Placental mRNA expression of α and β human chorionic gonadotrophin in early trisomy 18 pregnancies

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The placental expression of human chorionic gonadotrophin (HCG) I- and β-subunits was investigated in eight pregnancies presenting with trisomy 18 and in 30 normal pregnancies at 11–15 weeks gestation. In the control group, the median densitometric scores of placental β-HCG and I-HCG mRNA were 1.23 and 1.74 respectively. In the trisomy 18 group the median β-HCG mRNA was significantly lower (0.16, Z = 2.29, P < 0.05) but α-HCG (0.60, Z = 1.75, P = 0.08) was not significantly different from normal. These findings suggest that in trisomy 18 the decrease in maternal serum concentration of HCG subunits results from an impairment in the transcription of the corresponding gene which affects the β subunit to a greater extent than the I subunit.

Key words: gene expression/human chorionic gonadotrophin/trisomy 18

Introduction

The gene encoding for α-human chorionic gonadotrophin (HCG) is located on chromosome 6 (Fiddes and Goodman, 1979) and β-HCG is encoded by a cluster of at least six genes located on chromosome 19 (Talmadge et al., 1983). Of the β genes, only two are transcribed and expressed in placental tissue. Control of secretion of β-subunit is thought to be the rate-limiting step in the production of the dimer (Nagy et al., 1994).

In first trimester trisomy 18 pregnancies, the maternal serum concentration of free β-HCG is decreased (Aitken et al., 1993; Brizot et al., 1995a), whereas the concentration of the free α-subunit is increased (Jauniaux et al., 1996). This study investigates whether the placental mRNA expression of α- and β-HCG genes is influenced by an extra copy of chromosome 18.

Materials and methods

Sample collection

Placental villous tissue was collected at the time of surgical termination at 11–15 weeks gestation in eight pregnancies with fetal trisomy 18. Placental tissue was also collected from 30 normal pregnancies. These patients were undergoing pregnancy termination for psychosocial reasons. In all cases a regular fetal heart rhythm was present at the time of pregnancy termination. Written informed consent was obtained from the patients and the study was approved by the Research Ethics Committee of King’s College Hospital, London, UK.

Tissue collection was made in accordance with the Polkinghorne guidelines (1989) on the research use of fetal material. A 3 mm hypan dilator (DilapanK; Gynotech, Middlesex, NJ, USA) was inserted into the cervix 12 h pre-operatively for cervical ripening. Placental samples were snap frozen in liquid nitrogen and were stored at −70°C until assayed.

mRNA analysis

A modified version of the single step protocol of Chomczynski and Sacchi (1987) was used to extract total RNA from placental tissue. Northern blot analysis, slot blots and hybridization were performed as previously described (Brizot et al., 1995a,b). The probes used were a 320 bp α-HCG (corresponding to nucleotides 55–382 of exon 1), and a 400 bp β-HCG (corresponding to nucleotides 8–409 of exon 1). The probe for β-actin was used as a control for loading and transfer. The polymerase chain reaction (PCR) products were labelled with [32P]-dCTP as described by Feinburg and Volgelstein (1983). The membranes were exposed at −70°C on X-ray films for 2–20 h. The slot blot signals were scanned using a densitometer (GDS 2000; Mitsubishi Electric Corporation, Tokyo, Japan). To correct for any uneven loading of the RNA samples, the densitometric scores of β-HCG and α-HCG were normalized to β-actin mRNA by dividing the densitometric value of the target gene by the value of actin.

Statistical analysis

The Mann–Whitney U test was used to examine the significance of differences between the trisomy 18 pregnancies and the normal controls. P < 0.05 was considered to be statistically significant.

Results

In the control group, normalized densitometric scores for α-HCG and β-HCG mRNA did not change significantly with gestational age (r = 0.054 and r = -0.053 respectively) and the median scores were 1.74 and 1.23 respectively (Figure 1). In trisomy 18 the median placental β-HCG mRNA (0.16) was significantly decreased (Z = 2.29, P < 0.05), but the median α-HCG mRNA although lower (0.6) was not significantly different from normal (Z = 1.75, P = 0.08). The expression of α-HCG mRNA and β-HCG mRNA was decreased in 5 and 6 trisomy cases respectively. Figure 2 presents the results of a representative Northern blot analysis in four series of
placental samples from normal and trisomic pregnancies matched for gestational age.

**Discussion**

The results of this study suggest that in trisomy 18 pregnancies, changes in maternal serum concentration of HCG subunits are probably the consequence of impairment in the transcription of the corresponding genes which affects the β-subunit to a greater extent than the α-subunit.

Trisomy 18 is associated with an early onset fetal growth retardation (Kuhn et al., 1995), impaired development of the placental vasculature together with reduction in the number of small muscular arteries (Rochelson et al., 1990), and low concentrations of fetoplacental products in maternal serum (Staples et al., 1991; Brizot et al., 1994, 1995a,b). In trisomy 18, chorionic gonadotrophins and, in particular, serum free β-HCG, total α-HCG and total β-HCG concentrations are significantly lower whereas free α-HCG concentration is significantly higher compared with normal early pregnancies (Jauniaux et al., 1996). These findings suggest that the presence of an additional chromosome 18 affects both fetal and placental development. Conversely, in trisomy 21 fetal growth is normal (Kuhn et al., 1995) and the only maternal serum change observed in these cases is an increase in free β-HCG mean concentration (Jauniaux et al., 1996).
We have previously shown that placental tissue expression of \(\alpha\) - and \(\beta\)-subunits of HCG is similar in trisomy 21 and normal pregnancies at 11–15 weeks gestation (Brizot et al., 1996). Thus as placental mRNA expression is not altered in trisomy 21, the increase in maternal serum free \(\beta\)-HCG found in the majority of these cases is probably due to changes in the post-transcriptional phase of HCG protein biosynthesis. In normal early pregnancies, the total amount of \(\alpha\) - and \(\beta\)-HCG subunits increases similarly and reaches a peak at 8–10 weeks gestation. The \(\alpha\) - to \(\beta\)-HCG ratio is equimolar until the end of the first trimester of gestation and increases regularly during the second and third trimester as the rate of decrease of total \(\beta\)-HCG is faster than that of \(\alpha\)-HCG (Nagy et al., 1994). In trisomy 18, the decrease in total \(\beta\)-HCG is more pronounced than the decrease in total \(\alpha\)-HCG resulting in a significant increase in the total \(\alpha\)- to \(\beta\)-HCG subunit ratio (Jauniaux et al., 1996). These findings together with the present data support the concept that total \(\alpha\) - and \(\beta\)-HCG profiles approximately reflect the placental production of \(\alpha\) - and \(\beta\)-HCG monomers in both euploid and aneuploid early pregnancies.

In early embryonic failure of undetermined origin, the placental tissue expression of \(\alpha\) - and \(\beta\)-HCG is generally depressed when compared with normal pregnancies, and the \(\beta\)-HCG subunit appears to be down-regulated to a greater extent than the \(\alpha\)-subunit (Henderson et al., 1992). In some cases of early pregnancy failure, the placentation expression of \(\alpha\) - and \(\beta\)-HCG and the corresponding serum concentrations are within normal ranges suggesting that there are two subsets of early pregnancy failure: those that express apparently normal concentrations of placental proteins and those with severely depressed concentrations. The former group would be associated with abnormal fetal development and may be due to aneuploidy whereas the latter would reflect poor implantation and trophoblast differentiation (Henderson et al., 1992). We also found important variations in \(\alpha\) - and \(\beta\)-HCG placentation expression within the trisomy 18 group (Figure 1). This finding suggests that differences in HCG subunit expression in early pregnancy failure are not directly linked to the origin or the mechanism of the miscarriage but may be related in aneuploidies to the maternal versus paternal origin of the supplementary chromosomes.

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References


