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Short Communication

Placental glucocorticoid receptor isoforms in a sheep model of maternal allergic asthma

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A B S T R A C T

Maternal asthma increases the risk of adverse pregnancy outcomes and may affect fetal growth and placental function by differential effects on the expression of glucocorticoid receptor (GR) isoforms, leading to altered glucocorticoid signalling. Our aim was to examine the effect of maternal asthma on placental GR profiles using a pregnant sheep model of asthma. Nine known GR isoforms were detected. There was a significant increase in the expression of placental GR isoforms that are known to have low trans-activational activity in other species including GR A, GR P and GRγ which may result in a pro-inflammatory environment in the presence of allergic asthma.

1. Introduction

Asthma is an inflammatory disease that complicates 12% of human pregnancies [1] and associated with adverse perinatal outcomes including growth restriction and preterm delivery [2,3] especially in women with uncontrolled asthma during pregnancy [4]. Changes in fetal growth associated with maternal asthma may be the result of alterations in placental function and structure with specific changes in glucocorticoid regulated pathways mediated by differential expression of placental glucocorticoid receptor (GR) isoforms [5–7].

The glucocorticoid receptor (GR) is a ubiquitously expressed protein and a member of the nuclear receptor transcription factor super family [8–12]. Multiple GR isoforms are present in the placentae of humans [5,13], guinea pigs [14] and mice [15] and their abundance varies with glucocorticoid exposure, pregnancy complications, gestational age and fetal sex. The differential expression of GR isoforms may confer differences in placental glucocorticoid sensitivity to ensure fetal survival in an adverse maternal environment. We developed a pregnant sheep model of maternal allergic asthma that causes physiological changes in the maternal lung comparable to human asthma, reduces fetal growth and changes fetal lung development [16]. We aimed to identify whether the sheep placenta expresses multiple GR isoforms and if expression is altered by the presence of maternal allergic, asthma.

2. Methods

Experimental procedures were approved by the Animal Ethics Committees of Monash University (MARP/2013/133) and the University of Adelaide (M-2014-126). The breeding and treatment protocol for allergic, asthmatic (induced by house dust mite) (n = 7) or control (n = 5) singleton bearing Merino ewes (1–2 years of age) are reported in Clifton et al. [16]. All placentomes were removed from the endometrium at term delivery, individually weighed and classified for phenotype (Type A, B, C or D) [17]. Frozen whole placentome samples of each phenotype from control and asthmatic sheep were crushed and tissue was fractionated into cytosolic and nuclear components [5]. The volume density of placental tissue was quantified in formalin fixed sections of each phenotype from control and asthmatic sheep [18,19]. The surface area and barrier thickness were calculated using the weight of the individual placentome or total placental weight. GR isoforms were identified and visualised by Western blot using an affinity purified polyclonal rabbit anti-human total GR antibody (1:1000) (Bethyl Laboratories, Montgomery, TX, USA, A303-491A). The antibody is reactive to sheep antigens (Bethyl Lab website). A specific GR β antibody

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Table 1

n	Control	Asthma	p
	5	7	
Maternal Characteristics			
Post mortem weight (kg)	38.86 ± 2.07	39.60 ± 1.41	n.s.
Gestational age (days)	140.00 ± 1.10	139.57 ± 0.53	n.s.
Neonatal Characteristics			
Body weight (kg)	4.11 ± 0.28	3.69 ± 0.14	n.s.
Fetal: Maternal weight	0.11 ± 0.01	0.09 ± 0.00	0.038
Placenta characteristics			
Placental weight (g)	343.75 ± 50.98	322.90 ± 23.32	n.s.
Type-A placentome			
Proportion of placentomes (%)	49.42	21.05	n.s.
Average weight (g)	3.93 ± 0.58	3.46 ± 0.55	n.s.
Type-B placentome			
Proportion of placentomes (%)	22.59	45.95	0.037
Average weight (g)	4.18 ± 0.72	4.23 ± 0.25	n.s.
Type-C placentome			
Proportion of placentomes (%)	7.56	18.86	0.047
Average weight (g)	4.32 ± 1.07	3.97 ± 0.26	n.s.
Type-D placentome			
Proportion of placentomes (%)	20.43	14.15	n.s.
Average weight (g)	4.31 ± 1.01	5.38 ± 0.62	n.s.
Trophoblast volume density (Vd)			
Vd	0.527 ± 0.009	0.525 ± 0.006	n.s.
Volume of placenta (cm3)	213.876 ± 11.908	169.990 ± 5.905	0.0008
Fetal capillaries volume density (Vd)			
Vd	0.008 ± 0.001	0.007 ± 0.001	n.s.
Volume of placenta (cm3)	3.154 ± 0.263	2.395 ± 0.230	n.s.
Fetal connective tissue volume density (Vd)			
Vd	0.062 ± 0.005	0.057 ± 0.002	n.s.
Volume of placenta (cm3)	24.726 ± 1.509	18.392 ± 0.828	0.0004
Maternal epithelium volume density (Vd)			
Vd	0.185 ± 0.006	0.191 ± 0.003	n.s.
Volume of placenta (cm3)	74.897 ± 4.545	61.153 ± 1.829	0.0016
Maternal capillaries volume density (Vd)			
Vd	0.019 ± 0.001	0.016 ± 0.001	n.s.
Volume of placenta (cm3)	7.687 ± 0.549	5.290 ± 0.405	0.0027
Maternal connective tissue volume density (Vd)			
Vd	0.200 ± 0.007	0.204 ± 0.004	n.s.
Volume of placenta (cm3)	80.327 ± 3.483	65.751 ± 2.208	0.0013

Values displayed are means ± standard error of the mean. Significance $P < 0.05$.

(1:1000) was also used (Abcam Cambridge, MA, USA) [5]. Densitometry was used to quantify GR expression and normalised against β-actin [5]. Lamin A/C was used to ensure cytosolic fractions were clear of nuclear proteins [5]. Peptide pre-absorption was also conducted as an antibody specificity control using rabbit anti-human-GR total (1:1200) incubated with 10:1 concentration of control peptide (Bethyl Laboratories). Data analysed by Mann Whitney *U* test and Spearman's correlations using SPSS 22.0 for Windows (IBM, Armonk, USA).

3. Results

3.1. Maternal, neonatal and placental characteristics

Maternal plasma cortisol concentrations were decreased in pregnancies complicated by allergic asthma (Table 1). As previously published, fetal weight relative to maternal body weight was decreased in pregnancies complicated by asthma (n = 7) compared to control (n = 5) (p = 0.038). The proportion of Type-B and –C placentomes (%) was higher in pregnancies complicated by asthma compared to control (p = 0.037, p = 0.047, respectively) [16]. Placental volumes were reduced in asthmatic ewes (Table 1).

3.2. GR isoforms expressed in the ovine placenta

Thirteen bands were identified in cytosolic and nuclear protein extractions from sheep placentomes (n = 45) at molecular weights of 40–107 kDa (Fig. 1). GR peptide pre-absorption resulted in the inhibition of all bands specific to GR antibody (data not shown). Nine protein

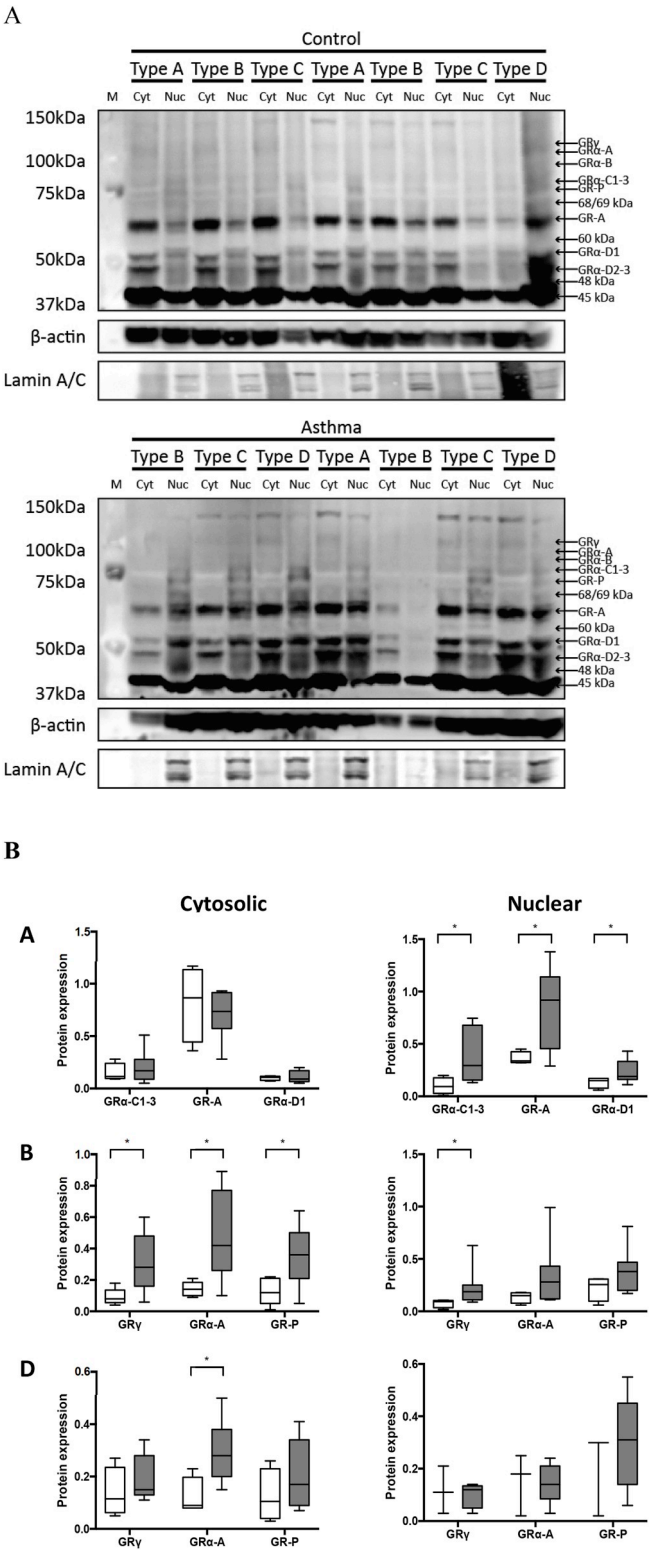

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Fig. 1. A: Sheep placentome glucocorticoid receptor isoforms of control pregnancies and pregnancies complicated by maternal allergic asthma Glucocorticoid receptor isoforms were measured in cytoplasmic and nuclear fractions of whole A, B, C and D type placentomes. Human placental tissue was used as a positive control. Blot is representative is of placental tissues from control (top) and asthmatic (bottom) animals. GR protein bands were normalised to β-Actin and LaminA/C was used to ensure nuclear fraction was specific. **B. Sheep placentome glucocorticoid receptor isoform expression of pregnancies complicated by maternal allergic asthma in placentomes A, B and D.** GR isoforms were significantly altered in A, B and D placentomes. The left panel shows isoforms that changed in the cytosolic fractions and the right panel shows isoforms that changed in the nuclear fractions of whole placentome extracts. Data is expressed as median with 25th-75th quartile range. The white bars are the controls group and the shaded bars are the asthmatic group. Significance was $P < 0.05$.

bands correspond to GRγ (95 kDa), GRα-A (94 kDa), GRβ (91 kDa), GRα-C1-3 (81–83 kDa), GR-P (70 kDa), GR-A (65 kDa) and GRα-D1-3 (50–55 kDa). The 91 kDa band was confirmed to be GRβ using a specific antibody. GR-A, GRα-D2, GRα-D3 and 45 kDa protein were the most abundant isoforms.

3.3. Effect of asthma and placentome type on GR isoform expression

Type-A placentomes from pregnancies complicated by asthma had higher expression of nuclear GRα-C1-3, GR-A and GRα-D1 relative to control (Mann-Whitney *U* Test P = 0.038) (Fig. 1). Type-B placentomes from asthmatic ewes had higher cytosolic and nuclear GRγ expression (P = 0.048, P = 0.024, respectively), higher cytosolic expression of GRα-A (p = 0.03) and GR-P (p = 0.048) (Fig. 1). There was higher expression of some of the unidentified GRs in Type B placentomes of asthmatic ewes relative to control, which included higher 48 kDa (p = 0.018), 45 kDa (p = 0.018) and nuclear expression of 68/69 kDa (p = 0.024) (data not shown). There were no significant differences in GR isoform expression between groups in Type-C placentomes (data not shown). Cytosolic GRα-A (p = 0.024) expression was higher in Type-D placentomes of asthmatic ewes (Fig. 1).

4. Discussion

Multiple isoforms of the GR are expressed in the sheep placenta and their levels were altered by the maternal allergic asthma. GR isoforms were also expressed differently in morphologically classified placentome types. GR expression has been previously reported in multiple organs of the ovine fetus including the placenta [20] but those studies only examined expression of GRα A and GRβ. Our study is the first to show there are 13 GR-immunoreactive proteins and demonstrate that the placental GR profile in the sheep is similar to the placenta of humans [5] and other animals [14,15] suggesting multiple GR isoforms are conserved across species. Multiple GR isoforms, expressed in many different combinations, may ensure only specific placental glucocorticoid-regulated genes are activated or repressed under different physiological conditions such as the presence of maternal asthma.

The low trans-activational GR isoforms; GR P, GRγ and GRα D1, all associated with glucocorticoid resistance in humans [21], and GRα C, known to mediate glucocorticoid-induced apoptosis [21], were localised to sheep placental nuclei suggesting downstream inflammatory pathways and apoptotic mechanisms may predominate in the presence of asthma. It appears that in the presence of maternal asthma, the ovine placenta adapts by reducing its glucocorticoid responsiveness. Previous work in pregnant women have identified sex differences in the placental response to maternal asthma [7] so further studies are required to assess the effect of sex in this experimental model. Our studies establish the value of our sheep model of maternal asthma in pregnancy for understanding the impact of maternal asthma on pregnancy.

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