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

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37

Abstract

38
39 Maternal asthma increases the risk of adverse pregnancy outcomes and may affect fetal growth
40 and placental function by differential effects on the expression of glucocorticoid receptor (GR)
41 isoforms, leading to altered glucocorticoid signalling. Our aim was to examine the effect of
42 maternal asthma on placental GR profiles using a pregnant sheep model of asthma. Nine known GR
43 isoforms were detected. There was a significant increase in the expression of placental GR isoforms
44 that are known to have low trans-activational activity in other species including GR A, GR P and
45 GR γ which may result in a pro-inflammatory environment in the presence of allergic asthma.

Introduction

46 Asthma is an inflammatory disease that complicates 12% of human pregnancies [1] and associated
47 with adverse perinatal outcomes including growth restriction and preterm delivery [2, 3] especially
48 in women with uncontrolled asthma during pregnancy[4]. Changes in fetal growth associated with
49 maternal asthma may be the result of alterations in placental function and structure with specific
50 changes in glucocorticoid regulated pathways mediated by differential expression of placental
51 glucocorticoid receptor (GR) isoforms[5-7] .
52

53
54 The glucocorticoid receptor (GR) is a ubiquitously expressed protein and a member of the nuclear
55 receptor transcription factor super family [8-12]. Multiple GR isoforms are present in the placentae
56 of humans[5, 13], guinea pigs[14] and mice[15] and their abundance varies with glucocorticoid
57 exposure, pregnancy complications, gestational age and fetal sex. The differential expression of GR
58 isoforms may confer differences in placental glucocorticoid sensitivity to ensure fetal survival in an
59 adverse maternal environment. We developed a pregnant sheep model of maternal allergic asthma
60 that causes physiological changes in the maternal lung comparable to human asthma, reduces fetal
61 growth and changes fetal lung development [16]. We aimed to identify whether the sheep placenta
62 expresses multiple GR isoforms and if expression is altered by the presence of maternal allergic,
63 asthma.
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68 Methods

69 Experimental procedures were approved by the Animal Ethics Committees of Monash University
70 (MARF/2013/133) and the University of Adelaide (M-2014-126). The breeding and treatment
71 protocol for allergic, asthmatic (induced by house dust mite) (n = 7) or control (n = 5) singleton
72 bearing Merino ewes (1-2 years of age) are reported in Clifton et al[16]. All placentomes were
73 removed from the endometrium at term delivery, individually weighed and classified for phenotype
74 (Type A, B, C or D)[17]. Frozen whole placentome samples of each phenotype from control and
75 asthmatic sheep were crushed and tissue was fractionated into cytosolic and nuclear components
76 [5]. The volume density of placental tissue was quantified in formalin fixed sections of each
77 phenotype from control and asthmatic sheep[18, 19]. The surface area and barrier thickness were
78 calculated using the weight of the individual placentome or total placental weight. GR isoforms
79 were identified and visualised by Western blot using an affinity purified polyclonal rabbit anti-
80 human total GR antibody (1:1000) (Bethyl Laboratories, Montgomery, TX, USA, A303-491A). The
81 antibody is reactive to sheep antigens (Bethyl Lab website). A specific GR β antibody (1:1000) was
82 also used (Abcam Cambridge, MA, USA) [5]. Densitometry was used to quantify GR expression
83 and normalised against β -actin[5]. Lamin A/C was used to ensure cytosolic fractions were clear of
84 nuclear proteins[5]. Peptide pre-absorption was also conducted as an antibody specificity control
85 using rabbit anti-human-GR total (1:1200) incubated with 10:1 concentration of control peptide
86 (Bethyl Laboratories). Data analysed by Mann Whitney U test and Spearman's correlations using
87 SPSS 22.0 for Windows (IBM, Armonk, USA).

Results

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 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 to 107 kDa (Fig. 1). GR peptide pre-absorption resulted in the inhibition of all bands specific to GR antibody (data not shown). Nine protein bands correspond to GR γ (95 kDa), GR α -A (94 kDa), GR β (91 kDa), GR α -C1-3 (81-83 kDa), GR-P (70 kDa), GR-A (65 kDa) and GR α -D1-3 (50-55 kDa). The 91 kDa band was confirmed to be GR β using a specific antibody. GR-A, GR α -D2, GR α -D3 and 45 kDa protein were the most abundant isoforms.

Effect of asthma and placentome type on GR isoform expression

Type-A placentomes from pregnancies complicated by asthma had higher expression of nuclear GR α -C1-3, GR-A and GR α -D1 relative to control (Mann-Whitney U Test $P = 0.038$) (Fig. 2). Type-B placentomes from asthmatic ewes had higher cytosolic and nuclear GR γ expression ($P = 0.048$, $P = 0.024$, respectively), higher cytosolic expression of GR α -A ($p = 0.03$) and GR-P ($p = 0.048$) (Fig. 2). 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 GR α -A ($p = 0.024$) expression was higher in Type-D placentomes of asthmatic ewes (Fig. 2).

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 GR α A and GR β . 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, GR γ and GR α D1, all associated with glucocorticoid resistance in humans [21], and GR α 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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Figure legend

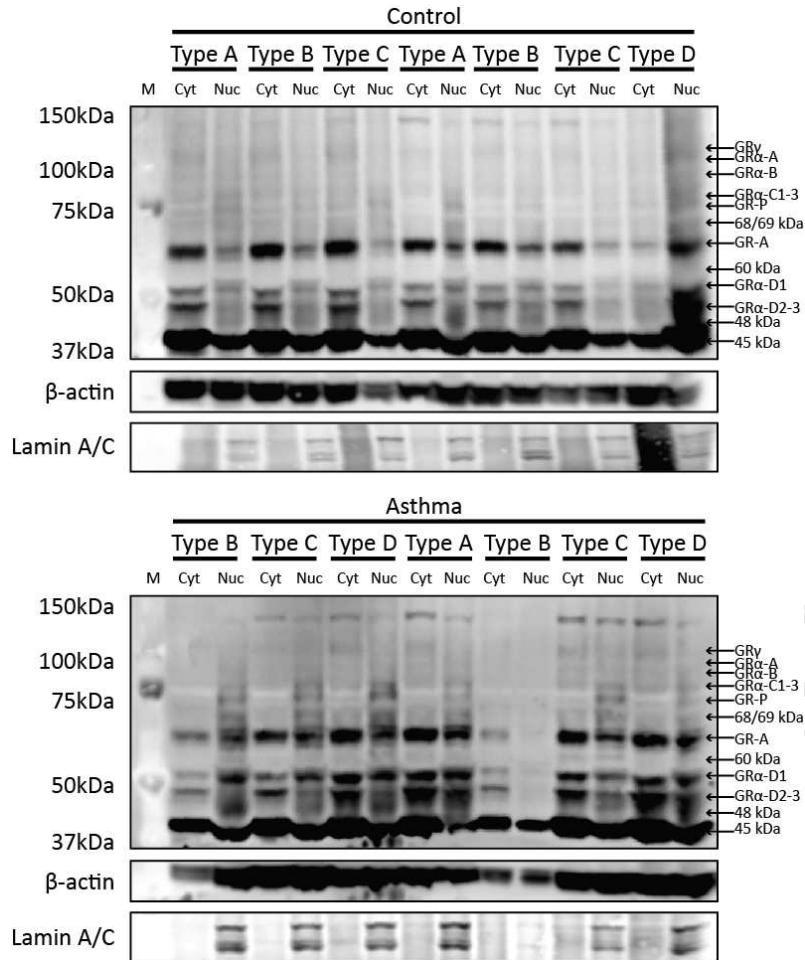
Figure 1: A: Sheep placentome glucocorticoid receptor isoforms of control pregnancies and pregnancies complicated by maternal allergic asthma Glucocorticoid receptor isoforms were measured in cytoplasmic and nuclear fractions of whole A, B, C and D type placentomes. Human placental tissue was used as 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 $P < 0.05$

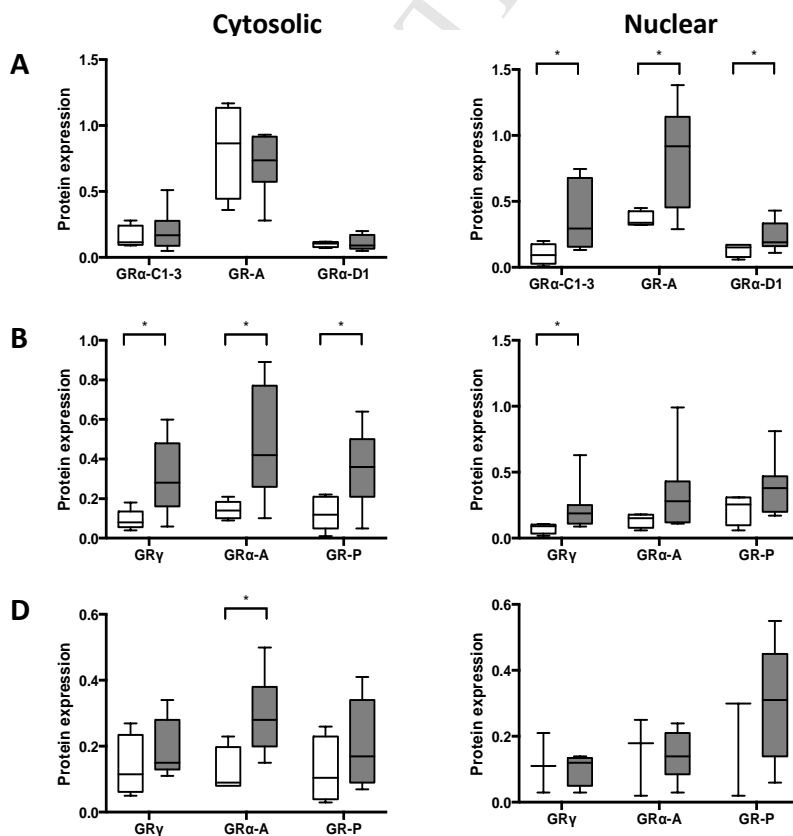
	Control	Asthma	p
n	5	7	
Maternal Characteristics			
Post mortem weight (kg)	38.86 ± 2.07	39.60 ± 1.41	n.s.
Gestational age (days)	140.00 ± 1.10	139.57 ± 0.53	n.s.
Neonatal Characteristics			
Body weight (kg)	4.11 ± 0.28	3.69 ± 0.14	n.s.
Fetal: Maternal weight	0.11 ± 0.01	0.09 ± 0.00	0.038
Placenta characteristics			
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 (cm ³)	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 (cm ³)	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 (cm ³)	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 (cm ³)	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 (cm ³)	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 (cm ³)	80.327 ± 3.483	65.751 ± 2.208	0.0013

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

A



B



Highlights

Sheep placenta contains 9 different glucocorticoid receptor (GR) isoforms

GR isoform expression varies in relation to placentome type

Low transactivational GR isoforms predominate in the presence of a sheep model of maternal allergic asthma

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COI statement

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There are no conflicts of interest to declare

Yours sincerely

Professor Vicki Clifton