

## Acid-Labile Subunit (ALS) Gene Expression and Protein Content in Human Placentas: Differences According to Birth Weight

Germán Iñiguez, Felipe Argandoña, Pablo Medina, Carolina González, Sebastián San Martín, Elena Kakarieka, M. Cecilia Johnson, and Fernando Cassorla

Institute of Maternal and Child Research (G.I., F.A., P.M., C.G., M.C.J., F.C.), School of Medicine, University of Chile, Santiago 8389100, Chile; Reproductive Biology Research Centre (S.S.M.), School of Medicine, Universidad de Valparaíso, Valparaíso 2362735, Chile; and Hospital Clínico San Borja-Arriarán (E.K.), Santiago 8320000, Chile

**Context:** In humans, IGF-I and -II have an important role in pre- and postnatal growth. The IGFs circulate in plasma principally as a ternary complex with the IGF binding protein-3 and an acid-labile subunit (ALS), which increases their half life.

**Objectives:** The objectives of the study were to determine whether the human placenta expresses the mRNA and protein for ALS and to evaluate any possible differences in the mRNA and protein for ALS in placentas from small (SGA) and appropriate (AGA) or gestational age newborns.

**Subjects/Methods:** We studied the placentas from 47 AGA and 42 SGA pregnancies. IGF-I, IGF-II, IGF binding protein-3, and ALS placental mRNA and protein contents were determined in both the basal and the chorionic plates of the placenta.

**Results:** We observed that the human placenta expresses the gene and protein for ALS. The ALS mRNA in SGA was higher compared with AGA placentas ( $0.15 \pm 0.01$  vs.  $0.12 \pm 0.01$  arbitrary units, respectively,  $P < 0.05$ ). In addition, the ALS protein content in SGA ( $31.7 \pm 3.3$  pmol/g) was higher compared with AGA ( $22.1 \pm 2.3$  pmol/g,  $P < 0.05$ ) placentas.

**Conclusion:** We describe that the human placenta expresses the mRNA and the protein for ALS, and we observed an increase in ALS mRNA expression and protein content in SGA compared with AGA placentas. (*J Clin Endocrinol Metab* 96: 187–191, 2011)

Fetal growth is under the control of genetic, environmental, and nutritional factors. Infants born small for gestational age (SGA) may experience metabolic derangements during intrauterine life, which may predispose them to increased fetal and neonatal mortality and morbidity (1) and an increased risk of disorders in adult life, such as cardiovascular disease, diabetes, and obesity (2).

Fetal growth restriction may be the consequence of maternal, fetal, or placental factors. The IGFs appear to be major determinants of fetal growth (3) and are expressed in the mother, fetus, and placenta in most species (4). We

recently showed that IGF-I and IGF-I receptor mRNA and protein are higher in placentas from SGA compared with appropriate for gestational age (AGA) placentas (5).

In postnatal human serum, most IGFs circulate as 150-kDa ternary complexes consisting of one molecule of IGF-I or IGF-II, IGF binding protein (IGFBP)-3, the predominant form in serum, and a 84- to 86-kDa glycoprotein, the acid-labile subunit (ALS). ALS is mainly produced by the liver and is encoded by the *IGFALS* gene (16p13.3) (6). The ternary complex serves to stabilize the IGFs by extending their half-life from 10 min in the free form to

more than 12 h in the complex (7). Recently several ALS mutations have been reported in patients presenting with impaired postnatal growth, but it is uncertain whether prenatal growth is affected by an *IGFALS* gene defect because limited data on birth weight have been reported in these patients (8).

To the best of our knowledge, no data on placental ALS gene expression and/or protein content have been published so far. Therefore, the aim of our study was to evaluate whether the human placenta expresses the ALS gene and its protein and to investigate any possible differences in these parameters in placentas from SGA and AGA newborns.

## Subjects and Methods

### Sample collection

We collected placentas from 47 AGA [birth weight =  $0.11 \pm 0.11$  SD score (SDS)] and 42 idiopathic SGA newborns (birth weight =  $-2.11 \pm 0.10$  SDS) from full-term pregnancies (37–40 wk of gestation) as previously described (6). All mothers gave their informed consent, and the protocol was approved by the Institutional Review Board of the San Borja Arriarán Clinical Hospital in Santiago, Chile.

Placental samples were rapidly processed as described by Wyatt *et al.* (9). The tissue was divided to obtain placental samples from the chorionic (CPs) and basal plates (BPs). The cord blood was centrifuged and the serum was aliquoted and stored at  $-20$  C until assayed.

### RNA preparation, cDNA synthesis, and RT-PCR

Total RNA was isolated from the frozen placentas (CPs and BPs) and cDNA was synthesized as previously described (5) (see Supplemental Materials and Methods, published on The Endocrine Society's Journals Online web site at <http://jcem.endojournals.org>).

### Gene amplifications

The *IGFBP3* (224 bp, gene ID 3486) upstream 5'-CTCCA-CATTCAGAGGCATCA-3' and downstream 5'-AGCCATTC-CTCCTTCCTGTT-3'; *ALS* (238 bp, gene ID 3438) upstream 5'-GTGCTCACGCTAGACCACAA-3' and downstream 5'-CCGTTGTCCTTGAGGAAGAG-3'. For *IGF*, *IGF2*, and *18S* rRNA, we used the primers previously described (5). The RT-PCR was developed as described in Supplemental Materials and Methods. Semiquantification of PCR products was performed by image analysis (Kodak 1D image analysis software; Rochester, NY). The amplified product identity of the ALS gene was confirmed by sequencing in purified PCR products (Macrogen Inc., Seoul, Republic of Korea).

### Assays

Total IGF-I and IGF-II serum concentrations and placental contents were determined in homogenates from frozen placentas as previously described (5).

IGFBP-3 concentrations were measured by IRMA (Diagnostic System Laboratories Inc., Webster, TX). The sensitivity of the

method was 100 ng/ml for cord serum samples and 10 ng/ml for placental homogenates. The intra- and interassay coefficients of variation were 1.1 and 1.8%, respectively.

ALS concentrations were measured by RIA (Bioclone, Sydney, New South Wales, Australia). The sensitivity of the method for serum and homogenates samples was 0.02 nmol/liter. The intra- and interassay coefficients of variation were 4.5 and 5.1%, respectively.

### Immunohistochemistry

Immunohistochemical staining for ALS was performed on 5- to 6- $\mu$ m formalin-fixed, paraffin-embedded placental sections using immunoperoxidase method using an anti-ALS host goat (Santa Cruz Biotechnology, Santa Cruz, CA) (see Supplemental Materials and Methods).

### Western blot analysis

Equal amounts (25  $\mu$ g) of different tissue proteins were resolved using 10% SDS-PAGE and subsequently transferred to nitrocellulose membranes. The membranes were blocked and then probed with an antibody against ALS (see Supplemental Material and Methods).

### Statistical analysis

Results are shown as mean  $\pm$  SEM. Differences were assessed by *t* test or Mann Whitney *U* test. To compare the results of the BP and CP in each placenta, we used the paired *t* test or Wilcoxon test. The studies of correlations were performed using the Pearson or Spearman tests. Statistics were performed using SPSS version 11.5, and a value of  $P < 0.05$  was considered significant.

## Results

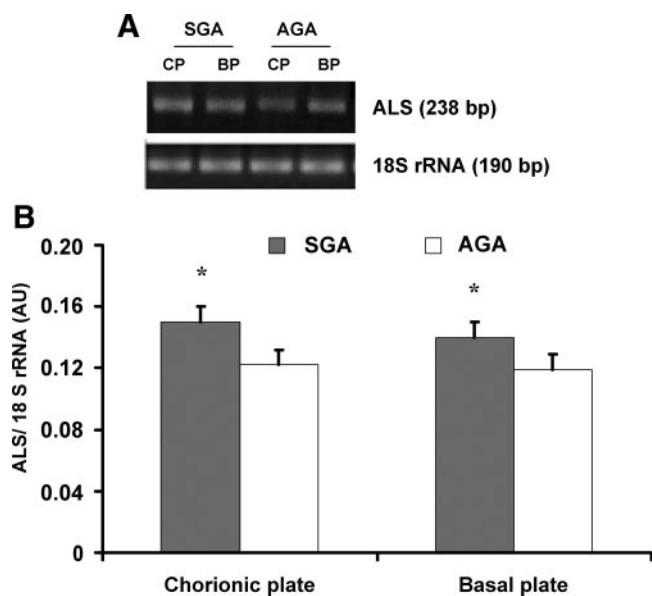
The clinical and hormonal data of the newborns are shown in Supplemental Table 1. As expected, the SGA newborns had significantly lower birth weight SDS, birth length SDS, head circumference SDS, and placental weight than the AGA newborns. In addition, mean IGF-I, IGF-II, IGFBP-3, and ALS cord blood levels were significantly lower in SGA compared with AGA newborns.

### Placental messenger of ALS mRNA

ALS mRNA amplification produced a single band of 208 bp (Fig. 1A). Semiquantitative RT-PCR showed that ALS is present in human placenta in both the CPs and BPs. The amplified product identity of the ALS gene was confirmed by sequencing in purified PCR products; the homology was 100% with human ALS in four samples analyzed.

### Placental mRNA level according birth weight

ALS mRNA level (Fig. 1B) was higher in both the CP and BP from the SGA placentas compared with the AGA placentas. As we previously reported, the mRNA expression of *IGF-I* was higher in the CP and BP from SGA



**FIG. 1.** Representative gel for ALS and mRNA expression in the CPs and BPs of the placentas (A). The ALS mRNA level in placentas from SGA ( $n = 42$ ) and AGA ( $n = 47$ ) pregnancies is shown (B). The values represent the mean  $\pm$  SEM of two different RT-PCRs. \*,  $P < 0.05$  SGA vs. AGA. The ALS ranges are: SGA CP (0.05–0.55), SGA BP (0.03–0.58), AGA CP (0.01–0.35), and AGA BP (0.02–0.34). \*,  $P < 0.05$  SGA vs. AGA.

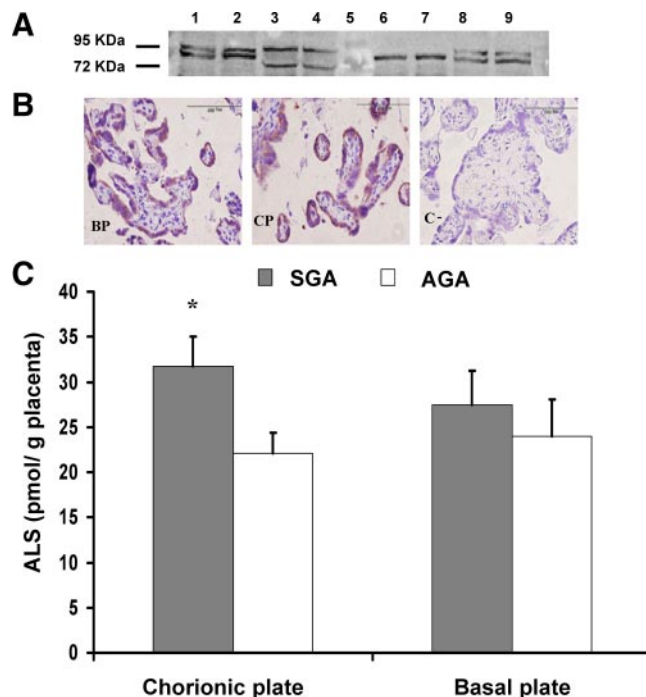
placentas compared with the same plates from AGA placentas (5) (Supplemental Table 1). The *IGFBP3* mRNA was slightly higher in the CPs and BPs of the SGA placentas compared with the AGA placentas, but this difference did not reach statistical significance (Supplemental Table 1).

### Placental protein content

We detected the ALS protein in human placentas by immunohistochemistry and Western blot (95 kDa, Fig. 2A). Positive immunostaining for ALS was observed in the cytoplasm of the cytotrophoblast and in the syncytiotrophoblast from the BPs (Fig. 2B, BP), and CPs (Fig. 2B, CP) of the placentas. No staining for ALS was observed in endothelial or mesenchymal cells from the placental villi.

The ALS protein content was higher in the CPs of the SGA compared with the AGA placentas. No differences were observed in the BP of the placentas between the SGA and AGA groups (Fig. 2C).

The IGF-I protein content was higher in the CP of the SGA placentas compared with the AGA placentas ( $89.3 \pm 6.2$  vs.  $75.4 \pm 4.7$  ng/g placenta, respectively,  $P < 0.05$ ). Similarly, the placental IGF-II protein content in the CP of the SGA placentas was higher compared with the AGA placentas ( $396 \pm 21$  vs.  $326 \pm 19$  ng/g placenta, respectively,  $P < 0.05$ ). Moreover, we observed a higher IGFBP-3 protein content in the CP compared with the BP from SGA placentas ( $704 \pm 42$  vs.  $546 \pm 35$  ng/g placenta, respectively,  $P < 0.05$ ).



**FIG. 2.** A, Representative immunohistochemistry for ALS in the CPs and BPs of the placenta. C, Negative control. B, Representative Western blot for ALS. Lanes 1 and 2, human fibroblasts; lanes 3 and 4, human placenta; lane 5, molecular weight marker; lanes 6 and 7, fetal hypothalamus; lanes 8 and 9, adult liver. C, ALS protein contents in the CPs and BPs of the placentas from SGA ( $n = 42$ ) and AGA ( $n = 47$ ) newborns. ALS content represents the average result of two different homogenates, from each placenta. \*,  $P < 0.05$  SGA vs. AGA. The ALS ranges are: SGA CP (6–135), SGA BP (4–95), AGA CP (6–65), and AGA BP (3–81). To convert ALS to picomoles per gram placenta, divide by 63.3. \*,  $P < 0.05$  SGA vs. AGA.

We observed an inverse correlation between birth weight with ALS mRNA and protein content in the CP of the placentas and directly with cord blood concentration ( $r = -0.385$ ,  $r = -0.237$ ,  $r = 0.386$ , respectively,  $P < 0.05$ ).

### Discussion

The placenta is an important organ for the supply of maternal substrates to the fetus. Fetal growth is a complex process regulated by various maternal, placental, and fetal factors. We have studied the ALS, an important component of the ternary complex of the IGF system.

Our study shows that the ALS is expressed in the human placenta. In addition, we show that placental ALS mRNA level was higher in the SGA compared with AGA placentas, particularly in their CP. In addition, we observed a significant increase in ALS protein content in the CP of the SGA compared with AGA placentas, suggesting that ALS may play a possible role in fetal growth. To our knowledge, this is the first study that describes ALS mRNA expression and protein content in human placentas according to birth weight.

The IGF-I and -II play crucial roles in growth and development. IGF-I is synthesized by a variety of cell types and is involved in linear growth, cell proliferation, and differentiation. Most of these effects are mediated by both endocrine and autocrine/paracrine mechanisms through the IGF-I receptor (10). In circulation, almost all the IGFs are present as 150-kDa ternary complexes comprised of one molecule of IGF, IGFBP-3, and an 85-kDa glycoprotein, the ALS (11). This ternary complex prolongs the half-life of the IGFs and allows them to remain in circulation, which facilitates their endocrine action. ALS is mostly produced by the liver, although ALS gene expression has been detected in some extrahepatic tissues, such as the kidney, bone, mammary gland, thymus, and lung (7). In humans, ALS is undetectable in fetal serum at 27 wk of gestation but is present at term (12). Proteolysis of IGFBP-3 and interactions of the ternary complex with proteoglycans have been shown to release IGFs (7). It is also possible that much of the released IGFs are the product of the equilibrium between the ternary complex and its individual components.

Inactivation of the *IGFALS* gene in mice produced a modestly growth deficient (13–20% smaller at 10 wk), despite a 62 and 88% reduction in IGF-I and IGFBP-3 plasma levels, respectively (8). It is uncertain whether prenatal growth in humans is affected by an *IGFALS* gene defect. However, some degree of postnatal growth restriction has been reported in patients with mutations of the *IGFALS* gene (8).

There are limited data on placental IGFBP-3 expression in humans. In our study, the *IGFBP-3* mRNA level was similar in SGA and AGA placentas, as previously reported in sheep (13). However, in placentas from pre-eclamptic pregnancies between 35 and 40 wk of gestation, a lower expression of IGFBP-3 compared with normal pregnancies has been reported (14). In addition, we documented higher IGFBP-3 protein content in the CP compared with the BP from the SGA placentas, suggesting a gradient for IGF-I, IGFBP-3, and ALS concentrations in the placenta.

As we previously reported (5), we also found a higher expression of *IGF-I* mRNA and protein content in SGA compared with AGA placentas. We have measured IGFBP-1 and IGFBP-2 in placental tissues, and we have observed that their placental concentrations are lower or similar to those observed in cord blood and in serum from prepubertal children (see Supplemental Table 2). Therefore, the possible interference by IGFBPs in our IGF-I assay is likely to be relatively minor.

The higher ALS, IGF-I, and IGFBP-3 mRNA expression and protein content in SGA compared with AGA placentas suggests a possible role for these important compo-

nents of the ternary complex in fetuses with growth restriction.

In conclusion, we have described for the first time the ALS mRNA and protein expression in human placentas, and we have shown increased mRNA and protein contents for IGF-I, IGFBP-3, and ALS in full-term placentas from SGA compared with AGA newborns. Our results suggest that the increase in IGF-I, IGFBP-3, and ALS mRNA expression and protein content observed in SGA compared with AGA placentas may represent a placental compensatory mechanism in response to fetal growth restriction.

## Acknowledgments

We are grateful for the generous contribution of all the patients who donated their placentas.

Address all correspondence and requests for reprints to: Germán Iñiguez, Institute of Maternal and Child Research, Faculty of Medicine, University of de Chile, Casilla 226-3, Santiago 6640750, Chile. E-mail: giniguez@med.uchile.cl.

This work was supported by Fondo Nacional de Ciencia y Tecnología (Grant 106 1082).

Disclosure Summary: The authors do not have any disclosure or conflict of interest relevant to this article.

## References

1. Kramer MS, Olivier M, McLean FH, Willis DM, Usher RH 1990 Impact of intrauterine growth retardation and body proportionality on fetal and neonatal outcome. *Pediatrics* 86:707–713
2. Barker DJ 2004 The developmental origins of well-being. *Philos Trans R Soc Lond B Biol Sci* 359:1359–1366
3. Liu JP, Baker J, Perkins AS, Robertson EJ, Efstratiadis A 1993 Mice carrying null mutations of the genes encoding insulin-like growth factor I (Igf-1) and type 1 IGF receptor (IGF-Ir). *Cell* 75:59–72
4. Fowden AL 2003 The insulin-like growth factors and fetoplacental growth. *Placenta* 24:803–812
5. Iñiguez G, González CA, Argandoña F, Kakarićka E, Johnson MC, Cassorla F 2010 Expression and protein content of IGF-I and IGF-I receptor (IGF-IR) in placentas from small, adequate and large for gestational age newborns. *Horm Res Paediatr* 73:320–327
6. Baxter RC 1990 Circulating levels and molecular distribution of the acid-labile subunit of the high molecular weight insulin-like growth factor-binding protein complex. *J Clin Endocrinol Metab* 70:1347–1353
7. Boisclair YR, Rhoads RP, Ueki I, Wang J, Ooi GT 2001 The acid-labile subunit (ALS) of the 150 kDa IGF-binding protein complex: an important but forgotten component of the circulating IGF system. *J Endocrinol* 170:63–70
8. Domené HM, Hwa V, Argente J, Wit JM, Camacho-Hübner C, Jasper HG, Pozo J, van Duyvenvoorde HA, Yakar S, Fofanova-Gambetti OV, Rosenfeld RG, International ALS Collaborative Group 2009 Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. *Horm Res* 72:129–141
9. Wyatt SM, Kraus FT, Roh CR, Elchalal U, Nelson DM, Sadovsky

- Y 2005 The correlation between sampling site and gene expression in the term human placenta. *Placenta* 26:372–379
10. Baker J, Liu JP, Robertson EJ, Efstratiadis A 1993 Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* 75:73–82
  11. Ooi GT, Boisclair YR 1999 Molecular biology of the insulin-like growth factor binding proteins. In: Rosenfeld R, Roberts JC, eds. *Contemporary endocrinology: the IGF system*. Totowa, NJ: Humana Press Inc.; 111–139
  12. Lewitt MS, Scott FP, Clarke NM, Baxter RC 1995 Developmental regulation of circulating insulin-like growth factor-binding proteins in normal pregnancies and in pre-eclampsia. *Progress in Growth Factor Res* 6:475–480
  13. de Vrijer B, Davidsen ML, Wilkening RB, Anthony RV, Regnault TR 2006 Altered placental and fetal expression of IGFs and IGF-binding proteins associated with intrauterine growth restriction in fetal sheep during early and mid-pregnancy. *Pediatr Res* 60:507–512
  14. Han JY, Kim YS, Cho GJ, Roh GS, Kim HJ, Choi WJ, Paik WY, Rho GJ, Kang SS, Choi WS 2006 Altered gene expression of caspase-10, death receptor-3 and IGFBP-3 in preeclamptic placentas. *Mol Cells* 22:168–174



**Sign up for eTOC alerts today**  
to get the latest articles as soon as they are online.

<http://jcem.endojournals.org/subscriptions/etoc.shtml>