BASIC SCIENCE: OBSTETRICS

# Altered gene expression patterns in intrauterine growth restriction: Potential role of hypoxia

Cathal McCarthy, PhD; Finbarr E. Cotter, FRCPath, PhD; Suzanne McElwaine, PhD; Anne Twomey, FAAP; Eoghan E. Mooney, MRCPath; Fergus Ryan, PhD; Joseph Vaughan, MSc

**OBJECTIVE:** Placental insufficiency is a primary cause of intrauterine growth restriction (IUGR). In our study, microarray technology was used to identify genes, which may impair placentation resulting in IUGR.

**STUDY DESIGN:** The RNA was isolated from both IUGR term placentas and normal term placentas. Microarray experiments were used to identify differentially expressed genes between the 2 cohorts. Real-time quantitative reverse transcriptase polymerase chain reaction and immunohistochemistry were used in follow-up experiments.

**RESULTS:** Microarray experiments identified increased expression of certain genes including leptin, soluble vascular endothelial growth factor receptor, human chorionic gonadotropin, follistatin-like 3, and hypoxia-inducible factor 2$\alpha$ in the IUGR. Real-time quantitative polymerase chain reaction confirmed these results.

**CONCLUSION:** The upregulation of soluble vascular endothelial growth factor receptor and hypoxia-inducible factor 2$\alpha$ at this period in pregnancy indicate that placental angiogenesis is altered in IUGR and that hypoxia is a major contributor to maldevelopment of the placental vasculature.

**Key words:** angiogenesis, hypoxia, intrauterine growth restriction, placentation

Cite this article as: McCarthy C, Cotter FE, McElwaine S, et al. Altered gene expression patterns in intrauterine growth restriction: Potential role of hypoxia. Am J Obstet Gynecol 2007:196:70.e1-70.e6.

Intrauterine growth restriction (IUGR) refers to a pathologic decrease in the rate of fetal growth.[1] An IUGR has an incidence of 4-7% of births and is linked to a 6- to 10-fold increased risk of perinatal mortality. It has been hypothesized that unsuccessful placentation is responsible for numerous perinatal complications varying from miscarriage to IUGR.[2] Placentation requires successful trophoblast invasion into the maternal vessels and the development of a uteroplacental circulation to cope with the increasing metabolic requirements of the developing fetus. It is widely accepted that shallow trophoblast invasion in the decidua and myometrium in collaboration with a failure to invade the spiral arteries and establish an efficient uteroplacental circulation can lead to fetal hypoxia and impaired growth.[3] Absence of trophoblast invasion of spiral arteries of the decidua has been reported in 45-100% of IUGR cases.[4]

There is a requirement for adequate angiogenesis during placentation. Angiogenesis is critical for fetal growth and development. Morphologic studies have described deficiencies in the structural make-up of villi in IUGR placentas, including reduced linear growth of villi and capillaries,[5] and IUGR terminal villi are thinner in comparison to villi in normal placentas.[6] Microarray technology allows the simultaneous examination of alterations in expression levels of thousands of genes. The purpose of this study was to identify the gene expression profile of IUGR term placentas compared with pathologically normal term placentas.

## Materials and Methods

### Tissue samples

The present investigation was performed on fresh placental tissue collected from vaginal and cesarean section deliveries at the National Maternity Hospital Dublin in the period 2004-2005. All samples were taken 10 minutes from delivery. The project had the approval of the hospital's ethics committee. The control group was chosen at random. All IUGR infants were small for gestational age with a birthweight that was designated as being below the 5th percentile as per the UK Cross Sectional Reference charts 1990.[7,8] The pathologically normal and IUGR cohorts respectively consisted of 4 placental samples each. The histology reports for 2 of the IUGR samples reported indications of uteroplacental insufficiency and 1 sample had histologic signs of villitis. The mean gestational age of delivery in the pathologically normal cohort was 39 weeks with a mean birthweight of 3558 g. The mean gestational age of delivery in the IUGR cohort was 36 weeks with a mean birthweight of 1935 g.

From the School of Biological Sciences, Dublin Institute of Technology, Dublin, Ireland (Drs McCarthy and Ryan, Mr Vaughan); the Department of Experimental Haematology, Barts and the London, Queen Mary's School of Medicine and Dentistry, London, England (Professor Cotter and Dr McElwaine); and the National Maternity Hospital, Dublin, Ireland (Drs Twomey and Mooney).

Received March 28, 2006; revised June 16, 2006; accepted Aug. 1, 2006.

Reprints not available from the authors.

0002-9378/$32.00

doi: 10.1016/j.ajog.2006.08.027

**TABLE 1**
**Thirty-eight genes selected with the use of the Venn diagram format from a gene list that had a 2-fold difference in expression between the 2 cohorts and a gene list that was selected by using the 1-way analysis of variance statistical parameter**

| Gene name | Accession number | Description |
|---|---|---|
| Upregulated genes in IUGR | | |
| LEP | NM_000230.1 | Leptin |
| FSTL3 | NM_005860.1 | Follistatin-like 3 (secreted glycoprotein) |
| sFlt-1 | U01134.1 | Soluble vascular endothelial growth factor receptor |
| SIGLEC6 | NM_001245.1 | Sialic acid binding Ig-like lectin 6 |
| LHB | NM_000894.1 | Luteinizing hormone beta polypeptide |
| BAP31BAP29 | AW276522 | Accessory proteins |
| 5T4 | NM_006670.1 | 5T4 oncofetal trophoblast glycoprotein |
| PLOD2 | NM_000935.1 | Procollagen-lysine, 2-oxoglutarate 5-dioxygenase |
| IL1RAP | AF167343.1 | Soluble interleukin-1 receptor accessory protein |
| IMPACT | NM_018439.1 | Hypothetical protein IMPACT |
| HIF2$\alpha$ | NM013332.1 | Hypoxia-inducible factor 2$\alpha$ |
| UBE3B | BE856776 | Ubiquitin protein ligase |
| KIAA0790 | AB018333 | KIAA0790 protein |
| SLC2A3 | NM_006931.1 | Solute carrier family 2 (facilitated glucose transporter) member 3 |
| HCG | NM_000737.1 | Human chorionic gonadotrophin |
| NDRG1 | NM_006096.1 | N-myc downstream regulated |
| Clone | AF130095.1 | Clone FLC0562 PRO2841 |
| PPL | NM002705.1 | Periplakin |
| SAR1 | BC003658.1 | SAR1 protein clone MGC:4824 |
| SLCA1 | AL162079.1 | Solute carrier family 16 member 1 |
| MYO6 | NM_004999.1 | Myosin VI |
| S6 | BE738425 | Ribosomal protein S6 |
| FN | X02761.1 | Fibronectin precursor |
| TP | Z24727.1 | Tropomyosin isoform |
| Clone | AI949687 | Clone transcription factor 7-like 2 (T-cell specific) |
| KIAA1438 | AB037859.1 | KIAA1438 protein |
| LXN | NM_020169.1 | Latexin |
| Clone | AI088622 | v-raf-1 murine leukemia viral oncogene homolog 1 |
| SSB | NM_003142.1 | Sjogren syndrome antigen B (autoantigen La) |
| Clone | BF689355 | Clone glyceraldehyde-3-phosphate dehydrogenase |
| HFE | AF149804.1 | Hemochromatosis protein splice variant 562-878del |
| Downregulated genes in IUGR | | |
| SIPA1 | NM_006747.1 | Signal-induced proliferation-associated gene 1 |
| KIAA0648 | AW991219 | KIAA0648 protein |
| FLJ23312 | NM_024752.1 | Hypothetic protein FLJ 23312 |
| Clone | BC002832.1 | Clone MGC3790 similar to butyrophilin subfamily 3 member A2 |
| DEFA1 | NM_004084.2 | Defensin alpha 1 myeloid-related sequence |
| HBD | NM_000519.2 | Hemoglobin delta |
| Clone | AW450911 | Clone membrane protein palmitoylated member 2 |