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Selected Abstracts of the 11th International Workshop on Neonatology

FROM THE WOMB TO THE ADULT

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The Workshop has been organized with the patronage of the Italian Society of Neonatology (SIN), the Italian Society of Pediatrics (SIP), the Italian Society of Perinatal Medicine (SIMP), The Italian Federation of Pediatricians (FIMP), the Union of European Neonatal and Perinatal Societies (UENPS), the Union of Mediterranean Neonatal Societies (UMENS), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), and lastly the Italian National Observatory of Residents in Paediatrics (ONSP).

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PRIMARY HCMV INFECTION IN PREGNANCY: PRELIMINARY METABOLOMIC DATA ON AMNIOTIC FLUID

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INTRODUCTION

Human cytomegalovirus (HCMV) is the most common cause of congenital viral infection leading to sensorineural hearing loss, and neurodevelopmental delay. Intrauterine transmission may follow either primary or recurrent infection. Primary maternal infection has a risk of vertical transmission of 30% to 35%, within the entire period of gestation.

Recently, transcriptomics, proteomics and metabolomics have been applied on biological samples in order to better understand the physiopathology or the mechanisms of the several diseases. In particular, metabolomics is the study of the entire range of low molecular weight molecules present in an organ, tissue, or biofluid. To our knowledge, only one paper was reported on the metabolic effects of HCMV on congenitally infected newborns. Such study dealt with the analysis of urine samples, collected within the first two weeks of life, applying ^{1}H-nuclear magnetic resonance (NMR) spectroscopy followed by multivariate statistical analysis [1]. In this study we propose a GCMS based metabolomics carachterization of the Amniotic Fluid (AF) in a population of pregnant women, which contracted primary HCMV infection during pregnancy.

A retrospective cohort study was conducted on 20 pregnant women, which transmitted the virus to the fetus ("transmitters"). Institution Review Board was requested for this retrospective study. A written informed consent was obtained from each woman undergoing invasive procedures. Amniotic fluid (AF) was collected from all pregnant women through amniocentesis. Results of prenatal diagnosis were confirmed from urine analysis at birth or from autopsy specimens obtained after the termination of pregnancy. AF not required for virological testing was aliquoted and stored at -80° until metabolomic analysis. In parallel, AF samples from 23 healthy pregnant women who underwent amniocentesis for cytogenetic-based diagnosis were included as controls. At enrolment, median age in the two study groups was 34 for transmitters, and 37 years for controls.

AF samples were thawed at room temperature and vortex mixed to homogenize. 200 µL were transferred in Eppendorf tubes (1.5 mL) and treated with 400 µL of acetone for protein precipitation. The mixture was vortexed for 30 s and centrifuged (1,400 rpm for 10 min). 400 µL of supernatant were transferred in glass vials and evaporated to dryness overnight in an Eppendorf vacuum centrifuge. 60 µL of a 0.24 M solution of methoxylamine hydrochloride in pyridine was added to each vial, samples were vortex mixed and left to react for 17 h at room temperature. Then 80 µL of MSTFA (*N*-Methyl-*N*-trimethylsilyltrifuoroacetamide) were added and left to react for 1 h at room temperature. The derivatized samples were diluted with hexane (100 µL) just before GC-MS analysis.

Samples were analyzed using a Agilent 5975C interfaced to the GC 7820 equipped with a DB-5ms column (J & W), injector temperature at 230°C, detector temperature at 280°C, helium carrier gas flow rate of 1 ml/min. The GC oven temperature program was 90°C initial temperature with 1 min hold time and ramping at 10°C/min to a final temperature of 270°C with 7 min hold time. 1 µL of the derivatized sample was injected in split (1:20) mode. After a solvent delay of 3 minutes mass spectra were acquired in full scan mode using 2.28 scans/s with a mass range of 50-700 Amu.

Each acquired chromatogram was analysed with the free software AMDIS (Automated Mass Spectral Deconvolution and Identification System): each peak was identified by comparison of the relative mass spectrum and retention time with those stored in an in-house made library of 255 metabolites. Other metabolites were identified using the database NIST08 (National Institute of Standards and Technology's mass spectral database) and the

Golm Metabolome Database (GMD). Through this strategy 58 compounds were detected and quantified: 50 identified, 4 unknown compounds matching equally unknown compounds contained in GMD, and 4 unknown metabolites which recurred in every sample.

Multivariate model based on Principal components analysis (PCA) was conducted in SIMCAp12 and used to overview the data variance structure in an unsupervised mode. Data have also been processed with the web tool MetaboAnalyst to confirm the previously obtained results from SIMCAP12+,and to investigate the canonical pathways involved. Subsequently, network mapping was used for the interpretation of multivariate results within a biological context: a biochemical and chemical similarity network was constructed among all the measured metabolites, applying KEGG and PubChem CID identifiers, and using a homemade routines for R.

RESULTS

A partial least squares discriminant analysis (PLS-DA) model was developed to identify important multivariate discriminant variables of control vs. transmitters amniotic fluid metabolomics profiles with a ROC curve (WEB Software MetaboAnalyst) reported in **Fig.1** with area under the curve of 0.9.

The most important identified metabolites of interest with VIP value > 1 for this model include: Serine, Urea and Glutamic acid.

Using these informations the MetaboAnalyst program calculates the most impacted human pathways involved in the discriminating model. Finally, using homemade routine in R and the Cytoscape software we have realized the network of metabolites of interested related to the differences between virus transmitters and control group. The primary contribution of the virus transmission is related to the "Alanine-Asparate" metabolism.

DISCUSSION AND CONCLUSIONS

Since more evidences are necessary to support the hypothesis about the condition for the virus transmission, metabolomics seems to be a suitable, powerful, and promising investigation tool. Indeed, strong correlations between specific metabolites and several gestational conditions (fetal malformations, preterm delivery, premature ruptures of membranes, gestational diabetes mellitus, preeclampsia, and so on) have been reported after the metabolomic analysis of AF, placenta, blood, and urine from pregnant women. To our knowledge, this is the first preliminary study on metabolomics profile of amniotic fluid in relation to HCMV infected fetuses.

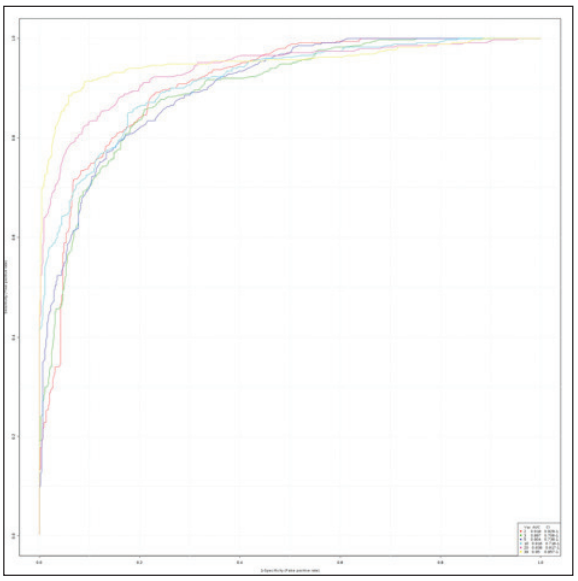

Figure 1 (ABS 33). Roc curve of the model for controls vs. transmitters (AUC = 0.9).

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ABS 34

NMR-BASED METABOLOMICS ANALYSIS OF URINARY CHANGES IN NEONATAL NECROTIZING ENTEROCOLITIS

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Necrotizing enterocolitis (NEC) is intestinal inflammation occurring mainly in preterm or sick neonates after enteral feedings have begun. The