# Expression of Glucocorticoid Receptor Messenger Ribonucleic Acid Transcripts in the Human Placenta at Term

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**Context:** Differential promoter use and alternative splicing generate a variety of glucocorticoid receptor (GR) mRNA transcripts, potentially altering the cortisol responsiveness of gestational tissues during pregnancy and labor.

**Objective:** We examined GR mRNA transcript expression in term placentae before and after labor, in association with fetal sex and after glucocorticoid treatment.

**Design:** RNA from 34 placentae and from eight placental explants incubated with glucocorticoids were analyzed for the GR mRNA variants GR-$\alpha$, GR-$\beta$, GR-P, and GR-$\gamma$ and the untranslated exon one variants 1A1, 1A2, 1A3, 1B, and 1C by quantitative RT-PCR.

**Main Outcome Measure:** mRNA expression was assessed.

**Results:** All GR mRNA variants examined were detected in the human placenta, with GR-$\alpha$ and GR-1C mRNA having the highest expression of GR splice variants and exon 1 variants, respectively. GR-P mRNA abundance decreased with spontaneous labor ($P < 0.01$). GR-1A3 mRNA abundance changed with fetal sex, with a higher level in placentae of male fetuses ($P < 0.05$). GR-1C was the preferential promoter for GR-$\alpha$, GR-$\gamma$, and GR-P mRNA. GR-$\beta$ mRNA was preferentially associated with GR-1A1. GR-P mRNA transcription switched to the GR-1A1 promoter after labor and to the GR-1A3 promoter in placentae from male fetuses. Glucocorticoid treatment significantly reduced transcription from promoters GR-1B and -1C and decreased GR-$\alpha$ and GR-P mRNA abundance.

**Conclusions:** The human placenta expresses a variety of GR mRNA transcripts. GR-$\alpha$ mRNA transcribed from the 1C promoter generates the majority of placental GR. However, alterations in promoter use and alternative splicing may modulate responses to cortisol during stressful events. **(*J Clin Endocrinol Metab* 93: 4887–4893, 2008)**

The glucocorticoid (GC) cortisol is produced from the adrenal cortex in response to ACTH and acts widely throughout the body. It is a key modulator of metabolic pathways, the stress response, and the immune and inflammatory pathways and is involved in fetal development. In the context of pregnancy, cortisol has a variety of roles including development and maturation of fetal organ systems such as the lungs, brain, and liver (1). Endogenous cortisol acts to promote fetal lung maturity before delivery (2, 3), and GCs are also known to regulate the production of hormones associated with the onset of labor including CRH and prostaglandins (4–6).

The actions of GCs are mediated by the GC receptor (GR). The GR is a member of the nuclear receptor subfamily 3 group C and is expressed in multiple cells of the body. It acts as a

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Abbreviations: GC, Glucocorticoid; GR, GC receptor; hnRNA, heterogeneous nuclear RNA.

transcription factor by binding to GC response elements in the promoter region of target genes and can also function as a *trans*-repressor of other transcription factors, such as nuclear factor-κB (7).

There are currently four known forms of the mature GR mRNA. These are GR-α, GR-β, GR-P (or GR-δ), and GR-γ. Alternative splicing of exons 9α or 9β gives rise to GR-α and GR-β mRNA, respectively (8). GR-P is a truncated version of the GR mRNA with intron G retained in the place of exons 8 and 9, and GR-γ is generated by the retention of three bases from intron C coding for an extra amino acid residue, arginine, between the third and fourth exons (9, 10). An additional five mRNA splice variants are produced by the actions of multiple promoters linked to untranslated exon 1 regions, including GR-1A1, GR-1A2, GR-1A3, GR-1B, and GR-1C (11, 12). Recently, Turner and Muller (13) have described an additional five promoters giving rise to another eight exon 1 regions, including two previously undescribed variants for the GR-1C promoter region. Similarly, Presul *et al.* (14) have also described an additional two alternative first exons, GR-1J and GR-J. Expression of the various mRNA splice variants and ultimately the mature GR mRNAs and protein mediate the response of different tissue systems to GCs.

The different GC splice variants may have different biological effects, and the different promoters may respond differently to physiological stimuli. Studies of differential GR promoter use in response to steroid-mediated apoptosis indicate that expression of GR-1A transcripts, in particular GR-1A3, is controlled by GC treatment in CEM-C7 and IM-9 cells (11, 15). Pedersen and Vedeckis (15) also suggest that GR-1A3 transcript expression may be involved in up-regulation of GR protein levels that control responsiveness to GCs in CEM-C7 cells. Furthermore, studies into hematological malignancies have implied that GR-P may reduce responsiveness to GCs (9). de Lange *et al.* (16) identified high levels of GR-P in GC-responsive as well as in GC-resistant cell lines. Additionally, they showed that transfection of a GR-P-containing plasmid into a number of cell lines, including HeLa and COS-1, enhanced the ability of a GR-α plasmid to activate an mouse mammary tumor virus luciferase reporter after dexamethasone treatment (16). This effect was cell line dependent with CHO cells showing a decrease in luciferase reporter expression after dexamethasone treatment when cotransfected with a GR-P plasmid (16). These data suggest possible roles for differential GR promoter use and splice variants controlling the response to GC in different cells systems.

In the context of pregnancy, multiple studies have detected GR mRNA and protein in the placenta. GR mRNA has been shown to be expressed in primary cultures of human cytotrophoblast cells (17), whereas Lee *et al.* (18) localized GR to villous fibroblast with low levels of expression in cytotrophoblasts. Johnstone *et al.* (19) found that GR-α protein was present in placenta from women delivering preterm, but its level was not altered by the presence of infection.

In both the porcine placenta (20) and the ovine placenta (21), GR-α mRNA levels increase during gestation, whereas in the rat, GR-α mRNA levels remained stable throughout gestation (22). These studies focused primarily on the mature mRNA splice variants, GR-α and GR-β. There are few data on the expression of the multiple GR mRNA variants in the human placenta, and there are no studies exploring differential promoter use as a potential mechanism influencing GR mature mRNA levels and splice variant distribution.

Given the data from cell lines showing the role of GR mRNA variants in the cellular response to GCs as well as the known role of cortisol in maturation fetal organ systems (2, 3) and regulation of placental hormone systems, alterations in GR promoter use and differential splicing may have a crucial impact on GC action in the placenta and the fetus. For example, we have shown that male and female fetuses exhibit altered responses to inflammatory stress, such as maternal asthma, resulting in fetal sex-associated differences in fetal growth and placental metabolism of cortisol (23).

The stress response, fetal growth, and placental cortisol metabolism are all influenced by GCs; it is therefore possible that the differential effects of stress are due at least partially to differences in GR gene expression in male and female fetuses. In this investigation we have determined the use of the different GR gene promoters in the placenta in relation to the mode of delivery and fetal sex to explore regulation at this level. We have also determined the distribution of the mature GR mRNA splice variants (GRα, -β, -P, and -γ) to assess labor-associated changes and the impact of fetal sex on GR mRNA isoform expression. The effects of GCs on GR promoter use, transcript variant distribution, and GR mRNA levels were determined in explant incubation experiments.

## Patients and Methods

### Patients and tissues

Placentae were obtained from 34 singleton pregnancies after spontaneous labor and vaginal delivery at term (n = 18), elective term cesarean section in the absence of labor (n = 10), and after vaginal delivery after induction at term (n = 6) at the John Hunter Hospital (Newcastle, New South Wales, Australia). All women included in the study were healthy and had no known defects in steroid synthesis or metabolism. Informed voluntary written consent was obtained from all participating women, and the collection and use of these tissues had been approved by the Hunter Area Health Service and the University of Newcastle Human Ethics Committees.

### Tissue collection and preparation of total RNA

Placental samples were collected from multiple cotyledons, blotted to remove blood, snap frozen in liquid nitrogen, and stored at −80 C.

Total RNA was extracted from 0.2 g crushed frozen placenta using TRIzol reagent (Invitrogen, Mt. Waverley, Australia) according to the manufacturer's protocol. After extraction, the RNA was purified and deoxyribonuclease-treated using QIAGEN Mini-Spin columns and the QIAGEN ribonuclease-free deoxyribonuclease kit (QIAGEN, Clifton Hill, Australia) according to the manufacturer's protocol. The integrity of purified RNA was confirmed by visualization of the 18S and 28S rRNAs after agarose gel electrophoresis.

### Quantitative real-time RT-PCR

Three micrograms of purified total RNA was reverse transcribed using the Invitrogen (Carlsbad, CA) first-strand synthesis kit for RT-PCR with random hexamers as primers, according to the manufacturer's in-

**TABLE 1.** Primer sequences for the GR

| Primer | Forward primer | Reverse primer | Concentration (nM) |
|---|---|---|---|
| GR-1A1 | CCAAATCACTGGACCTTAGAAGTTG | GGAGTTAATGATTCTTTGGAGTCCAT | 800 |
| GR-1A2 | TCCAACGGAAGCACTGGG | CATCAGTGAATATCAACCTCTTTCTGTT | 400 |
| GR-1A3 | CATTAAAGTGTCTGAGAAGGAAGTTGAT | TACCAGGAGTTAATGATTCTTTGGAGT | 800 |
| GR-1B | CCCGGGCCCAAATTGA | GGAGTTAATGATTCTTTGGAGTCCAT | 800 |
| GR-1C | TTGTTTATCTCGGCTGCGG | CCATCAGTGAATATCAACTCTGGC | 200 |
| GR-$\alpha$ | CTTGGATTCTATGCATGAAGTGGTT | TTGGAAGCAATAGTTAAGGAGATTTTC | 800 |
| GR-$\beta$ | ACTCTTGGATTCTATGCATGAAAATG | TGTGTGAGATGTGCTTTCTGGTTT | 200 |
| GR-$\gamma$ | TTCAAAAGAGCAGTGGAAGGTAGA | TTTATCGATGATGCAATCATTCCT | 400 |
| GR-P | TTACTGCTTCTCTCTTCAGGTTGGT | CATGCTTTTGACATAAGGTGAAAAG | 800 |
| GR hnRNA | CAAGTGATTGCAGCAGTGAAATG | TTTATGTTTTGCATCTTACCTGGTATTG | 400 |

structions. Primers for the quantitative real-time PCR were designed using the Primer Express version 1.0 computer software (PE Biosystems, Branchburg, NJ). The primer sequences for the GR variants are shown in Table 1. For each primer pair, optimal primer concentrations and template amounts required to produce constant and equal amplification and efficiency of the target and reference sequences were determined in preliminary experiments. Real-time PCR was performed using an ABI Prism 7700 sequence detector (Applied Biosystems, Warrington, UK). Each mRNA sequence was amplified in triplicate in 25-$\mu$l reactions containing SYBR green PCR master mix (Applied Biosystems), cDNA corresponding to 40 ng reverse-transcribed RNA, and forward and reverse primers at concentrations between 200 and 800 nM depending on the optimal primer concentration (Table 1). The placement of primers for the GR mRNA variants can be seen in supplemental Fig. 1 (published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org). Primers for the housekeeping gene 18S rRNA were used at 10 nM, and primer sequences were as described (24). Controls (no template and no reverse transcriptase) were included for each amplification reaction, and the homogeneity of the amplified products was confirmed routinely by melting-curve analysis and agarose gel electrophoresis.

### Tissue incubations

An additional eight placentae were collected, and the effect of GC treatment on the expression of the GR was determined in 48-h tissue incubations. After delivery, the placenta was collected and samples were taken from multiple cotyledons, washed with PBS, and separated into 0.5-g lots. Each sample was incubated in DMEM containing antibiotics at 37 C for 24 h. After 24 h incubation, samples were treated with either DMEM alone or DMEM containing 100 nM cortisol or 100 nM dexamethasone for an additional 24 h. After the incubation, the samples were blotted and snap frozen in liquid nitrogen. Total RNA was extracted from the samples as described above, and expression of GR mRNA variants was determined using quantitative real-time PCR.

### Data analysis

The abundance of the target sequences was calculated relative to 18S rRNA using the following formula: relative abundance $= 2^{-\Delta CT}$, where $\Delta C_T$ is calculated as the difference between the $C_T$ (threshold cycle) of the test sequences and of the reference 18S rRNA sequence. Comparisons between groups were performed using the Student's $t$ test with a Welch correction. Where multiple groups were compared, data were analyzed using ANOVA followed by the Tukey-Kramer multiple comparisons test if a significant effect was found. Relationships have been determined by robust regression. In all statistical tests, $P < 0.05$ was considered significant.

## Results

### Alternate promoter use and GR transcript expression in the placenta

The use of alternate GR promoters in the human placenta was examined by quantitative real-time RT-PCR. mRNA for all five untranslated exon 1 regions examined was detected in the human placenta with varying levels of expression (Table 2). The highest expression was observed for exon 1C with the lowest expression observed for exon 1A1, which was detectable in only 25 of the 34 placentae examined.

Similarly, the abundance of the GR mRNA transcripts produced by alternative splicing was determined in the placenta. All currently known mRNA splice variants of the GR, GR-$\alpha$, GR-$\beta$, GR-$\gamma$, and GR-P were detected in the human placenta (Table 2). Levels of GR-$\alpha$ mRNA were the highest with the level of GR-$\beta$ mRNA the lowest expressed. Of note, the presence of GR-P mRNA was detected in only 22 of the 34 placentae.

### Influence of mode of delivery on GR promoter use and expression of GR mRNA

The influence of mode of delivery on GR mRNA abundance was determined by quantitative real-time RT-PCR. There was a significantly lower abundance of GR-P mRNA level in placentae of women who delivered after spontaneous labor at term [Fig. 1; $F_{(2,31)} = 5.307; P < 0.01$] compared with women delivering after

**TABLE 2.** Average relative abundance of the placental GR mRNA transcripts

| | Average abundance[a] | SD | % Detectable[b] (%) |
|---|---|---|---|
| GR-1A1 | 0.0002 | 0.0006 | 74 |
| GR-1A2 | 0.0006 | 0.0014 | 100 |
| GR-1A3 | 0.0058 | 0.0045 | 100 |
| GR-1B | 1.3652 | 0.8501 | 100 |
| GR-1C | 5.1247 | 2.7970 | 100 |
| GR-$\alpha$ | 4.0620 | 2.1473 | 100 |
| GR-$\beta$ | 0.0001 | 0.0001 | 100 |
| GR-$\gamma$ | 0.3072 | 0.1959 | 100 |
| GR-P | 0.2218 | 0.2675 | 65 |
| hnRNA | 0.2476 | 0.1384 | 100 |

[a] Average abundance relative to 18S rRNA.

[b] Percentage detectable in tissues from a total number of 34 samples.

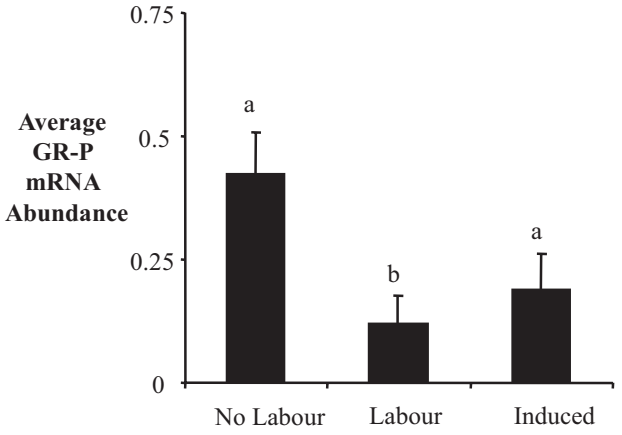


**FIG. 1.** Influence of mode of delivery on GR-P mRNA levels. GR-P mRNA levels were measured in placenta by quantitative real-time RT-PCR after spontaneous vaginal delivery (n = 18), induced vaginal delivery (n = 6), and elective cesarean section (n = 10). There was a significant difference between GR-P mRNA levels between the groups, with a lower abundance of GR-P after spontaneous vaginal delivery compared with induced delivery or cesarean section. Significance of a *vs.* b was $P < 0.01$ by ANOVA.

induction of labor or after cesarean section. However, there was no other effect of mode of delivery on GR promoter use or on other transcript expression.

### Influence of fetal sex on GR promoter use and expression of GR mRNA transcripts

We examined the influence of fetal sex on GR promoter use and expression of GR mRNA transcripts in the human placenta from 17 women delivering a male and 17 women delivering a female fetus. Of all the GR mRNA transcripts examined, only the untranslated exon 1A3 region showed any significant difference with fetal sex. There was a significantly lower GR-1A3 transcript level in women delivering a female fetus when compared with women delivering a male fetus (Fig. 2; $t = 2.127$; $df = 31$; $P < 0.05$).

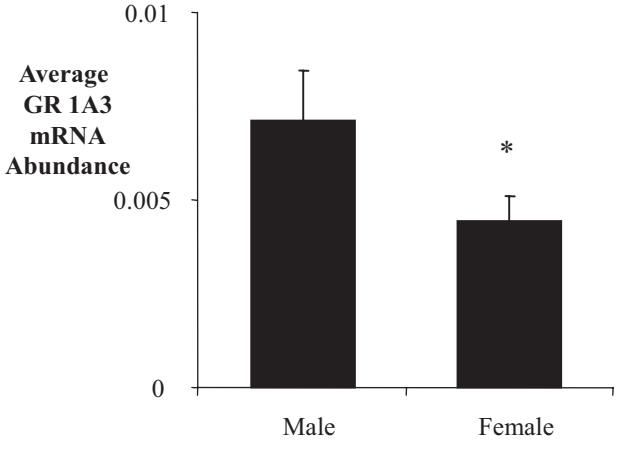


**FIG. 2.** Influence of fetal sex on GR-1A3 mRNA levels. GR-1A3 mRNA levels were measured in placenta by quantitative real-time RT-PCR from women carrying either a female (n = 17) or male (n = 17) fetus. There was a significant difference between GR-1A3 mRNA levels in placenta from women carrying a female fetus compared with a male fetus. Significance was determined as $P < 0.05$ by *t* test.

### Relationship between GR gene activity, promoter use, and mRNA transcript expression

The relationship between GR gene activity, as indicated by GR heterogeneous nuclear RNA (hnRNA) expression, and GR promoter use, indicated by the GR exon 1 transcripts, was analyzed by robust regression. As seen in Table 3, there was a significant regression between all of the GR exon 1 transcripts and GR hnRNA, with the exception of GR-1A2, suggesting that the GR promoters tested were active. The strongest regression was observed between GR hnRNA and the GR-1C transcript followed by the GR-1B transcript, suggesting that these are the most active promoters. We also examined the relationship between GR promoter use and the mature GR mRNA splice variants using robust regression. The regression analysis suggested that the GR-$\alpha$, GR-P, and GR-$\gamma$ mRNA variants were strongly related to GR-1C transcript expression followed closely by the GR-1B transcript (Table 3). The GR-$\beta$ mRNA variant was most strongly related to the GR-1A1 transcript (Table 3). There was no difference in the relationship between GR hnRNA and the GR exon 1 transcripts with mode of delivery or fetal sex. Similarly, there was no difference in the relationships between GR-$\alpha$ or GR-$\gamma$ and the exon 1 transcripts with mode of delivery or fetal sex. However, for the GR-P mRNA transcript in the spontaneous labor group, the strongest regression was observed between GR-P and GR-1A1 [$F_{(1,4)} = 18.28$; $P < 0.02$] rather than between GR-P and GR-1C [$F_{(1,6)} = 0.00$, $P = 0.9983$]. Similarly in women delivering a male the strongest relationship was observed between GR-P and GR-1A3 [$F_{(1,7)} = 19.33$; $P < 0.01$] rather than between GR-P and GR-1C [$F_{(1,7)} = 4.37$; $P = 0.07$].

### Effect of GC treatment on GR promoter use and expression of GR mRNA transcripts

The effect of cortisol and dexamethasone treatment on GR mRNA transcript expression was examined *in vitro*. RNA from eight placentae treated with DMEM, 100 nM cortisol, or 100 nM dexamethasone for 24 h were collected and examined for GR promoter sequences or GR mRNA transcript expression. There was a significant alteration in GR promoter use and GR transcript expression in placentae after treatment with dexamethasone compared with DMEM alone (Table 4). Specifically, there was a significant decrease in mRNA levels for GR-$\alpha$ and GR-P after treatment with dexamethasone. Furthermore, GR-P mRNA levels were significantly lower in placentae treated with dexamethasone compared with placentae treated with cortisol (Table 4). Moreover, GR-1B promoter-derived transcript levels decreased significantly and GR-1C derived transcript levels decreased nearly significantly ($P = 0.05$) in response to GC treatment (Table 4).

## Discussion

The placenta is exposed to increasing amounts of cortisol as pregnancy progresses, and the effect of this cortisol is primarily mediated through the GR. However, there are currently no data concerning the expression of different GR promoters or mRNA splice variants in placenta. Our study currently represents the

**TABLE 3.** Analysis of relationship between GR promoter use and GR mRNA splice variant expression in the placenta

| | GR hnRNA | GR-α | GR-β | GR-γ | GR-P |
|---|---|---|---|---|---|
| GR-1A1 | $F_{(1,32)} = 18.41$; $P < 0.001$ | $F_{(1,31)} = 1.42$; $P = 0.243$ | $F_{(1,31)} = 44.13$; $P < 0.001$ | $F_{(1,31)} = 0.21$; $P = 0.651$ | $F_{(1,16)} = 35.32$; $P < 0.001$ |
| GR-1A2 | $F_{(1,31)} = 2.60$; $P = 0.117$ | $F_{(1,31)} = 2.56$; $P = 0.120$ | $F_{(1,31)} = 40.28$; $P < 0.001$ | $F_{(1,31)} = 0.08$; $P = 0.775$ | $F_{(1,21)} = 16.43$; $P < 0.001$ |
| GR-1A3 | $F_{(1,31)} = 38.46$; $P < 0.001$ | $F_{(1,32)} = 23.55$; $P < 0.001$ | $F_{(1,32)} = 7.05$; $P < 0.05$ | $F_{(1,32)} = 22.19$; $P < 0.001$ | $F_{(1,21)} = 37.81$; $P < 0.001$ |
| GR-1B | $F_{(1,32)} = 118.42$; $P < 0.001$ | $F_{(1,31)} = 180.58$; $P < 0.001$ | $F_{(1,32)} = 8.57$; $P < 0.01$ | $F_{(1,32)} = 115.81$; $P < 0.001$ | $F_{(1,22)} = 43.21$; $P < 0.001$ |
| GR-1C | $F_{(1,32)} = 195.63$; $P < 0.001$ | $F_{(1,32)} = 601.06$; $P < 0.001$ | $F_{(1,32)} = 13.43$; $P < 0.001$ | $F_{(1,32)} = 160.32$; $P < 0.001$ | $F_{(1,22)} = 83.80$; $P < 0.001$ |

most complete picture of GR transcript expression in the human placenta at term and examines differences in GR mRNA transcripts and differential promoter use with mode of delivery and fetal sex.

All of the GR mRNA mature and untranslated exon 1 splice variants examined were detected in the human placenta, although levels of expression of each of the different transcripts differed markedly. Of the mature mRNA variants, GR-$\alpha$ mRNA was detected at the highest level with very low expression of GR-$\beta$. Only GR-P mRNA levels were shown to change significantly with a reduction in GR-P after spontaneous labor (Fig. 1). This result is interesting because studies in hematological malignancies, in particular multiple myeloma, suggest that GR-P may be associated with GR resistance (9). Recent studies by de Lange *et al.* (16), however, have suggested that the role of GR-P may be more complex and may involve up-regulation of GR-$\alpha$ activity in response to GC treatment. It is important to note that this effect is seen only in certain cell types and has not been tested in the human placenta. However, if GR-P in the placenta acted to increase GR-$\alpha$ activity in the presence of GCs, the concentration of which increase with gestation, such a reduction in GR-P mRNA may reduce responsiveness of the placenta to GCs and thus relieve the GR repression of cytokine production (25) and promote synthesis of regulatory factors such as prostaglandins.

All of the untranslated exon 1 GR mRNA splice variants examined in the study were observed in the human placenta. We examined the major exon 1 mRNA transcripts, 1A1, A2, A3, 1B, and 1C, because these are the most well characterized and data from previous studies suggest that these exon 1 mRNA transcripts may play a role in responsiveness of cells and tissues to GCs (11, 15). Recent studies have identified a number of additional exon 1 mRNA transcripts (13), but there are currently no data on their possible functions. Overall, our data indicate that the three alternative promoters, 1A, 1B, and 1C, are all active in the human placenta. Products from the GR-1C and -1B promoters were the most highly expressed. The GR-1A3 transcript was the most highly expressed from the 1A promoter, and it was the only difference in transcript expression seen between women carrying a male or female fetus (Fig. 2). The GR-1A3 transcript has been previously shown to be up- or down-regulated by GC depending on the cell line studied (11, 15), and it has been suggested that the GR-1A3 transcript may up-regulate GR protein levels to control responsiveness to GCs (15). Previous data from our group have provided evidence for altered male and female fetal growth in response to stress produced by untreated maternal asthma, due in part to alterations in placental metabolism of cortisol (23). Differences in the abundance of the GR-1A3 mRNA transcript may contribute to altered responsiveness to placental cortisol.

To determine how changes in the abundance of the GR mRNA transcripts may be controlled in the placenta, we examined the relationship between GR promoter use, GR hnRNA, and GR mRNA abundance. Consistent with the overall mRNA abundance levels, our regression data suggested that the GR-1C and -1B promoters are the most active and are the main contributors to the expression of the GR transcripts GR-$\alpha$, GR-$\gamma$, and GR-P (Table 3). This is consistent with data obtained by Russcher

**TABLE 4.** Effect of cortisol and dexamethasone treatment on GR mRNA expression in the placenta

| | DMEM | Cortisol | Dexamethasone | *P* value |
|---|---|---|---|---|
| GR-$\alpha$ | 5.35 ± 2.90 | 1.42 ± 0.74 | 0.74 ± 0.53 | $F_{(2,19)} = 4.378$; $P < 0.05$[a] |
| GR-$\beta$ | 4.45 ± 1.97 ($\times 10^{-5}$) | 1.85 ± 1.24 ($\times 10^{-5}$) | 0.37 ± 0.16 ($\times 10^{-5}$) | $F_{(2,19)} = 2.557$; $P = 0.104$ |
| GR-$\gamma$ | 0.30 ± 0.15 | 0.11 ± 0.06 | 0.04 ± 0.02 | $F_{(2,19)} = 2.470$; $P = 0.114$ |
| GR-P | 0.05 ± 0.02 | 0.03 ± 0.02 | 0.01 ± 0.01 | $F_{(2,19)} = 5.567$; $P < 0.02$[b] |
| GR-1A1 | 2.24 ± 0.98 ($\times 10^{-5}$) | 10.90 ± 8.16 ($\times 10^{-5}$) | 0.73 ± 0.20 ($\times 10^{-5}$) | $F_{(2,19)} = 0.729$; $P = 0.496$ |
| GR-1A2 | 0.004 ± 0.002 | 0.013 ± 0.009 | 0.002 ± 0.001 | $F_{(2,19)} = 0.637$; $P = 0.539$ |
| GR-1A3 | 0.013 ± 0.009 | 0.006 ± 0.004 | 0.004 ± 0.003 | $F_{(2,19)} = 3.003$; $P = 0.074$ |
| GR-1B | 1.24 ± 0.73 | 0.48 ± 0.32 | 0.14 ± 0.08 | $F_{(2,19)} = 4.343$; $P < 0.05$[a] |
| GR-1C | 2.51 ± 1.20 | 1.18 ± 0.63 | 0.53 ± 0.34 | $F_{(2,19)} = 3.521$; $P = 0.05$ |

Data are shown as average ± SE.

[a] Significant difference only between DMEM and dexamethasone.

[b] Significant difference between DMEM and dexamethasone and between cortisol and dexamethasone.

*et al.* (26) who showed that GR-$\alpha$ was preferentially produced through the GR-1C promoter, whereas GR-P preferentially used the GR-1B promoter in a number of human tissues and hematopoietic cells. However, our data also showed that there is a switch in promoter use for the GR-P mRNA variant from predominantly GR-1C to GR-1A1 after spontaneous labor and to GR-1A3 in placenta from women delivering a male fetus. Interestingly, we see a decrease in GR-P after spontaneous labor, which may result from this switch to the GR-1A1 promoter that has markedly lower activity than the GR-1C promoter. Furthermore, the use of the GR-1A3 promoter in placenta from women carrying a male fetus for the GR-P mRNA variant may play a role in altering GR responsiveness in these placentae.

Finally, we examined changes in GR mRNA transcript expression after treatment with the GCs cortisol or dexamethasone. We observed a decrease in GR-$\alpha$ and GR-1B levels in response to dexamethasone, whereas the response to cortisol was not significantly different from the effects of vehicle or dexamethasone (Table 4). It should be noted that the concentrations of dexamethasone and cortisol used in the study were equal; however, dexamethasone is a more potent GC than cortisol. Thus, the difference in these responses to the two GCs may also be related to differences in metabolism of the two GCs by the placenta (27). Notably, GR-P responded to dexamethasone with significantly lower GR-P mRNA levels than after either vehicle or cortisol treatment (Table 4). This is particularly interesting because GR-P has been implicated in increasing cellular responsiveness to dexamethasone in several cell culture models (16). Moreover, GR-P exhibited the only difference in mRNA transcript abundance between mode of delivery (Fig. 1), showing a decrease with labor. Furthermore, some of the transcripts appear to be resistant to GC treatment, particularly GR-1A1 (Table 4). This suggests that the switch in promoter use for GR-P mRNA generation from highly active GR-1C to the relatively inactive GR-1A1 promoter after spontaneous labor may act to reduce GR-P variant levels at labor, potentially decreasing GC responsiveness mediated by GR-$\alpha$ (Fig. 1).

In conclusion, our study has shown that all of the major GR exon 1 mRNA transcripts and GR mRNA splice variants are expressed in the human placenta at term and has demonstrated differences in the GR transcripts implicated in the GR responsiveness of tissues, GR-P and GR-1A3, with mode of delivery and fetal sex, respectively. It provides the most complete picture to date of the GR transcript expression in the placenta at term and suggests that there are differences in the GR transcript expression and GR promoter use in the human placenta at term that may control the way in which the placenta responds to changes in cortisol during pregnancy and the differential responses of placentas associated with male and female fetuses.

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