Δ).

Figure 3. A) KCNQ and B) KCNE mRNA expression in placentae from women with preterm (n=8) and term (n=14) preeclampsia (PE) *versus* normotensive controls (NC, n=24). Data is expressed as median [IQR] normalised to GAPDH. KCNQ3 and KCNE5 expression was significantly up-regulated in both preterm and term PE placentae. KCNQ1 expression was increased in preterm PE alone, whereas KCNQ4 and KCNE1 were down-regulated in term samples. KCNQ5 expression was significantly decreased in preterm PE samples only (* $p<0.05$, ** $p<0.001$).

Figure 4. Expression of A) KCNQ3 and B) KCNE5 encoded proteins in placentae from women with preeclampsia (PE, n=6) *versus* normotensive controls (NC, n=6). KCNQ3 and KCNE5 expression was significantly up-regulated in PE placentae (A2/B2) *versus* normotensive controls (A1/B1). Positive staining was localised mainly to stromal areas and syncytiotrophoblast (black arrow). Some staining was detected in blood vessels (white arrow). Data is expressed as median [IQR], arbitrary units. x 400 magnification, positive controls (human midbrain) are shown in panels A3/B3 and negative controls in A4/B4 (* $p<0.05$, ** $p<0.001$).

Parameter	Normotensive (n = 24)	Preeclampsia (n = 22)
Maternal age (years)	30 ± 7.0	32 ± 6.0
Primipara	14 (58%)	15 (65%)
Booking body mass index (Kg/m^2)	26.2 ± 5.3	26.8 ± 4.7
Max. systolic blood pressure outside labour (mmHg)	116 ± 5.0	157 ± 8.0 †
Max. diastolic blood pressure outside labour (mmHg)	76 ± 3.0	98 ± 4.0 †
Proteinuria (g/L)	-	1.0 [0.4, 1.7]
Gestation age at delivery (weeks)	40.1 ± 1.2	36.8 ± 3.6 †
Nº of deliveries < 37 weeks	0	8 (36%)
Caesarean section	4 (16.6%)	8 (16.6%) *****
Birthweight (Kg)	3.51 [3.25, 3.76]	3.14 [2.14, 3.52] †
Birthweight centile	45 [23, 65]	35 [3, 82]

Table 1. Demographic, pregnancy, clinical and biochemical data of subject groups. Data are presented as mean ± SD or median [IQR] as appropriate, except for proteinuria: [median (min, max)] and parity, Caesarean sections and nº of deliveries < 37 weeks [number (percentage)]. These are a subgroup of our previous publication[28]. $^{*}P < 0.05$, $^{\dagger}P < 0.001$, between normal and pre-eclamptic pregnancies.

Gene	Accession number	Primers	Length (bp)
KCNQ1	NM_000218.2	5'-catcacccacatctcacagc-3' 5'-gtcccgcacatcgtaagg-3'	124
KCNQ2	NM_004518.3	5'-gacaaggaccgcaccaag-3' 5'-caccaggaagtccagcttct-3'	123
KCNQ3	NM_004519.2	5'-cgagtttgctttgaggatctg-3' 5'-aggcaatcagcacaaagatgt-3'	119
KCNQ4	NM_004700.2	5'-tgccccctcagaggaagt-3' 5'-ccaccaggaacttgagaatcc-3'	119
KCNQ5	NM_019842.2	5'-agtcccaccaaagtgcaga-3' 5'-agtgccaagggctgtgtc-3'	120
KCNE1	NM_000219.2	5'-gtcctttgatgcaagggtcta-3' 5'-ctggtctcttcctcctgagc-3'	123
KCNE2	NM_172201.1	5'-atcctgcccacacactgc-3' 5'-cgccaattgtccatataagtaataaaa-3'	114
KCNE3	NM_005472.4	5'-tccagagacatcctgaagagg-3' 5'-ggggaagactcggtagaagc-3'	123
KCNE4	NM_080671.1	5'-cctcttggactggacgattt-3' 5'-gtgctgttcagaggctccat-3'	107
KCNE5	NM_012282.2	5'-aactctgggccgtctaactg-3' 5'-aaggcaactggaagctgga-3'	113
GAPDH	NM_002046.3	5'-ggaagcttgtcatcaatggaa-3' 5'-tggactccacgacgtactca-3'	102

Table 2. Primers used in qPCR.