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The Role of Skeletal Muscle Ryanodine Receptor Type 1 (*RYR1*) in Uterine Vascular and Myometrial Smooth Muscle Function During Pregnancy

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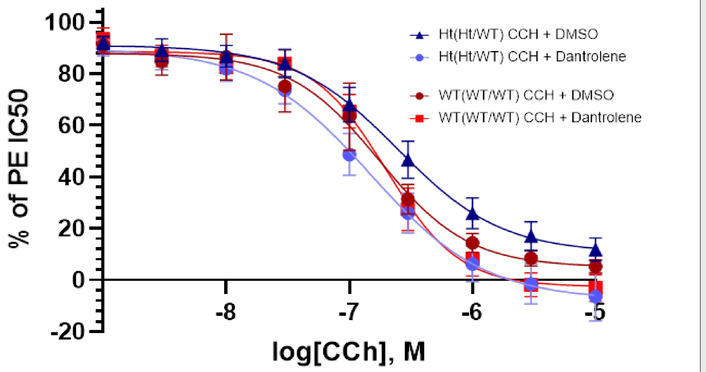

Figure 1. Concentration response curves in isolated uterine artery segments from pregnant wildtype (wildtype litter) and Ryr1$^{Y522S/+}$ heterozygous (mixed litter) mothers to cumulative concentrations of carbachol with dantrolene (30 μM) or vehicle control DMSO treatment. P= 0.0457, N=20-22.

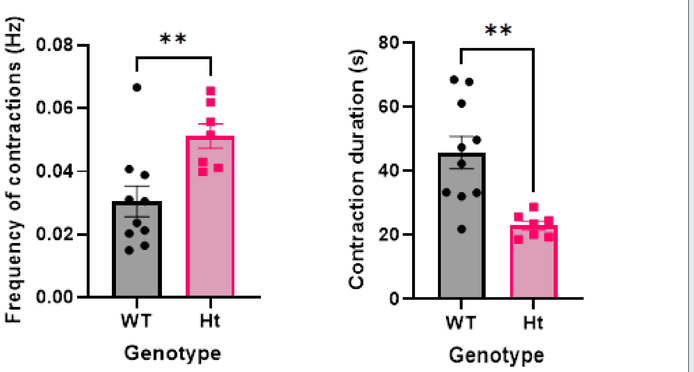

Figure 2. Spontaneous myometrial contractions in non-pregnant Ryr1$^{Y522S/+}$ animals (4 animals, 7 tissue strips) were more frequent (P=0.0046) and of shorter duration (P=0.0014) than wildtype (5 animals, 10 tissue strips).

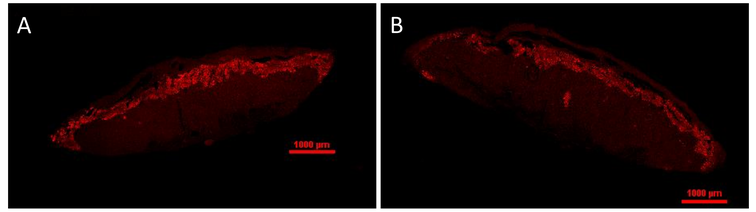

Figure 3. Representative immunofluorescent images of RyR1-positive stained (**A**) wildtype and (**B**) heterozygous Ryr1$^{Y522S/+}$ gestation day 18.5 placentas (n=10).

Background

Mutations in *RYR1* encoding the skeletal muscle ryanodine receptor are a common cause of neuromuscular disorders but have also been implicated in a mild bleeding disorder characterised by severe menorrhagia, post-partum and postoperative bleeding. Excessive bleeding suggests a role of RyR1 in vascular smooth muscle function, an observation experimentally tested by Lopez *et al*. (2016).

Hypothesis

The presence of a gain-of-function *Ryr1* mutation in pregnancy will lead to enhanced vasorelaxation of vascular smooth muscle cells and altered contractility of myometrium. This will impact on fetal and placental development and influence the length of gestation and parturition.

Methods

Ryr1$^{Y522S/+}$ mouse model in late-stage pregnancy (gestation day 18.5).
Vascular smooth muscle function pharmacologically investigated using wire myography (phenylephrine, carbachol and dantrolene).
Myometrial function studied using video recordings to determine gestation length, isometric tension recordings of spontaneous myometrial contractions and RNAseq for gene expression changes in the pregnant myometrial tissue.
Fetal and placental weight measurements were made on gestation day 18.5. Histological techniques were used to study placental morphology. Two-tailed unpaired t-tests were used for pairwise comparisons. Data: mean ±SEM.

Results

Paradoxically, uterine artery **vasodilatory capacity (log_{IC50}) was reduced** in vessels from heterozygous *Ryr1*$^{Y522S/+}$ (mixed litter) dams ($10^{-6.597}$±0.135, n=20) compared to vessels from wildtype (wildtype litter) dams ($10^{-7.275}$±0.292, n=22), P=0.0457, but was reversed by dantrolene. **Frequency of non-pregnant myometrial contractions was greater** in Ryr1$^{Y522S/+}$ tissue (0.0512±0.00389 Hz, 4 animals, 7 tissue strips) compared to wildtype tissue (0.03046±0.00488 Hz, 5 animals, 10 tissue strips), P=0.0046. **Duration of non-pregnant myometrial contractions decreased** in heterozygous tissue (22.91±1.416 s) compared to wildtype tissue (45.71±5.106 s), P=0.0014.

Gestation length of *Ryr1*$^{Y522S/+}$ mouse pregnancies was not different to that of the wildtype mouse (P=0.8452). The *Ryr1*$^{Y522S/+}$ mouse had **fewer fetuses per litter** (7.364±0.305, n=22) compared to wildtype littermates (8.481±0.223, n=27), P=0.0248, and **lower fetal:placental weight** ratios (9.36±0.420, n=11) compared to wildtype (10.88±0.2445, n=16), P=0.0132. The RyR1 protein localised to the **junctional zone** of the placenta (n=10).

Conclusions

Through these studies we have shown that the maternal *Ryr1* Y522S influences uterine artery vasodilation and myometrial contraction, suggesting a physiological role of RyR1 in smooth muscle function, which contributes to fetal-placental growth and attachment.

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