Metabolomics in Prenatal Medicine: The Sardinian Experience

Giovanni Monni¹, Federica Murgia², Valentina Corda³, Ambra Iuculano⁴, K Joseph Hurt⁵, Luigi Atzori⁶

ABSTRACT

In recent years, the holistic view is becoming the dominant perspective for the resolution of medical issues. Metabolomic approaches offer new opportunities to enhance our understanding of fetal biology and the multifactorial impact of perinatal diseases. Although metabolomics in maternal-fetal biology is increasing, it is not yet widely applied in perinatal medicine. Our group has been at the forefront of applying these powerful approaches to understanding pregnancy and fetoplacental development. Dynamic interactions among the fetus, the placenta, and the mother have been characterized by metabolite studies in various reports since the 1960s. Readily accessible maternal and fetal samples of blood, urine, vaginal secretions, amniotic fluid, cord blood, and placenta enabled investigation of complex maternal-fetal physiology, but metabolite assays and statistical approaches were rudimentary. Nonetheless, several publications highlighted correlations between biomarkers in these compartments and pregnancy complications such as fetal malformations, preterm labor, premature rupture of membranes, preeclampsia, and gestational diabetes mellitus. However, metabolomics offers a far more powerful method, tracking many biomarkers simultaneously in a single experiment and identifying changes in whole pathways or networks of pathways, thus emphasizing physiologic and clinical findings that may more rapidly explain the underlying biology.

Compared with traditional biochemical assays and single metabolite approaches, metabolomics may yield more rapid progress. Here, we report our metabolomics studies in three separate prenatal investigations utilizing chorionic villus sampling and amniocentesis samples obtained in our busy prenatal diagnostic center. In our first study, we used metabolomics to characterize first-trimester placentae obtained by transabdominal chorionic villus sampling (TA-CVS). The CVS specimens are an ideal source for these studies as they can be obtained from the undisturbed, sterile placenta at an early time point in pregnancy (in contrast to placental tissue obtained from term birth, miscarriage, or termination). We obtained both normal and aneuploid placentae.

Normal controls included women with advanced maternal age above 35 years as their only indication for TA-CVS and euploid fetal karyotype. Aneuploid cases included fetuses with trisomy 21, trisomy 18, and trisomy 13 confirmed by cytogenetics. We extracted the CVS tissue and performed nuclear magnetic resonance (NMR), gas chromatography-mass spectrometry (GC-MS), and high-performance liquid chromatography (HPLC) analyzes. In the multivariate statistical analysis, we found significant correlations between the metabolic profile of normal euploid fetuses and their crown-rump length (CRL). Particularly, myoinositol and inositol levels correlated linearly with CRL, suggesting a developmental role in early pregnancy and possible involvement in glucose homeostasis, which has been reported by others. We also identified correlations between CRL and increased levels of glutamine, citrate, glycerol, dehydroascorbic acid, and ribitol, and decreased levels of xylitol, 1,5-anhydro-D-sorbitol, D-fructose, and D-mannose. These findings suggested the crucial role of the energetic and polyols pathways that, interestingly, we observed as fundamental also in the following

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analysis. Indeed, next we compared the metabolome of the euploid and aneuploid specimens at matched gestational ages. We identified a unique metabolic signature in the aneuploid CVS tissue, with prominent differences in energy metabolic pathways such as glycolysis and gluconeogenesis, the pentose phosphate pathway (PPP), pyruvate metabolism, and the tricarboxylic acid cycle. Moreover, polyol pathways appeared to be excessively activated in the aneuploid placenta, perhaps indicating increased oxidative stress. Indeed, when we compared aminothiol levels, the aneuploid tissue showed decreased antioxidant glutathione and increased dehydroascorbic acid.

These findings may suggest that metabolomic study of TA-CVS samples could be helpful in establishing placental/fetal maturity, diagnosing aneuploidy, or identifying early placental dysfunction (e.g., risk for fetal growth restriction in otherwise normal fetuses).

Our second study of prenatal metabolomics expanded on the findings above by examining metabolic changes in relation to fetal nuchal translucency (NT) measurement. The NT measurement in the first trimester from 11 to 14 weeks’ gestational age in combination with maternal blood hormone screening is highly effective in identifying abnormal fetuses. The presence of a thickened NT increases the risk of fetal aneuploidy, genetic syndrome, congenital infection, and anatomic malformation. However, thickened NT is not inherently pathological as it is found in some normal fetuses. This may mean that several different mechanisms may produce this sonographic marker, and metabolomic investigation could help identify unique underlying pathogenic processes. We collected amniotic fluid samples (AFS) from 16 to 18 weeks of gestation from women with the only indication of advanced maternal age above 35 years and women diagnosed with increased NT greater than the 95th percentile during first trimester screening. We excluded fetuses with abnormal karyotype or structural anomalies. Comparing control and increased NT AFS groups showed significant differences by NMR profiling and the HPLC aminothiol analysis. The most altered pathways in the increased NT group involved alanine, aspartate, glutamate, nitrogen, arginine, pyruvate, and proline metabolism. Glycolysis and gluconeogenesis were also abnormal in the increased NT fetuses. These findings imply increased glycolysis in otherwise normal fetuses with increased NT compared with gestational age-matched controls. Those fetuses persistently maintained glycolytic pathways as their primary energy source. Moreover, increased lactate and decreased antioxidant metabolites in the increased NT fetuses suggests increased oxidative stress, which was confirmed by decreased levels of GSH and ascorbic acid. These findings may have important implications for developmental changes in fetuses with increased NT and perhaps altered fetal programming leading to future health effects.

In our third perinatal metabolomics study, we evaluated metabolic changes in a cohort of pregnancies affected by β-thalassemia. In early gestation, phenotypic and genetic heterogeneity create challenges in the β-thalassemia assessment. Pathologic or environmental stressors can influence gene expression and may have widespread effects on metabolic markers. Therefore, we compared the CVS metabolome of three groups of fetuses using GC-MS: homozygous β-thalassemia, heterozygous β-thalassemia, and unaffected normal. Statistical comparisons demonstrated significant differences between the controls and of both the β-thalassemia groups, but surprisingly there was no difference between the heterozygous and homozygous β-thalassemia fetuses. We identified a specific “metabolic fingerprint” for each group, showing that the most abnormal pathways in the homozygotes involved arachidonate, glutamine and glutamate, alanine and aspartate, PPP, glycolysis, and gluconeogenesis. Altered PPP in β-thalassemia suggests high cellular demand for ribose 5-phosphate (for nucleotide synthesis and embryonic development) and nicotinamide adenine dinucleotide phosphate production (for antioxidant maintenance). Oxidative stress is an
important mechanism for β-thalassemia pathophysiology, so changes associated with oxidation and cellular redox protection are not surprising.

As illustrated by the three reports described above, omics approaches and network analysis in perinatal physiology hold promise for a new generation of prenatal biomarkers and potential therapeutics. Complex fetal metabolic pathways cannot be studied in isolation or via selected individual metabolite measurements.

Metabolic approaches offer new opportunities to enhance our understanding of fetal biology and the multifactorial impact of perinatal diseases.

Further investigation of prenatal samples using these high-throughput techniques is likely to enhance our knowledge of fetal pathology and improve our ability to diagnose and treat diseases of pregnancy.

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REFERENCES
1. Monteiro MS, Carvalho M, Bastos ML, et al. Metabolomics analysis for biomarker discovery: advances and challenges. Curr Med Chem 2013;20(2):257–271. DOI: 10.2174/092986713804806621.
2. Baker M. Metabolomics: from small molecules to big ideas. Nat Methods 2011;8(2):117–121. DOI: 10.1038/nmeth0211-117.
3. Heazell AEP, Brown M, Worton SA, et al. Review: the effects of oxygen on normal and pre-eclamptic placental tissue—insights from metabolomics. Placenta 2011;32:S119–S124. DOI: 10.1016/j.placenta.2010.12.001.
4. Desì A, Marincola FC, Fanos V. Metabolomics and the great obstetrical syndromes – GDM, PET, and IUGR. Best Pract Res Clin Obstet Gynaecol 2015;29(2):156–164. DOI: 10.1016/j.bpobgyn.2014.04.023.
5. Woodham PC, O’Connell T, Grimes J, et al. Metabolomics to predict severe preeclampsia in early pregnancy. Am J Obstet Gynecol 2012;206(1):S348. DOI: 10.1016/j.ajog.2011.10.809.
6. Tissot van Patot M, Murray AJ, Beckey V, et al. Human placental metabolic adaptation to chronic hypoxia, high altitude: hypoxic preconditioning. Am J Physiol—Regul Integrat Comparat Physiol 2010;298(1):R166–R172. DOI: 10.1152/ajpregu.00383.2009.
7. Díaz SO, Pinto J, Graça G, et al. Metabolic biomarkers of prenatal disorders: an exploratory NMR metabolomics study of second trimester maternal urine and blood plasma. J Proteome Res 2011;10(8):3732–3742. DOI: 10.1021/pr200352m.
8. Akoleshar R, Mandal R, Bahado-Singh RO, et al. First-trimester metabolicomic detection of late-onset preeclampsia. Am J Obstet Gynecol 2013;208(1):58.e1–58.e7. DOI: 10.1016/j.ajog.2012.11.003.
9. Kenny LC, Broadhurst DI, Dunn W, et al. Robust early pregnancy prediction of later preeclampsia using metabolomic biomarkers. Hypertension 2010;56(4):741–749. DOI: 10.1161/HYPERTENSIONAHA.110.157297.
10. Romero R, Mazaki-Tovi S, Vaisbuch E, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. J Matern Fetal Neonatal Med 2010;23(12):1344–1359. DOI: 10.3109/14767058.2010.482818.
11. De Seymour JV, Conlon CA, Sulek K, et al. Early pregnancy metabolic profiling discovers a potential biomarker for the subsequent development of gestational diabetes mellitus. Acta Diabetol 2014;51(5):887–890. DOI: 10.1007/s00592-014-0626-7.
12. Graca G, Diaz SO, Pinto J, et al. Can biofluids metabolic profiling help us to improve health care during pregnancy? Spectroscopy 2012;27:515–523. DOI: 10.1155/2012/128367.
13. Monni G, Mur gia F, Corda V, et al. Metabolomics Application in Fetal Medicine. Perinatology - Evidence-Based Best Practices in Perinatal Medicine. Springer Publishers; 2020. In press.
14. Monni G, Corda V, Iuculano A, et al. The decline of amniocentesis and the increase of chorionic villus sampling in modern perinatal medicine. J Perinat Med 2020(4). DOI: 10.1515/jpm-2020-0035 Online ahead of print.
15. Monni G, Peddes C, Iuculano A, et al. From prenatal to preimplantation genetic diagnosis of β-thalassemia. Prevention model in 8748 cases: 40 years of single center experience. J Clin Med 2018;7(2):35. DOI: 10.3390/jcm7020035.
16. Monni G, Iuculano A. Re: ISUOG practice guidelines: invasive procedures for prenatal diagnosis. Ultrasound Obstet Gynecol 2017;49(3):414–415. DOI: 10.1002/uog.17375.
17. Murgia F, Iuculano A, Peddes C, et al. Metabolic fingerprinting of chorionic villous samples in normal pregnancy and chromosomal disorders. Prenat Diagn 2019(10):1–11. DOI: 10.1002/pd.5461.
18. Catalano PM, Roman-Drago NM, Amini SB, et al. Longitudinal changes in body composition and energy balance in lean women with normal and abnormal glucose tolerance during pregnancy. Am J Obstet Gynecol 1998;179(1):156–165. DOI: 10.1016/S0002-9378(98)70267-4.
19. Iuculano A, Murgia F, Peddes C, et al. Metabolic characterization of amniotic fluids of fetuses with enlarged nuchal translucency. J Perinat Med 2019;47(3):311–318. DOI: 10.1515/jpm-2018-0314.
20. Nicolaides KH, Azar G, Byrne D, et al. Fetal nuchal translucency: Ultrasound screening for chromosomal defects in first trimester of pregnancy. Br Med J 1992;304(6831):867–889. DOI: 10.1136/bmj.304.6831.867.
21. Snijders RJ, Noble P, Seb ire N, et al. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency thickness at 10–14 weeks of gestation. Lancet 1998;351(9125):343–346. DOI: 10.1016/S0140-6736(98)70180-6.
22. Shipp TD, Benacerraf BR. Second trimester ultrasound screening for chromosomal abnormalities. Prenat Diagn 2002;22(4):296–307. DOI: 10.1002/pd.307.
23. Benacerraf BR. The role of the second trimester genetic sonogram in screening for fetal down syndrome. Semin Perinatol 2005;29(6):386–394. DOI: 10.1053/j.semperi.2005.12.003.
24. Kagan KO, Wright D, Baker A, et al. Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free beta-human chorionic gonadotropin and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 2008;31(6):618–624. DOI: 10.1002/uog.5331.
25. Monni G, Zopp MA, Ibbra RM, et al. Fetal nuchal translucency test for Down’s syndrome. Lancet 2007;350(9091):1631–1632. DOI: 10.1016/ S0140-6736(05)64050-0.
26. Monni G, Murgia F, Corda V, et al. Metabolic investigation of β-thalassemia in chorionic villi samples. J Clin Med 2019;8(6):798. DOI: 10.3390/jcm8060798.