

## SHORT COMMUNICATION

## Increased mRNA expression of glucocorticoid receptor-P in placenta is associated with a decreased risk of allergen sensitisation in the child

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Foetal programming is an emerging concept that links environmental conditions during foetal development with a variety of adult diseases, including obesity, hypertension and type 2 diabetes (1). The scope of this hypothesis has been broadened in recent years to include the theory that foetal exposure to maternal stress can influence the development of diseases originating in childhood, such as allergies (2). The glucocorticoid and stress hormone cortisol generally increases in the maternal circulation during pregnancy, and it plays an important role in foetal growth and organ maturation (3). In the placenta, the actions of cortisol are mediated by the different glucocorticoid receptor (GR) variants (4), while the enzyme 11 $\beta$ -hydroxysteroid dehydrogenase type 2 (HSD11B2) converts active glucocorticoids (cortisol) into inactive glucocorticoids (cortisone), thus protecting the foetus from excessive glucocorticoid exposure (5). As the cortisol levels are comparably much higher in the mother than in the foetus, even small changes in the placental activity of HSD11B2 may significantly change the foetal exposure to cortisol (5). All different GR variants have been detected on the messenger ribonucleic acid (mRNA) level in term placentas (4), and 12 protein isoforms were recently described in human placentas (6). Some of the GR differ in expression according to foetal sex (4,6,7). GR $\alpha$  is the most active isoform, whereas GR $\beta$  is considered a negative regulator of the receptor and little is known about the function of GR-P (8).

We recently showed that increased salivary cortisol levels at 6 months of age were associated with the risk of allergen sensitisation and eczema during the first 2 years of life (9).

In this study, we investigated if placental expression of one or more of the GRs and/or HSD11B2 would contribute to a programming effect, resulting in the subsequent development of allergy in the child.

Placentas from the Assessment of Lifestyle and Allergic Disease During Infancy (ALADDIN) prospective birth cohort (10) were investigated for the mRNA expression of GR $\alpha$ , GR $\beta$ , GR-P and the HSD11B2 enzyme in relation to allergen sensitisation in children at 24 months of age. Because of the effect of sex on placental GR expression (4,6,7), only placentas from women pregnant with a female foetus in the ALADDIN study (10) were included. All children were born at term with a weight that was appropriate for their gestational age and had been tested for allergen sensitisation at 24 months of age, as detailed below. None of their mothers were using any medications containing glucocorticoid during pregnancy. In addition, the placentas were selected from mothers whose saliva cortisol levels were known, measured in saliva samples collected over the course of a day – morning, afternoon and evening – when their child was six months old and then again when they were 12 months old (11). We created a composite rank measure of the six saliva samples and selected mothers with low, medium and high cortisol values. The rationale behind this was that we wanted to increase the contrast in cortisol exposure from the mothers. Based on all these inclusion criteria, 29 placentas and children were included. The study was approved by the Research Ethical Committee at Huddinge University Hospital, Stockholm, Sweden, and the parents gave their written informed consent.

Plasma from the children collected at 24 months of age was analysed by ImmunoCAP<sup>®</sup> (Thermo Fischer Scientific, Uppsala, Sweden) for seven allergens: hen's eggs, cow's milk, peanuts, cats, dogs, birch and timothy. In addition, a skin prick test was performed at 24 months of age using four allergens: hen's eggs, cow's milk, cats and birch (ALK, Hørsholm, Denmark). A child was classified as sensitised if at least one of the seven immunoglobulin E (IgE) levels was  $\geq 0.35$  kU<sub>A</sub>/L and, or, if at least one of the four allergens in the skin prick test gave rise to a wheel of flare bigger than the control. Parental blood samples were collected during the third trimester of pregnancy, and allergen sensitisation was defined by ImmunoCAP Phadiatop<sup>®</sup> (Thermo Fischer Scientific) with an allergen-specific plasma IgE measurement  $\geq 0.35$  kU<sub>A</sub>/L.

Total RNA was isolated from both the foetal and maternal side of the placenta from biopsies collected and placed into RNeasy (Qiagen; Applied Biosystems, Foster City, CA, USA) immediately after birth by the midwives. RNA isolation and complementary deoxyribonucleic acid (cDNA) synthesis were performed under coded conditions as previously described (12). The reaction was performed in a Rotor-Gene Q (Qiagen, Hilden, Germany) using Rotor-Gene SybrGreen (Qiagen). Primers for the genes of interest were as follows: GR $\alpha$ : Fw-CAAACTCTTGGATTCTA TGCATGAA, Rv-ATTTTCAG CTAACATCTCGGGGAAT, efficiency 1.95. GR $\beta$ : Fw-AGCACATCTCACACATTAATC, Rv-CTATAGTTGTTCGATGAGCATC, efficiency 1.81. GR-P: Fw-TGTTTTGCTCCTGATC TGA, Rv-CCTTTGTTTCTAGG CCTTC (7), efficiency 1.94. 11 $\beta$ HSD2: Fw-ATTAGCCGC GTGCTAGAGTTC, Rv-CCGCATCAGCAACTACTTCAT, efficiency 1.77. Primers for the reference genes were bought as QuantiTect Primer Assay (Qiagen) with the following efficiencies GAPDH 2.00, EEF1A1 1.94 and YWHAZ 1.85. The relative gene expression (R) was calculated using the mean of duplicate measurements and the Pfaffl method (13). Gene expression levels were normalised using the geometric mean of three reference genes (GAPDH, EEF1A1 and YWHAZ) as a reference (14). Relative expression levels were not normally distributed, and nonparametric statistics were used as indicated. Exact logistic regression was performed using STATA 11.  $P \leq 0.05$  was accepted as being significant.

Of the 29 children, eight were allergen sensitised and 21 nonsensitised at 24 months of age. In the group of eight sensitised children, one father was allergen sensitised. In the group of 21 nonsensitised children, six mothers and nine fathers were allergen sensitised. We analysed whether GR $\alpha$ , GR $\beta$ , GR-P or HSD11B2 expression at both the maternal and foetal side of the placenta was associated with the development of sensitisation at 24 months using exact logistic regression. This revealed a significant association (OR = 0.04,  $P = 0.02$  and CI 0.0009–0.678) between the mRNA expression of GR-P at the maternal side of the placenta and the outcome of allergen sensitisation in the child at 24 months of age. See also Table 1 for odds ratios,  $P$  values and confidence intervals for all genes. When adding maternal or paternal allergen sensitisation as covariates to the exact logistic regression, no

**Table 1** The association between gene expression in the placenta and the development of allergen sensitisation in the child at 24 months of age, analysed by means of exact logistic regression

	OR	$P$ -value	95% Conf. interval
GR $\alpha$ maternal ( $n = 29$ )	0.50	0.44	0.063–1.824
GR $\alpha$ foetal ( $n = 29$ )	0.38	0.33	0.043–2.058
GR $\beta$ maternal ( $n = 29$ )	0.33	0.21	0.046–1.653
GR $\beta$ foetal ( $n = 29$ )	2.46	0.16	0.731–10.907
GR-P maternal ( $n = 29$ )	0.04	0.02	0.0009–0.678
GR-P foetal ( $n = 29$ )	1.15	0.82	0.265–4.564
HSD11B2 maternal ( $n = 29$ )	0.52	0.22	0.168–1.441
HSD11B2 foetal ( $n = 28$ )	0.79	0.72	0.242–2.195

substantial effects were seen on the odds ratios (data not shown).

It is known that early life events can affect the development of allergy later in life. High cortisol levels during infancy seem to be more common in children at risk of allergy (15). A previous study from the ALADDIN programme showed an association between increased levels of salivary cortisol at 6 months of age and the risk of allergen sensitisation and eczema during the first 2 years of life (9). This suggests that hypothalamic–pituitary–adrenal (HPA) axis related events or changes may have a role in the development of allergic disease. In this study, we examined, at an even earlier time point, if the placental gene expression of the glucocorticoid receptors and, or, the HSD11B2 enzyme might have had an effect on the later development of allergy in the child. Our data suggest that for GR-P, the risk of developing allergen sensitisation was significantly decreased with increased mRNA expression at the maternal side of the placenta. This may indicate that an increased expression of GR-P may protect the foetus from elevated cortisol levels and thereby decrease the risk of allergy development during infancy.

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**CONFLICT OF INTEREST**

None of the authors have any conflict of interests to disclose.

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