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**Review Article**

# The Use of New Technologies in the Study of Pregnancy Disorders: The OMICS Approach

## Abstract

The "omics" technologies represent a new model of approach in the study of human disease. Metabolomics is defined as the quantitative measurement of the dynamic metabolic response of living systems to genetic, physical, pathological or developmental factors. Proteomics analysis of biological samples has the potential to identify novel protein expression patterns and/or changes in protein expression patterns in different developmental or disease states. In this manuscript we present the omics technologies applicated in obstetrics. The management of different pregnancy diseases could be improved by knowing their metabolic background. In this review we focused our attention on omics application for intrauterine growth restriction (IUGR), preterm birth and preeclampsia. Omics in IUGR field could help to discover novel biomarkers for early diagnosis, the molecular link between nutrient deprivation in utero and the increase in risk of developing cardiovascular illness and metabolic syndrome in adults. It could identify one or more therapeutic targets that allow to minimize the organ damage. Recently, the use of metabolomics permitted to discover significant differences in 70 proteins within the trophoblastic cells of women with pre-eclampsia when compared with healthy control women. Recently were identified proteins in cervical–vaginal fluid that could be useful to predict preterm labor in asymptomatic women: thioredoxin and interleukin-1 receptor antagonist. In conclusion, this approach can lead to new hypothesis-based medicine and provide a "shortcut" to obtain new biological insight.

## Abbreviations

IUGR: Intrauterine growth restriction; AGA: Appropriate for gestetional age; LC–HRMS: Liquid chromatography high resolution mass spectrometry

## Introduction

### The Use of the "OMICS"

The "omics" technologies represent a new model of approach in the study of human disease. It consists of general approaches on molecules (such as genes, transcripts, proteins and metabolites) of which a cell, tissue or organism is made. Although the interactions between the 'omes' mean that omic strategies are complementary, there are differences in terms of their molecular properties, and therefore, the technologies required for preparation and analysis [1]. Metabolomics is defined as the quantitative measurement of the dynamic metabolic response of living systems to genetic, physical, pathological or developmental factors. The technology has emerged in numerous fields of research, including fetal medicine aiming to facilitate the understanding of fetal disease pathophysiology and discovery of predictive biomarkers [2]. Another kind of omic is "proteomic". Proteomics analysis of biological samples has the potential to identify novel protein expression patterns and/or changes in protein expression patterns in different developmental or disease states. The difficulty is that each organ is composed of multiple cell and tissue types [3]. That's why several limits must be considered. Often altered expression of a gene is not always causally related to a phenotype or a process and so a change in transcription is not always followed by protein synthesis. Furthermore, other modifications may also play a role, such as post–transcriptional, translational and post–translational one [4].

### "OMICS" in Obstetrics

Most frequent "–omics" applications are in physiology, functional genomics, pharmacology, toxicology and nutrition. We know that physiological variables and pathological conditions can affect metabolic profiles of different biofluids in pregnant women. The management of different pregnancy diseases could be improved if we know their metabolic background. The literature on obstetrical and gynecological diseases has expanded exponentially in recent years, with

an increasing focus on fetal pathologies [5]. Many of the "-omics" publications relating to reproductive medicine have assessed single class of "-omics" data, utilizing genomics, transcriptomics, proteomics or metabolics in isolation. The results of many of these "-omics" publications have failed to replicate and their practical value has been limited, failing to translate into clinical practice. The limited successes of singular approaches emphasize the need for integrated approaches to investigate complex phenotypes across "-omics" categories. A wide variety of fluids or tissues such as cervical mucus, blood, urine, saliva, amniotic fluid, vaginal discharge, myometrium and placenta are appropriate depending on the investigation. Recent studies have evaluated how physiological variables or pathological conditions can affect metabolomic profiles of different tissues in fetuses. Vascular alterations in pregnancy are the primum movens of different diseases in pregnancy, such as maternal thromboembolism, preeclampsia syndrome, fetal growth restriction, fetal loss, and abruption, that manifest a shared etiopathogenesis and predisposing risk factors [6]. Recent studies demonstrated that these pregnancy's diseases, involving cardiovascular mechanisms, seem to increase six fold the risk of maternal cardiovascular events [7]. Moreover, seems also to be present a fetal programming for cardiovascular events (i.e. stroke, cardiac failure, etc) and metabolic syndrome for offspring of affected pregnancies in adult later life [8]. Our knowledge about the physiopathology of these vascular diseases should be increased to be able to identify people who are at higher risk to develop cardiovascular diseases. "Omics" technologies and biomarkers development have been largely based on advances in vascular biology, improved understanding of the molecular basis and biochemical pathways responsible for the clinically relevant diseases. The development of omics technologies, allowing a global analysis of biological or molecular system, raised the prospect that classical, hypothesis-driven and single gene-based approaches may soon become obsolete.

## "OMICS" in Preterm Birth

Preterm birth (birth before 37 weeks gestation) is the leading cause of neonatal mortality and morbidity [9] and the rates of this condition have been increasing over the past three decades in developed Countries [10]. Its etiology is multifactorial and this complicates our understanding of preterm birth. Among factors associated with increased risk of preterm birth are maternal smoking, maternal age, decimal thrombosis, cervical insufficiency, and a variety of environmental and genetical factors [11]. The evidence increases for a genetic contribution to preterm birth, so the need to explore genomics, transcriptomic, proteomics and metabolomics in literatures [12]. Romero et al. reported for the first time the analysis of the inflammatory-related protein network in the amniotic fluid of patients with spontaneous preterm labor and intact membranes [13]. Little is known about the overall metabolic status of the term and preterm neonate. On the other hand, the management of sick or preterm newborns might be improved if more information on perinatal/neonatal maturational processes and their metabolic background were available [14]. Liong et al recently identified proteins in cervical–vaginal fluid that could be useful to predict preterm labor in asymptomatic women: thioredoxin and interleukin-1 receptor antagonist. They were significantly reduced for up to 90 days before onset of preterm labor when compared with women who delivered at term [15]. Further, in a recent systematic review, Nápoles Méndez reminded us of other classic proteomic markers described in the literature that help to predict preterm birth, such as alpha-fetoprotein, C-reactive protein, interleukin-1, interleukin-6, and interleukin-8, tumor necrosis factor, matrix metalloproteinase-8, and fetal fibronectin [16].

## "OMICS" in Intrauterine Growth Restriction

Intrauterine growth retardation (IUGR) is defined as a fetus that does not reach its growth potential and is characterized at birth by a weight and a body mass index lower than normal for the number of gestational weeks [17]. IUGR is the major cause of foetal death and morbidity, causing the 52% of stillbirths and 10% of perinatal mortality [18]. IUGR is also associated with foetal complications (hypoxia/acidosis, malformations), neonatal complications (hypoglycemia, hypocalcemia, hypoxia/acidosis, hypothermia, meconium aspiration, polycythemia, hyperbilirubinemia, sepsis, congenital malformations, apneic spells, intubation sudden infant death syndrome), and long-term complications (such as lower IQ, learning and behavior problems, major neurological handicap seizures, cerebral palsy, mental retardation, syndrome X, a condition associating obesity with hypertension and type 2 diabetes) [19]. Omics technologies pave the way to face challenges concerning the pathogenic mechanism of IUGR. In which way? Discovering novel biomarkers for early diagnosis, molecular link between nutrient deprivation in utero and the increase in risk of developing cardiovascular illness and metabolic syndrome in adults and identifying one or more therapeutic targets that allow to minimize the organ damage. Although it is well established that the origins of IUGR are in early pregnancy and that the placenta plays an integral role in pregnancy outcome, the exact etiologies of these multifactorial diseases remain poorly defined. Decades of research have not translated into a clear understanding of the underlying patho-physiologies or effective identification of women who are at high risk of developing IUGR. Through metabolomic and proteomic investigation of cord blood in IUGR and control neonates, significant changes in the serum metabolic profiles have been identified, thus providing the evidence on the critical role of placental transportation and nutrient deficiency in the pathophysiology of IUGR. It was seen in a study conducted by Cecconi et al who supported that the IUGR condition alters the expression of proteins some of which are involved in the coagulation process, immune mechanisms, blood pressure and iron and copper homeostasis control. The identification of the altered expression of proteins may contribute to improve to discover candidate proteins utilizable as biomarkers [20]. Moreover, the study conducted by Favretto et al. on IUGR fetuses through LC-HRMS analysis in serum obtained from cord blood, shows statistically significant differences about 22 metabolites, of which seven are alpha-amino acids. In humans, phenylalanine and tryptophan are both essential amino acids which must be supplied in dietary proteins. Although

in Literature there is no agreement about their metabolomic espression, the up-regulation of phenylalanine described may be the result of the small size of the placenta in IUGR fetuses, which in turn may lead to altered placental metabolism, with increased fetal protein catabolism and decreased amino acid transfer across the basal membrane, with a final accumulation of phenylalanine [21-22]. As regards tryptophan, a serotonin precursor, in presence of malnutrition, there is an acceleration in the brain synthesis of serotonin, starting in the fetal period and coinciding with elevation of the free fraction of tryptophan in plasma and brain [23]. The nutrient deficiency, investigated by metabolomics, may play an important role in the pathophysiology of IUGR and suggest a novel point of view for understanding the pathogenesis of IUGR. Twin pregnancies and selective intrauterine growth restriction (sIUGR) represent a model of unique opportunity to mimic a scientific experiment to study IUGR and, reflecting nutritional stresses within a similar genetic fetal background, to distinguish between genetic and environmental causes of phenotypic variations in the human population [24]. The cord blood metabolomic profiles in sIUGR twins with Doppler abnormalities showed a trend for increase of sphingosine compared with AGA co-twins. Sphingosine is an aliphatic amino alcohol component of all sphingoglycolipids; it is also expressed in the cardiovascular system and may be involved in the pathophysiology of diseases associated with endothelial dysfunction, expressed by an increase of aorta intima media thickness [25]. The results were confirmed in twins. A metabolomic analyis performed on fetal blood samples of monochorionich twins (obtained from the umbilical vein immediately after fetal extraction) showed an up-regulation of phenylalanine, sphingosine, glycerophosphocholine and choline, and a down-regulation of valine, tryptophan, isoleucine, and proline in selective IUGR compared with AGA twins [26]. Moreover, in a study on preterm infants [27], metabolomics showed how the myoinositol content was higher in urine of IUGR fetuses compared to controls. The increase in myoinositol concentration in plasma and urine, which has often been associated with glucose intolerance and insulin resistance in adults, could also be considered as a valid marker of altered glucose metabolism during fetal development in IUGR [28].

### "OMICS" in Preeclampsia

Finally, omics technique is useful in another obstetric disease as preeclampsia, which is characterized by gestational hypertension, proteinuria, and other systemic disturbances, occurring after the 20th weeks of gestation. It affects up to 8% of all pregnancies and it is an important cause of maternal and perinatal morbidity and mortality worldwide. Its pathophysiology is unknown, but high levels of neurokinin B in cytotrophoblast seems to suppress the activity of other proteins like thioredoxin, cyclophilin A, cytokeratin 1 and peroxiredoxin 5, that play an important role in regulating cell antioxidant defenses, and to decrease levels of cytokeratin 1 and annexin 11, that inhibit intravascular coagulation [29]. Another study performed by Gharesi-Fard et al showed that chloride intracellular channel 3, apolipoprotein A-I, transthyretin, and protein disulfide isomerase are up-regulated in the placental tissue of women with pre-eclampsia, with decreased expression of other proteins, including peroxiredoxin 2, peroxiredoxin 3, Cu/Zn-superoxide dismutase, actin gamma 1 propeptide, and chain A of enoyl-coenzyme A hydratase. These authors hypothesized that all those protein dysfunctions can lead to disorders in antioxidant activities, playing a role in the pathogenesis of preeclampsia [30]. Recently, the use of metabolomics permits to discover significant differences in 70 proteins within the trophoblastic cells of women with pre-eclampsia when compared with healthy control women. Thirty-one of these proteins were down-regulated and 39 were up-regulated, helping in the early diagnosis of pre-eclampsia and classification of the patients in the first trimester [31]. Furthermore, as preeclampsia has been now linked to higher incidence of cardiovascular disease, diabetes, and several other disorders in later life, so the offspring also exhibits an elevated risk of cardiovascular disease, stroke, and mental disorders during adulthood. This suggests that preeclampsia not only exposes the mother and the fetus to complications during pregnancy but also programs chronic diseases [32].

In conclusion, Omics covers a variety of methodologies and procedures whose aim is the identification of molecules in specific biological samples. This approach can lead to new hypothesis-based medicine and provide a ''shortcut'' to obtain new biological insight. Metabolomic technologies give us the possibility to measure several metabolites in biological fluids, becoming a possible diagnostic and/or prognostic tool that has the potential to significantly drive the management of cardiovascular disease and to represent the therapeutic target.

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