

Research Article

Expression of KCNH2 (hERG1) and KCNE2 Correlates With Expression of Key Myometrial Genes in Term Pregnant Human Myometrium

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Abstract

Background

Loss of activity of $K_{v11.1}$ potassium channels (encoded by *KCNH2*) facilitates labor, and is associated with expression of an inhibitory subunit encoded by *KCNE2*.

Objective

To determine whether *KCNH2* and *KCNE2* expression was linked to expression of key genes involved in myometrial contractility, including the oxytocin receptor (*OXTR*), estrogen receptor 1 (*ESR1*), progesterone receptor (*PGR*) and prostaglandin-endoperoxidase synthase 2 (*PTGS2*). We further aimed to examine *KCNH2* and *KCNE2* expression in preterm samples.

Study Design

Biopsies of term or preterm, non-laboring human myometrium were analysed by qPCR to determine *KCNH2* and *KCNE2* mRNA abundance, as well as abundance of *OXTR*, *ESR1*, *PGR* and *PTGS2* mRNAs.

Results

KCNH2 and *KCNE2* expression were significantly correlated at term, but not correlated with BMI. *KCNH2* expression significantly correlated with *OXTR*, *ESR1*, *PGR* and *PTGS2* expression, while *KCNE2* expression correlated with the expression of *ESR1*, *PGR* and *PTGS2*. Preterm samples revealed no difference in *KCNH2* and *KCNE2* expression compared to term.

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Conclusion

KCNH2 and *KCNE2* expression correlate with the expression of key myometrial genes implicated in parturition, further strengthening a role for $K_{v11.1}$ channels in human pregnancy. Neither gene was correlated with BMI, suggesting that previously reported effects of obesity likely affect protein levels rather than gene expression.

Keywords: *ESR1*; hERG; *KCNE2*; *KCNH2*; Key myometrial genes; Myometrium; *OXTR*; *PGR*; *PTGS2*

Introduction

A number of studies have demonstrated that ion channels encoded by the ether-à-go-go-related gene 1 (ERG1) play a role in regulating the contractile activity of cardiac myocytes (reviewed by Vandenberg *et al.* [1]). Moreover, there is increasing evidence demonstrating a role for *ERG1* in regulating smooth muscle cell contractility. *ERG1* encodes the pore-forming α -subunit of the delayed rectifier voltage-gated potassium channel, $K_{v11.1}$. Herein we refer to the *ERG* gene and the cognate mRNA as '*KCNH2*', and '*KCNH2* mRNA', respectively, to the channel protein as ERG and to the functional channel as $K_{v11.1}$. In addition to ERG, $K_{v11.1}$ channels contain regulatory β -subunits [2,3], such as the single transmembrane domain protein potassium voltage-gated channel subfamily E members 2, encoded by the gene *KCNE2* (mRNA referred to as '*KCNE2* mRNA', and channel protein referred to as *KCNE2*).

$K_{v11.1}$ channels are activated following action potentials and function to repolarize the cell membrane by conducting potassium ions (K^+) out of the cell, which constitutes the rapid component of the delayed rectifier current I_{Kr} [4,5]. Repolarization terminates the action potential and the associated contraction.

$K_{v11.1}$ channels have been shown in rat stomach and murine portal vein [6-8], as well as in opossum oesophagus [9]. In addition, selective $K_{v11.1}$ channel blockers have been shown to increase contractility in rat stomach [6], mouse portal vein [8], opossum oesophagus [9], mouse and guinea pig gall bladder [10], bovine epididymis [11] and human and equine jejunum [12,13]. These smooth muscles all exhibit spontaneous contractile activity, which is also a property of uterine smooth muscle [14].

$K_{v11.1}$ channels were initially linked to myometrial contractility by Aaronson *et al.*, who demonstrated that tetraethylammonium (TEA)- and 4-aminopyridine (4-AP)-sensitive voltage-dependent K^+ (K_v) channels played a role in regulating action potential duration in rat myometrium [15]. Greenwood *et al.* later examined mouse ERG (mERG) expression and function, and confirmed the presence of both the ERG1a and ERG1b splice variants, with ERG1a expression being more abundant than that of ERG1b [16]. They found that *KCNH2* mRNA abundance did not change throughout gestation or with the onset of labor, however, mRNAs encoding auxiliary subunits, namely *KCNE2*, were significantly up-regulated approaching term [16].

More recently, our group reported that human ERG1 (hERG1) and *KCNE2* were present in pregnant human myometrium during late gestation and labor [17]. We found that $K_{v11.1}$ activity suppressed the amplitude and duration of contractions prior to labor, thereby

supporting a role for $K_{v11.1}$ activity in helping to maintain uterine quiescence [17]. Previous reports have indicated that *KCNE2* co-expression with *hERG1* was not necessarily inhibitory of $K_{v11.1}$ activity [18], however, we found that the onset of labor was associated with the increased expression of *KCNE2* and the decreased responsiveness of myocytes to the $K_{v11.1}$ inhibitor, dofetilide [17]. Together these findings support an inhibitory role for *KCNE2* in the context of regulating *hERG1* in the myometrium. The evidence also suggests that $K_{v11.1}$ activity contributes to the electrophysiological mechanisms that regulate uterine contractions and that inhibition of the α -subunit, *ERG1*, by the β -subunit, *KCNE2*, may facilitate labor [17]. Interestingly, the mechanism for reducing $K_{v11.1}$ activity to facilitate labor appears to be dysregulated in obese women. Maternal obesity is associated with increased rates of labor induction, dysfunctional labor requiring Caesarean section (CS) delivery, longer pregnancies, as well as postpartum haemorrhage [19-21]. We reported that high body mass index (BMI) was associated with increased levels of *hERG1* and reduced levels of *KCNE2* in the myometrium [17]. Furthermore, high BMI was associated with heightened $K_{v11.1}$ activity *in vitro*, suggesting that the delayed and protracted labor often observed in obese women is linked to elevated $K_{v11.1}$ activity in the myometrium [17].

Genes encoding the progesterone receptor (*PGR*) [22-24], estrogen receptor 1 (*ESR1*) [23,25], oxytocin receptor (*OXT*) [24,26,27] and prostaglandin-endoperoxidase synthase 2 (*PTGS2*) [24,28] have been identified as key genes involved human parturition. There is now extensive published literature linking the myometrial regulation of these genes, among others, to the maintenance of uterine quiescence and the transition to a contractile phenotype at labor [29-31]. Uncovering an association between expression of these key genes and genes encoding the $K_{v11.1}$ channel would ascertain the involvement of this channel in the increased contractility of the myometrium at term and as part of the myometrial transformation leading to labor.

The aim of this study therefore was to determine whether expression of *KCNH2* and *KCNE2* in term non-laboring human myometrium correlate with the expression of *PGR*, *ESR1*, *OXT* and *PTGS2*. We report that in term pregnancy, *KCNH2* and *KCNE2* are expressed coordinately with these key parturition-associated genes, thus strengthening the link between uterine contractility and K^+ channel abundance in myometrial cells. Furthermore, this study examined the relationship of myometrial *KCNH2* and *KCNE2* mRNA abundance with BMI, and explored the expression of both genes in myometrial biopsies from preterm deliveries.

Materials and Methods

Consumables and reagents

Superscript III First Strand Synthesis System, Ultrapure Glycogen, Ultra Pure Agarose and Trackit 100 BP DNA ladder were purchased from Invitrogen (Carlsbad, USA). TRizol Reagent and Turbo DNA-free 50 reactions were from Ambion (Austin, USA). Alien QRT-PCR Inhibitor Alert 400 Reactions were purchased from Integrated Sciences Pty (Sydney, Australia). PCR primers were purchased from Sigma (St Louis, USA). The 2 mL 2.8 mm CK28-R Ceramic Bead Kit for the Precellys homogenizer (Bertin Instruments, France) were purchased from Thermo Fischer Scientific (Melbourne, Australia). L-Glutamine, Sodium Pyruvate, Gentamicin, HEPES and Dulbecco's Modified Eagle Medium (DMEM) were obtained from Gibco (Carlsbad, USA). SYBR Green 2x Master mix was from Applied Biosystems (Carlsbad, USA).

Myometrial tissue acquisition

These studies were approved by the Hunter and New England Area Human Research Ethics Committee and the University of Newcastle Human Ethics Committee (02/06/12/3.13). All participants gave informed written consent. Human myometrial samples ($5 \times 5 \times 10$ mm) were obtained from the lower uterine segment during elective CS of singleton pregnancies. Preterm samples ranged from 31 - 34 weeks gestation while term samples were 38.2 - 39.6 weeks gestation. Patient BMI range was 18.3 - 38.0, and all patients were not-in-labor. The indications for elective not-in-labor term CS were previous CS, placenta praevia, fetal distress or breach presentation. The indications for elective not-in-labor preterm CS were placenta increta, pre-eclampsia and low levels of amniotic fluid. Following delivery of the placenta, 5 units of syntocinon were administered directly into an intravenous line as part of standard care for the prevention of postpartum hemorrhage. Samples were therefore exposed to oxytocin for a brief period of time (3 min). All myometrial samples were placed on ice in a serum-free media containing DMEM with high glucose, 2 mM L-glutamine, 1 mM sodium pyruvate, 40 μ g/mL gentamicin and 10 mM HEPES for the transfer to the laboratory. Myometrial tissues were then cleared of serosa, fibrous or damaged tissue and visible blood vessels before being dissected into smaller pieces and washed in serum-free media to remove excess blood. Approximately 100 mg of tissue was snap frozen in liquid nitrogen for subsequent analysis.

RNA extraction, Reverse transcription and Real-time quantitative PCR

RNA was extracted from 100 mg of tissue using TRizol Reagent (Ambion, USA) according to the manufacturer's protocol. Homogenization of tissue in TRizol Reagent was performed using a Precellys24 homogenizer (Bertin Instruments, France). Following extraction, RNA samples were purified using the TURBO DNA-free kit (Ambion, USA). An ND-1000 spectrophotometer (NanoDrop Technologies, Inc., USA) was used to measure RNA concentration (absorbance at 260 nm (A_{260}) and 280 nm (A_{280})) and purity. RNA integrity was checked by agarose gel electrophoresis. Each RNA sample (0.5 μ g of total RNA) was spiked with 0.5×10^7 copies of Alien RNA (Integrated Sciences Pty, Australia) and reverse-transcribed using the Super Script III First-Strand Synthesis System with random hexamer primers (Invitrogen, USA). The Alien RNA transcript is an *in vitro* transcribed RNA molecule that is non-homologous to any known nucleic acids and as such was used as a housekeeping gene for these studies [32-34]. Quantitative real-time PCR was performed using an ABI 7500 Sequence Detector (Applied Biosystem, USA). No-reverse transcription (no-RT) negative controls were prepared for each sample to ensure there was no DNA contamination. The final volume of each PCR reaction was 20 μ L containing 10 μ L of 2x SYBR Green PCR Master Mix (Applied Biosystems, USA), master mix cDNA template (corresponding to 10 ng of reverse transcribed RNA), target cDNA-specific forward and reverse primers, and MilliQ water. For the reference gene (Alien primer), the final volume was also 20 μ L with 1.0 μ L of 2.5 μ M of Alien Primer Mix, 10 μ L of 2x SYBR Green PCR and the same amount of cDNA as the target genes and MilliQ water. No-template control samples (NTCs) were included in each PCR plate to detect any contamination and primer-dimers. PCR primers were designed using Primer Express, optimized and validated by confirming that single amplicons of appropriate size and sequence were generated (Table 1).

| Primer | Primer Sequence (5'-3') | Amplicon Size | GenBank # |
|--------|---|---------------|-------------|
| KCNH2 | F: ACCTCATCGTGGACATCA R: CTCCTCGTTGGCATTGAC | 77 | NM_000238.3 |
| KCNE2 | F: CACGAGGCAATCCAAAT R: CTCCTCAAGCAAGCATAA | 141 | NM_172201.1 |
| OXTR | F: CTGGACGCCTTCTCTTCGT R: GAAGGCCGAGGCTTCCTT | 101 | NM_000963 |
| ESR1 | F: TGAAAGGTGGGATACGAAAAGAC R: CATCTCTCTGGCGCTTGTGT | 66 | NM_000125.3 |
| PGR | F: GTGGGAGCTGTAAGGTCTCTTTAA R: AACGATGCAGTCATTCTTCCA | 83 | NM000926.4 |
| PTGS2 | F: ATGTTCCACCCGAGTACAGA R: CAGCATAAAGCGTTTGCAGTA | 73 | NM_000916.3 |

Table 1: cDNA primer sequences for KCNH2, KCNE2, OXTR, ESR1, PGR and PTGS2.

KCNH2: Potassium Voltage-Gated Channel Subfamily H Member 2
KCNE2: Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 2
OXTR: Oxytocin Receptor
ESR1: Estrogen Receptor 1
PGR: Progesterone Receptor
PTGS2: Prostaglandin-Endoperoxide Synthase 2

Data and statistical analysis

All mRNA abundance data were expressed relative to the Alien reference RNA. The relative mRNA abundance was calculated using the delta C_t (ΔC_t) method [35]. All mRNA relative abundance values were then logarithmically transformed to approach normal distribution. Statistical analyses were conducted with Graph Pad Prism software (San Diego, CA, USA). Graphical data are presented as mean ± SEM. For comparison between two groups, Student's *t*-test was used. For correlation studies Pearson's product moment correlation coefficient was used (Pearson's *r*). Preterm and term samples were compared by 1-way ANOVA with Bonferroni's multiple comparisons test. *P*-values ≤ 0.05 were considered statistically significant.

Results

KCNH2 and KCNE2 expression in human myometrium at term pregnancy

Relative abundance of KCNH2 and KCNE2 mRNA was measured in non-laboring term myometrial tissues (n=18). Expression of KCNH2 was much higher than of KCNE2 (over two orders of magnitude, Figure 1A). A statistically significant positive correlation was found between KCNH2 and KCNE2 mRNA abundance (*r*²=0.34, *p*=0.01) (Figure 1B).

There was no statistically significant relationship between KCNH2 mRNA abundance and gestational age (*r*²=0.06, *p*=0.31) (Figure 1C), or KCNE2 mRNA abundance and gestational age (*r*²=0.04, *p*=0.41) (Figure 1D) within the term gestation range of 38.2-41.0 weeks.

Since we have found previously that hERG1 levels increase whilst KCNE2 levels decrease with increasing BMI of term pregnant women, we have correlated KCNH2 mRNA as well as KCNE2 mRNA abundance to BMI. The BMI in our patients group ranged from 18.3 to 38.0. There was no statistically significant correlation between KCNH2 mRNA abundance (*r*²=0.0002, *p*=0.96) (Figure 1E) or KCNE2 mRNA abundance and BMI (*r*²=0.07, *p*=0.29) (Figure 1F).

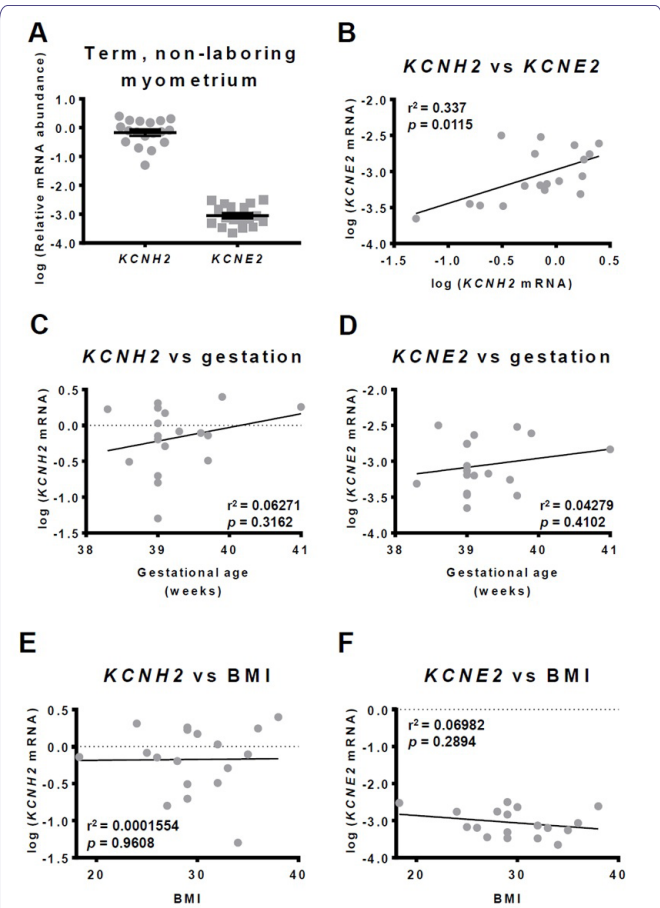
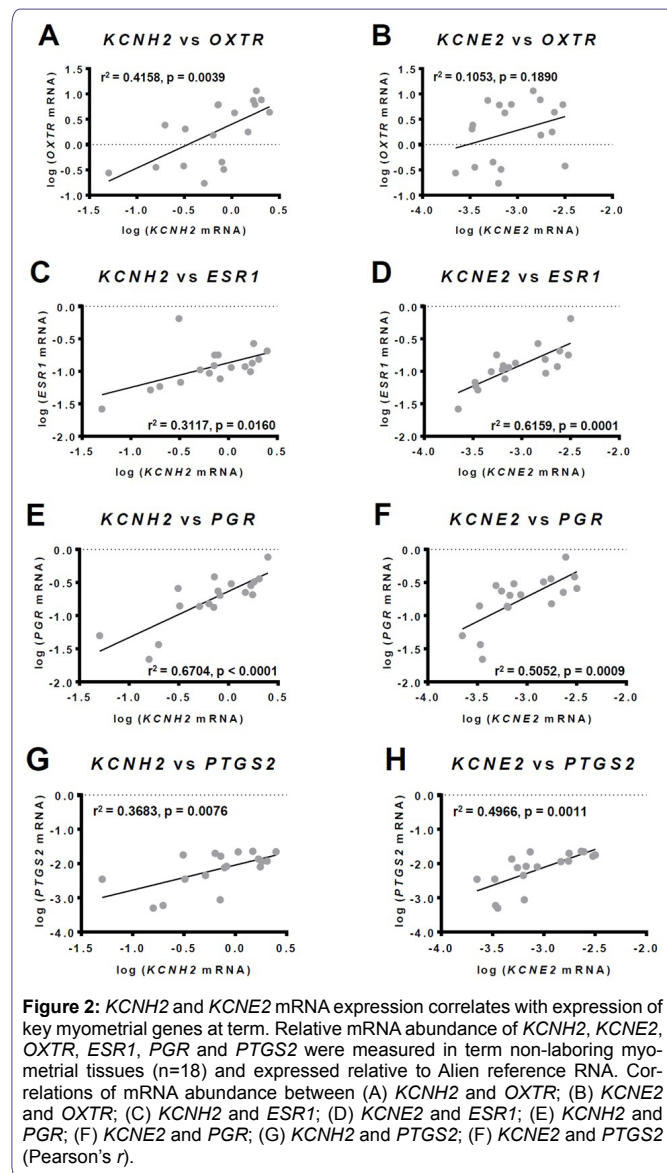


Figure 1: Expression of KCNH2 and KCNE2 mRNA in pregnant human myometrium. Relative abundance of KCNH2 and KCNE2 mRNA was measured in term non-laboring myometrial tissues (n=18) and expressed relative to Alien reference RNA. (A) KCNH2 and KCNE2 mRNA abundance at term. (B) Correlation between KCNH2 and KCNE2 mRNA abundance. (C) KCNH2 mRNA abundance across term gestation time points. (D) KCNE2 mRNA abundance across term gestation time points. (E) KCNH2 mRNA abundance against BMI. (F) KCNE2 mRNA abundance against BMI (Pearson's *r*).

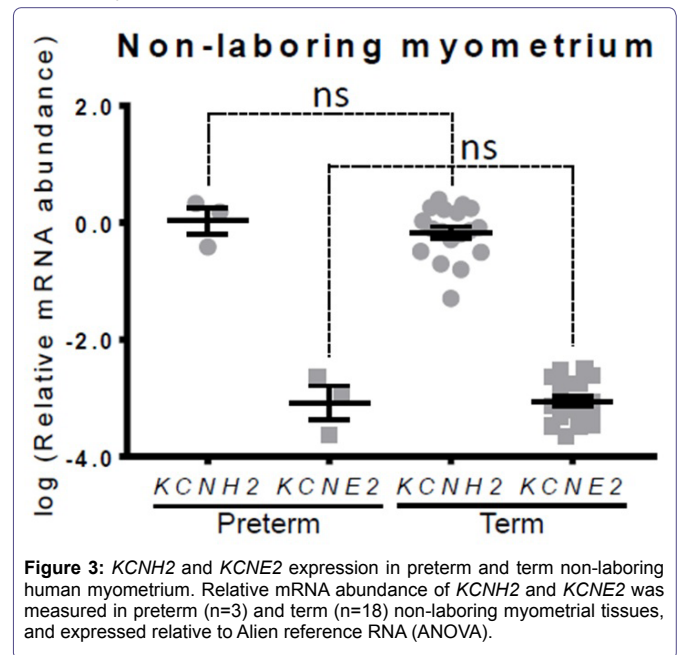
Correlations between the expression of *KCNH2*, *KCNE2* and contraction-associated myometrial genes

We measured the relative abundance of *OXTR*, *ESR1*, *PGR* and *PTGS2* mRNAs ($n=18$ each) and correlated their abundance with *KCNH2* and *KCNE2* mRNA abundance. A statistically significant positive correlation was found between *KCNH2* and *OXTR* ($r^2=0.42$, $p=0.0039$) (Figure 2A) but not between *KCNE2* and *OXTR* mRNA abundance ($r^2=0.11$, $p=0.19$) (Figure 2B). *ESR1* mRNA abundance was positively correlated with *KCNH2* ($r^2=0.3117$, $p=0.0160$) (Figure 2C) and *KCNE2* mRNA abundance ($r^2=0.62$, $p=0.0001$) (Figure 2D). Moreover, *PGR* mRNA also correlated positively with *KCNH2* ($r^2=0.67$, $p<0.0001$) (Figure 2E) and *KCNE2* mRNA abundance ($r^2=0.51$, $p=0.0009$) (Figure 2F). In our term non-laboring samples progesterone receptor A (*PR-A*) expression was barely detectable, meaning correlations with *PGR* were predominantly in relation to progesterone receptor B (*PR-B*). Finally, the key contraction-associated gene product, *PTGS2* mRNA, also exhibited significant positive correlation with *KCNH2* ($r^2=0.37$, $p=0.0076$) (Figure 2G) and *KCNE2* mRNA abundance ($r^2=0.50$, $p=0.0011$) (Figure 2H).



KCNH2 and *KCNE2* expression in preterm human myometrium

The relative abundance of *KCNH2* and *KCNE2* mRNA was determined in a limited number of non-laboring preterm myometrial tissues ($n=3$). No difference was found for *KCNH2* ($p=0.47$) or *KCNE2* ($p=0.92$) mRNA abundance compared to term non-laboring samples ($n=18$) (Figure 3).



Discussion

We previously reported the presence of *hERG1* and *KCNE2* in pregnant human myometrium in late gestation, and demonstrated that labor onset is associated with diminished $K_{11.1}$ activity in association with enhanced expression of the inhibitory subunit, *KCNE2* [17]. Furthermore, we reported a significant positive correlation between $K_{11.1}$ activity and BMI, which was attributable to increased *hERG1* levels and decreased *KCNE2* levels [17]. In follow up to that functional study, we examined *KCNH2* and *KCNE2* gene expression in term non-laboring myometrium and found that *KCNE2* expression was relatively low, and correlated with *KCNH2* expression. Moreover, the levels of both mRNAs correlated positively with the expression of myometrial genes that are key regulators of parturition, but showed no relationship with the BMI of the participants. Patients comprising our term non-laboring cohort ranged from underweight (BMI 18.3) to Class II obese (BMI 38.0) (World Health Organisation guidelines). The lack of correlation suggests that within the examined BMI range, the effect of obesity is not on *KCNH2* or *KCNE2* gene expression, but rather on protein levels. This finding refines the link between $K_{11.1}$ activity and BMI, and suggests that future studies should focus on the effect of obesity on *KCNH2* and *KCNE2* mRNA translation as well as protein turnover.

Another potassium channel that plays a crucial role in pregnancy is the ATP-sensitive K^+ (K_{ATP}) channel. K_{ATP} -mediated K^+ efflux plays a role in maintaining the resting membrane potential of myocytes [36]. Several studies have found that in late pregnancy, the number of myometrial K_{ATP} channels is reduced, which increases uterine excitability, thereby promoting the establishment of labor [37-39]. Du *et al.* [40]

found that all K_{ATP} channel subunits, apart from the SUR2A subunit, were down-regulated in the late pregnant uterus compared to the non-pregnant uterus. More importantly, expression of SUR2B/Kir6.1 in term pregnant human myometrium was found to be increased in women older than 35 years [40]. Advanced maternal age is associated with increased obstetric risks in general, as well as increased risk of elective and emergency CS [41,42]. The study therefore suggests that increased risk of birth complications in women aged over 35 years may be linked to reduced myocyte excitability attributable to increased numbers of K_{ATP} channels in the myometrium [40]. This is consistent with our previous study on *hERG1*, which similarly links poor labor outcomes for obese women with the elevated activity of a potassium channel [17]. Both studies illustrate the importance of understanding how different potassium channels regulate uterine contractility, as dysregulation can lead to obstetric complications.

In pregnant human myometrium, we observed a significant positive correlation between *KCNH2* and *KCNE2* mRNA abundance at term (Figure 1B). This correlation, if it translates to protein abundance, could suggest an underlying subunit stoichiometry in myometrial $K_{11.1}$ channels. Given that all term samples analysed were non-laboring, preservation of the high *KCNH2*:*KCNE2* ratio may play a role in maintaining quiescence in non-laboring tissue.

The uterine quiescence, which persists for the majority of pregnancy, is maintained by inhibitors of uterine contraction, such as progesterone [24,43-46]. As term approaches there is a shift from progesterone to estrogen dominance and the uterus undergoes a phenotypic transition. This transition is characterised by up-regulated expression of a series of contraction-associated genes (reviewed by Smith [47]), including receptors for oxytocin and prostaglandins, increased expression of genes encoding myometrial gap junctions, such as connexin 43, which facilitates synchronous contractions, and alterations in resting membrane potential of myocytes, which renders myocytes more prone to excitation [24,43-46,48]. Collectively these changes increase the likelihood that sporadic contractions of the uterus will propagate in synchrony and lead to established labor [49]. In fulfilling a role as a regulator of myometrial contractility, it would therefore be reasonable to expect $K_{11.1}$ activity, and thus *KCNH2* and *KCNE2* expression, to correlate with the expression of key genes that are known to play a role in this phenotypic transition.

OXTR expression in the myometrium is reportedly constant between 24 - 36 weeks gestation, but rises significantly in term samples (>37 weeks) prior to the onset of labor [48,50]. Within this latter time frame (term), we found that *KCNH2* expression correlated positively with *OXTR* expression, whereas *KCNE2* did not. Given that *KCNH2* mRNA abundance is significantly correlated with both *KCNE2* and *OXTR* mRNA abundance, a correlation should be expected between *KCNE2* and *OXTR* mRNA levels. It is possible that with greater sample numbers a positive correlation would be demonstrated between *KCNE2* and *OXTR* expression. Given that the role of $K_{11.1}$ within the myometrium is to rapidly terminate contractions in order to prevent propagation of uterine contractility and the establishment of labor, it is reasonable to conjecture that *KCNH2* expression is co-regulated with *OXTR* expression as a counter-measure to offset increased sensitivity to oxytocin.

Similar considerations may apply to the observed positive correlation of *KCNH2* and *KCNE2* with *ESR1* and *PTGS2*. *ESR1* encodes estrogen receptor α (ER α), which drives the estrogen-dependent expression of contraction-associated proteins, such as connexin 43 [51-54].

PTGS2 is responsible for the biosynthesis of prostaglandins (PGs). Prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$) is produced mainly by the maternal decidua and is involved in the up-regulation of *OXTR* levels and gap junctions in the myometrium, thus promoting uterine contractions [55]. Prostaglandin E_2 (PGE $_2$) is produced by the fetus and placenta and is involved in collagen degradation and dilation of small blood vessels in the cervix, thus promoting cervical ripening as well as spontaneous rupture of the fetal membranes [56]. It is important to remain mindful, however, that non-laboring tissues were analysed in our study suggesting the possibility that women with high expression of *ESR1* and *PTGS2* may require higher levels of *KCNH2* in order to maintain uterine quiescence than women with lower *ESR1* and *PTGS2* expression. Moreover, in the heart *KCNE2* expression is up-regulated by estrogen [57]. Taking into consideration that in humans circulating levels of estrogen are high for most of pregnancy, and remain elevated during parturition [46,58], it is likely that estrogen regulation of *KCNE2* expression, and thus $K_{11.1}$ activity, plays an important role in modulating the transition from quiescence to contractility.

KCNH2 and *KCNE2* mRNA levels were both positively correlated with *PGR* expression. Consistent with previous studies we report *PGR* total expression [30] as within our term non-laboring cohort *PR-A* expression was barely detectable. The strong positive correlations between *KCNH2* and *KCNE2* expression with *PGR* expression are therefore in relation to *PR-B*, which is consistent with the observed *KCNH2*:*KCNE2* ratio potentially contributing to pregnancy maintenance. A follow up analyses determining whether *KCNH2* and *KCNE2* expression continue to correlate with *PGR* expression in laboring tissue would provide valuable insight into whether progesterone regulates *hERG1* and *KCNE2* levels as means of maintaining uterine quiescence.

In mice, *KCNH2* expression remains constant across gestation and there is no change in *ERG1* protein levels [16]. *KCNE2*, however, is gestationally regulated in mice in that mRNA abundance is significantly up-regulated by day 14 of a 20 day pregnancy, and *KCNE2* protein levels are significantly up-regulated by day 17 [16]. Our analyses of preterm non-laboring samples showed no change in *KCNH2* or *KCNE2* gene expression earlier in gestation suggesting that, unlike mice, *KCNE2* gene expression may not be gestationally regulated in humans. If confirmed by analysis of additional preterm samples, this could indicate that alternative auxiliary subunits need to be explored in the context of human myometrium. To date, studies exploring the role of $K_{11.1}$ channels in human myometrium have focused on *KCNE2* co-expression alongside *hERG1*. However, a variety of auxiliary subunits are known to modulate $K_{11.1}$ activity [1], and studies by Greenwood *et al.* [16] indicate that *KCNE4* expression is gestationally regulated in mouse uterine tissue in addition to *KCNE2*. As such it is possible that *KCNE4*, or another regulatory subunit, is gestationally regulated in humans.

Conclusion

We have previously provided functional data examining the role of $K_{11.1}$ in regulating myometrial contractility at term labor. Here we have followed up the functional study with an analysis of *KCNH2* and *KCNE2* gene expression in term non-laboring women. We have shown that *KCNH2* and *KCNE2* expression correlate with the expression of key myometrial genes implicated in parturition. Our data suggest that *KCNH2* and *KCNE2* are participants in the gene network controlling myometrial contractility at term. Uncovering this association advances our understanding of the mechanisms that underpin

myometrial transformation, and reiterates the complexity of the parturition process. A complementary analysis using laboring myometrium would assist with the further interpretation of these results as alterations of the correlative network with the onset of labor would provide insight into which relationships are critical in maintaining uterine quiescence. We have also performed an initial investigation into *KCNH2* and *KCNE2* expression in preterm samples, which did not show any robust association between the preterm status and *KCNH2* or *KCNE2* expression. This investigation should be followed up with additional preterm samples to strengthen the study, as well as the analysis of preterm in-labor samples. Furthermore, future studies include examining whether these correlations translate into correlations at the protein level.

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Conflict of Interests Disclosure

Roger Smith holds a patent through the University of Newcastle in relation to the hERG potassium channel, titled "Compositions and methods for modulating uterine contractions". The remaining authors declare that there is no conflict of interest regarding the publication of this paper.

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