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Profili proteici di campioni urinari nelle malattie respiratorie

Rossana Rossi, ITB-CNR Milano

EUROPEAN BIOTECH WEEK
INNOVATION IS IN OUR GENES

FEDERCHIMICA
ASSOBIOTEC
Associazione nazionale per lo sviluppo delle biotecnologie

PART OF GLOBAL BIOTECH WEEK

Coordinated by
CVBF
Consorzio per Valutazioni Biologiche e Farmacologiche

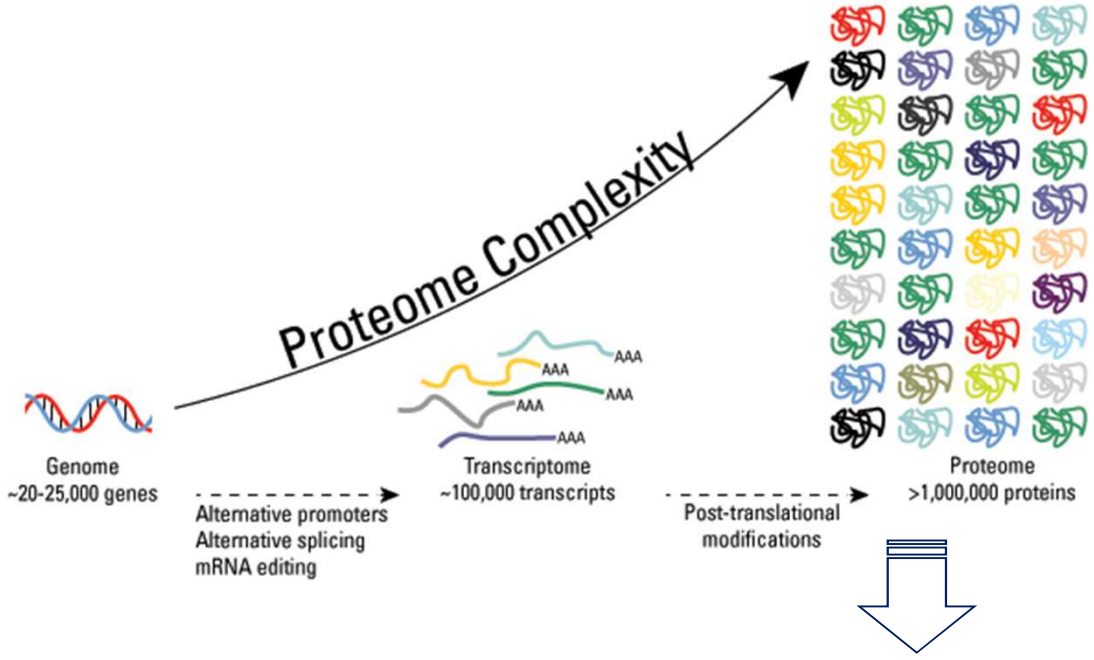
General Assembly led by
National Research Council of Italy

Scientific Committee led by
INCiPiT Italian Network for Paediatric Clinical Trials

Proteomics Mission

PROTEOMICS

large-scale comprehensive **study** of a specific proteome, including information on protein **abundances**, their variations and **modifications**, along with their **interacting** partners and **networks**, in order to understand cellular processes.



- one gene can encode more than one protein
- proteins are dynamic
- proteome differs from cell to cell and from time to time
- proteins are post-translationally modified
- proteins exist in a wide range of concentrations

WHY PROTEOMICS ?

genes are only the "recipes" of the cell, while the **proteins** encoded by the genes are ultimately the **functional players** that **drive both normal and disease physiology**

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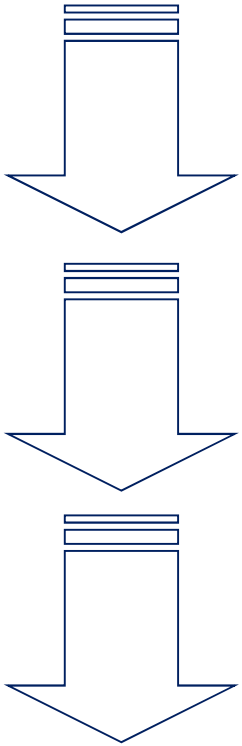
Clinical proteomics

CLINICAL PROTEOMICS

sub-discipline of proteomics that involves the **application of proteomic technologies** on clinical specimens

SHOTGUN PLATFORMS

- ↑ confidence
- ↑ sensitivity
- ↑ dynamic range



LC miniaturization

- more separative techniques
- easy automation
- high reproducibility

MS evolution

- Speed
- High Resolution
- Mass Accuracy

Bioinformatic

- Data handling
- Data mining
- Statistical Analysis

BIOMARKER DISCOVERY

- ✓ **patient stratification**
- ✓ **diagnosis** and **therapeutic monitoring**
- ✓ characterization of the behavior of **candidate drugs**

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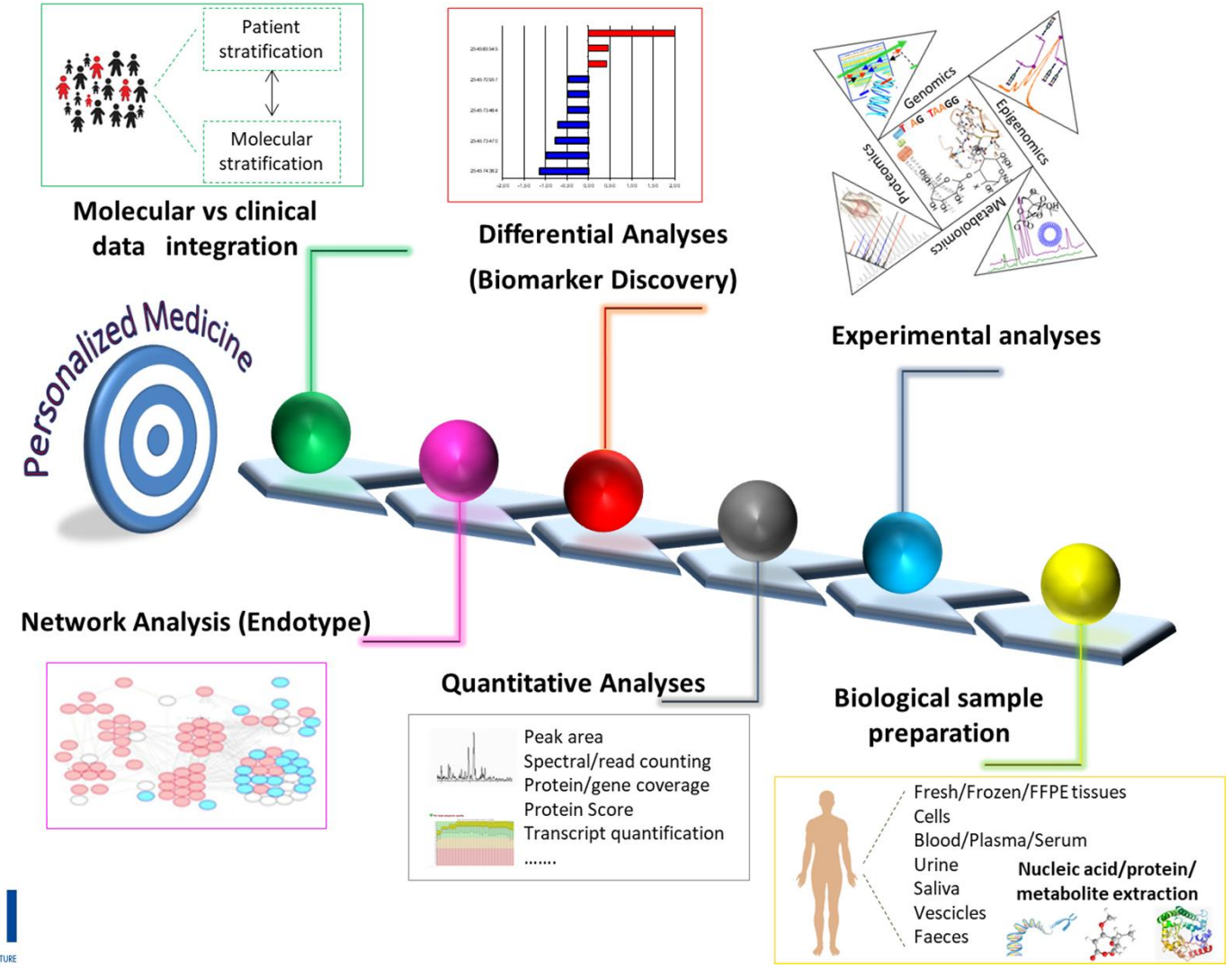
Clinical Proteomics research workflow



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Roffia V, et al. Pharma Horizon 2017; 1:59-64.

Samples and Fields of Application

Biospecimens

- antibody / IgG
- pull-down
- tears
- venom
- exosomes
- vescicles
- organelles
- saliva
- balf
- sputum
- EBC
- CSF
- urine
- cell lines
- blood
- tissues

Sources

- Human
- Pig
- Mice/Rats
- Microrganisms:
  - Yeasts
  - Bacteria
- Apis
- Parasites
- Plants

Applications

- Cancer
- Respiratory diseases
- Neurodegenerative diseases
- Cardiovascular diseases
- Amyloidosis
- Microorganisms

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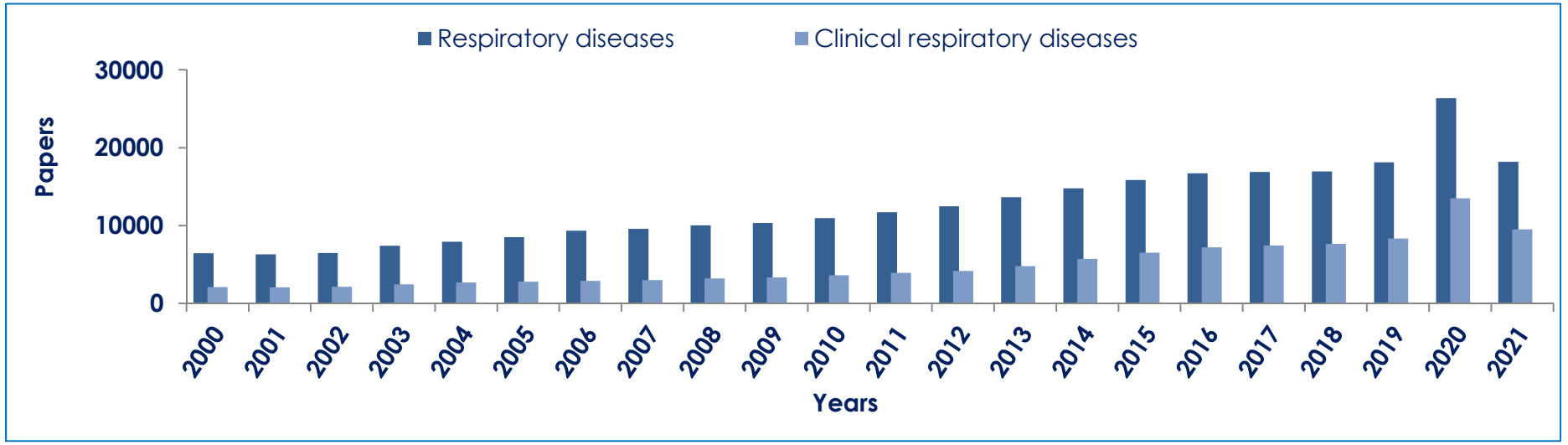
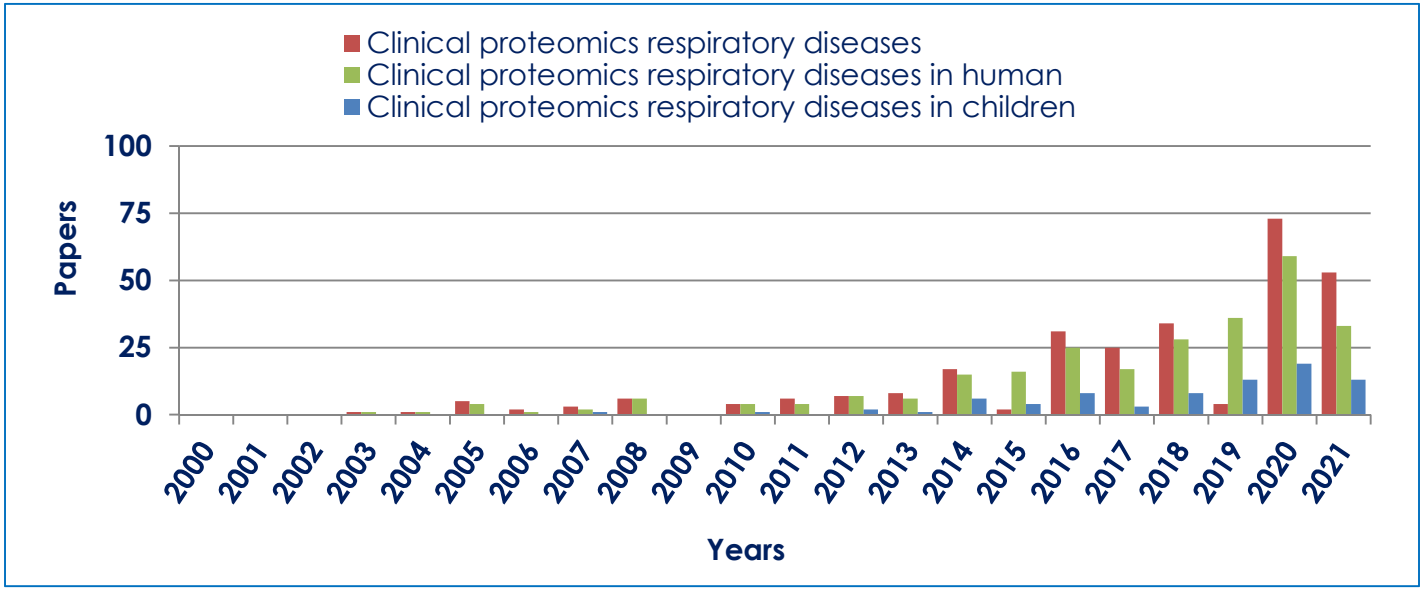
Respiratory diseases





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Proteomic approaches

Human Sample source

- → **Bronchial fluids**
  - → BALF
  - → exhaled breath condensate
  - → Induced sputum
- → **Body fluids**
  - → serum
  - → plasma
  - → Blood
  - → **urine**
- → **Cell cultures**
  - → nasal epithelial cell
- → **Biopsies**

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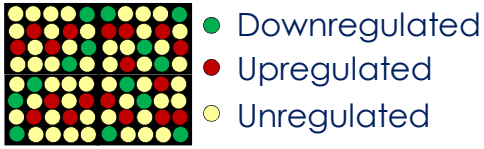
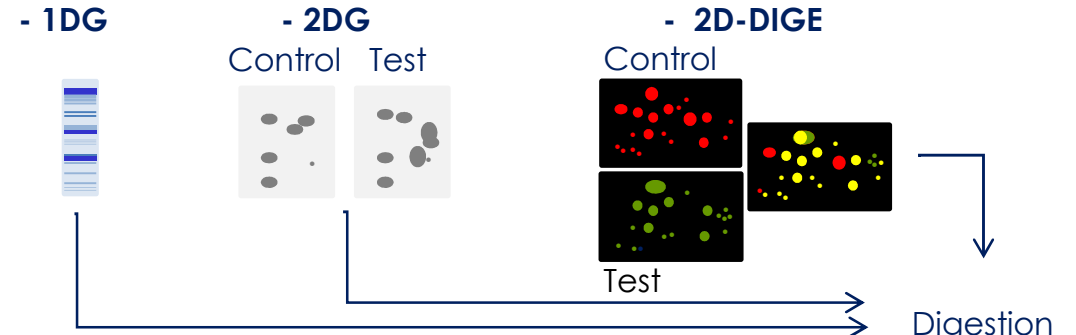
Proteomic approaches

A) Antibody-based methods



B) Peak profiling-MS based

C) Gel-MS based methods



D) Gel-free methods (LC-MS-based methods)

Urinary Proteomics

Review > Mol Cell Proteomics. 2008 Oct;7(10):1850-62. doi: 10.1074/mcp.R800001-MCP200.
Epub 2008 Jul 30.

Urine in clinical proteomics

Stéphane Decramer [1], Anne Gonzalez de Peredo, Benjamin Breuil, Harald Mischak, Bernard Monsarrat, Jean-Loup Bascands, Joost P Schanstra

Review > Adv Exp Med Biol. 2015;845:31-42. doi: 10.1007/978-94-017-9523-4_4.

Human urine proteome: a powerful source for clinical research

Lili Zou [1], Wei Sun

Review > Biochim Biophys Acta. 2014 May;1844(5):884-98. doi: 10.1016/j.bbapap.2013.06.016.
Epub 2013 Jul 2.

Urine as a source for clinical proteome analysis: from discovery to clinical application

Eva Rodríguez-Suárez [1], Justyna Siwy [2], Petra Zürbig [1], Harald Mischak [3]

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Sleep Medicine 7 (2006) 221–227

Urinary protein expression patterns in children with sleep-disordered breathing: Preliminary findings

Jyoti Krishna [a,1], Zahoor A. Shah [a,1], Michael Merchant [b], Jon B. Klein [b], David Gozal [a,*]

ELSEVIER

SLEEP MEDICINE

www.elsevier.com/locate/sleep

Abstract

Background: Obstructive sleep apnea (OSA) is the most common form of sleep-disordered breathing, with almost 15 million Americans affected and many more at risk. Current diagnostic approach to OSA requires polysomnography, which is laborious, onerous, and time-consuming. There is ample evidence that inflammatory responses to the perturbations associated with OSA trigger a variety of genes and signaling cascades that ultimately lead to end-organ injury and changes in kidney function and protein expression. The aim of this study was therefore to analyze proteins in human urine and assess whether differential expression of particular proteins is associated with the presence of OSA.

Methods: Eleven OSA and 11 control children between the ages of three and 14 (males = 17; females = 5) underwent overnight sleep studies followed by a first-morning urine sample. Proteomic analysis of urine samples yielded a unique map of proteins, of which, five spots were selected for further analysis due to their significant intensity differences between OSA and control groups.

Results: Mass spectrometry followed by peptide mass fingerprinting conclusively identified four of the five spots as gelsolin, perlecan (a heparan sulfate proteoglycan), albumin, and immunoglobulin ($P < 0.05$ and protein scores > 67).

Conclusions: Overall, increased expression of gelsolin and perlecan in the urinary proteome of children with OSA suggests that the episodic hypoxia associated with OSA may lead to changes in protein permeability or alternatively to increased catabolism of these proteins and their excretion in urine.

Table 2
Peptide mass fingerprinting identifications for proteins differentially expressed in morning urine samples from children with obstructive sleep apnea (OSA), and controls

Spot #	Most likely candidate	NCBI gi number	Observed pI range	Observed MW (kDa)	pI	MW (KDa)	Number peptides	Percent coverage	Spot volumes *P* value[a]
1	Gelsolin	4,504,165			5.9	85.7	11		
			4.5–4.8	43–47	5[a]	41.3[a]	11	26	0.00008
2	Perlecan	184,427			6.1	479.2	8	2	
			5.3–5.6	21.0	5.7[b]	19.92[b]	8	49	0.002
3	Albumin	4,389,275	4.8–5.3	67–68	5.7	66.0	19	30	NS
4	Tropomyosin	339,958	5.0–5.3	25–27	4.6	26.6	6	28	NS
5	IgG kappa chain	4,176,418	7.0–7.5	22–23	6.9	23.7	7	41	NS

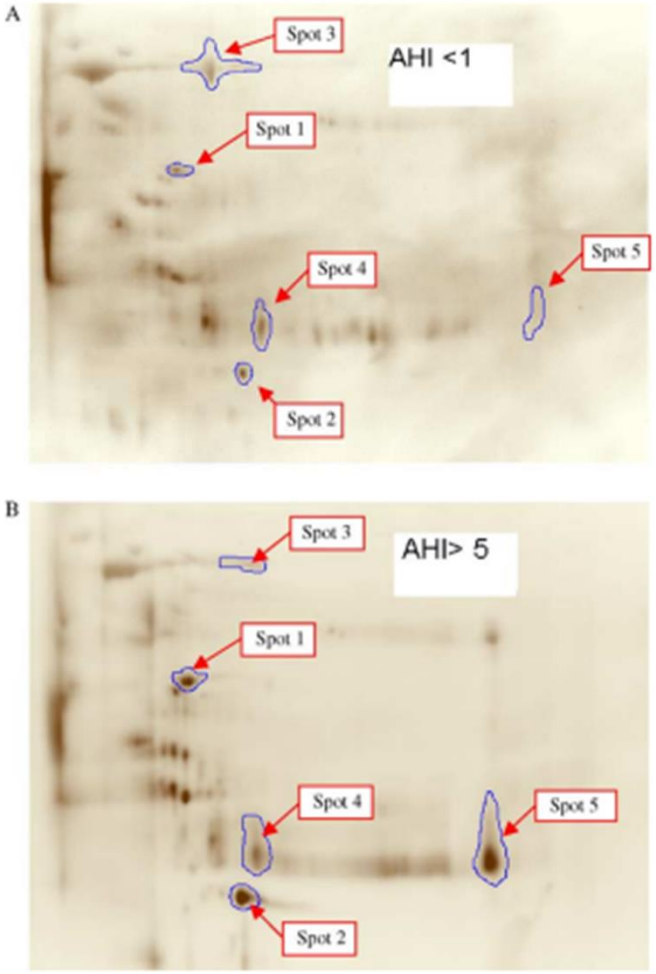

Fig. 1. Representative 2D gels of in a control subject (A) and an age- and gender-matched subject with OSA (B). (Note: spot five volume also appears increased in B; however, this was not consistent across all subjects.)

L'aumentata espressione sia di **gelsolina** che di **perlecan** nel proteoma urinario dei bambini con **OSA** suggerisce che l'ipossia episodica (diminuzione della saturazione di ossiemoglobina) può portare a **cambiamenti nella permeabilità delle proteine** o in alternativa ad un **aumento del catabolismo di queste proteine** e **della loro escrezione nelle urine.**

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Two-Dimensional Differential In-Gel Electrophoresis Proteomic Approaches Reveal Urine Candidate Biomarkers in Pediatric Obstructive Sleep Apnea

David Gozal[1], Saeed Jortani[2], Ayelet B. Snow[3], Leila Kheirandish-Gozal[1], Rakesh Bhattacharjee[3], Jinkwan Kim[3], and Oscar Sans Capdevila[3]

Am J Respir Crit Care Med Vol 180. pp 1253–1261, 2009
Originally Published in Press as DOI: 10.1164/rccm.200905-0765OC on September 24, 2009

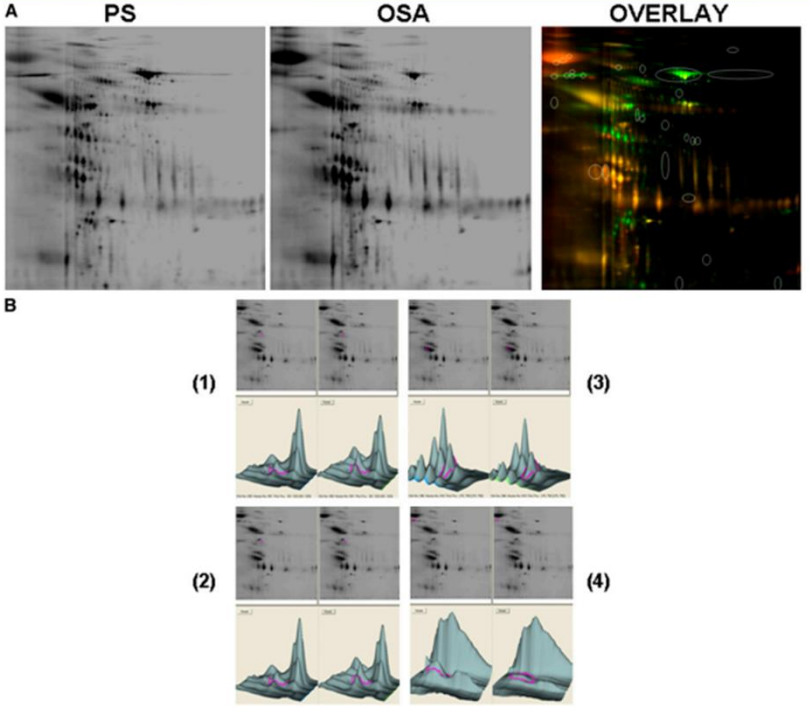
Rationale: Sleep studies are laborious, expensive, inaccessible, and inconvenient for diagnosing obstructive sleep apnea (OSA) in children.

Objectives: To examine whether the urinary proteome uncovers specific clusters that are differentially expressed in the urine of children with OSA.

Methods: Two-dimensional differential in-gel electrophoresis (2D-DIGE) and mass spectrometry proteomics followed by validation with western blot of ELISA.

Measurements and Main Results: Morning urine proteins from 60 children with polysomnographically confirmed OSA and from matched children with primary snoring (n = 30) and control subjects (n = 30) were assessed. A total of 16 proteins that are differentially expressed in OSA were identified, and 7 were confirmed by either immunoblots or ELISA. Among the latter, receiver–operator curve analyses of urinary concentrations of uromodulin, urocortin-3, orosomucoid-1, and kallikrein assigned favorable predictive properties to these proteins. Furthermore, combinatorial approaches indicated that the presence of values beyond the calculated cutoff concentrations for three or more of the proteins yielded a sensitivity of 95% and a specificity of 100%.

Conclusions: Proteomic approaches reveal that pediatric OSA is associated with specific and consistent alterations in urinary concentrations of specific protein clusters. Future studies aiming to validate this approach as a screening method of habitually snoring children appears warranted.



ure 1. (*A*) Two-dimensional differential in-gel electrophoresis (2D-DIGE) gels of urinary proteins of a child with obstructive sleep apnea (OSA) d a matched child with primary snoring (PS) and fluorescence image overlay. (*B*) Candidate spot analysis showing spot location on gel, differential nsity analysis for two spots showing increased expression in OSA (spots 1 and 2), and two spots in which decreased expression occurred (spots 3 d 4).

Figure 2. Representative Western blots for tenascin, tribbles homolog protein-2, and zinc finger protein-81 in urinary proteins from children with obstructive sleep apnea (OSA), those with primary snoring (PS), and healthy control subjects (CO).

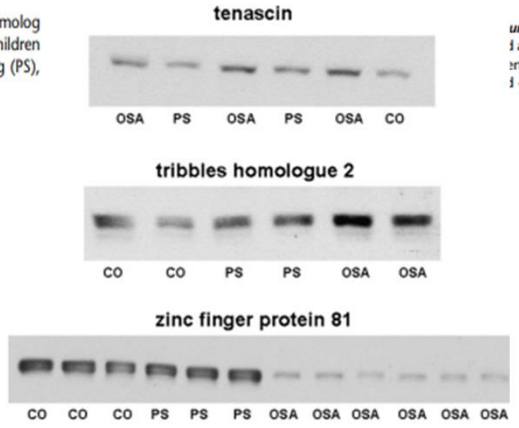


TABLE 3. URINARY PROTEINS ALTERED IN PEDIATRIC OBSTRUCTIVE SLEEP APNEA

Increased	Decreased
Uromodulin	Kallikrein
Urocortin-3	Zinc finger protein-81
Bikunin	Zinc finger protein-36/1
Tenascin	
Human Tribbles homolog-2	
Orosomucoid-2	
α_1-Microglobulin	
PCAF histone acetylase	
Prolyl hydroxylase domain	

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Two-Dimensional Differential In-Gel Electrophoresis Proteomic Approaches Reveal Urine Candidate Biomarkers in Pediatric Obstructive Sleep Apnea

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Am J Respir Crit Care Med Vol 180. pp 1253–1261, 2009
Originally Published in Press as DOI: 10.1164/rccm.200905-0765OC on September 24, 2009

→ **Anti-infiammatory activity**

- → Bikunin
- → Tenascin

→ **Oxidative stress and infiammatory activity**

- → Human Tribble homolog-2
- → Orosomucoid-2
- → PCAF Hystone acetylase:

→ **Protective activity**

- → Prolyl hydroxylase domain
- → Kallikrein-1
- → Zinc finger protein-81
- → Zinc finger protein-36/1

→ **Renal dysfunction**

- → Uromodulin
- → Urocortin-3
- → α1-Microglobulin (A1M)

Sulla base dei risultati precedenti (Krishma et al., Slep medicin 2006) e dei risultati attuali, è ragionevole presumere che **l'ipossia intermittente** e l'**aumento** globale dello **stress ossidativo dei processi infiammatori** attivati dall'OSA possono portare a una **lieve disfunzione renale.**

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Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

Investigation of urine proteome of preterm newborns with respiratory pathologies

Natalia L. Starodubtseva [a,b,1], Alexey S. Kononikhin [a,e,b,1], Anna E. Bugrova [a,c], Vitaliy Chagovets [a], Maria Indeykina [c,e], Ksenia N. Krokhina [a], Irina V. Nikitina [a], Yury I. Kostyukevich [b,c,e], Igor A. Popov [a,b,c,e], Irina M. Larina [d,b], Leila A. Timofeeva [a], Vladimir E. Frankevich [a], Oleg V. Ionov [a], Dmitry N. Degtyarev [a], Eugene N. Nikolaev [b,c,e,*], Gennady T. Sukhikh [a]

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[b] Moscow Institute of Physics and Technology, Moscow, Russia
[c] Emanuel Institute for Biochemical Physics, Russian Academy of Sciences, Moscow, Russia
[d] Institute of Biomedical Problems – Russian Federation State Scientific Research Center, Russian Academy of Sciences, Moscow, Russia
[e] V.L. Talrose Institute for Energy Problems of Chemical Physics, Russian Academy of Sciences, Leninskij pr. 38 k.2, 119334 Moscow, Russia

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Respiratory tract disorders
Intensive care

ABSTRACT

A serious problem during intensive care and nursing of premature infants is the invasiveness of many examination methods. Urine is an excellent source of potential biomarkers due to the safety of the collection procedure. The purpose of this study was to determine the features specific for the urine proteome of preterm newborns and their changes under respiratory pathologies of infectious and non-infectious origin. The urine proteome of 37 preterm neonates with respiratory diseases and 10 full-term newborns as a control group were investigated using the LC-MS/MS method. The total number of identified proteins and unique peptides was 813 and 3672 respectively. In order to further specify the defined infant-specific dataset these proteins were compared with urine proteome of healthy adults (11 men and 11 pregnant women) resulting in 94 proteins found only in infants. Pairwise analysis performed for label-free proteomic data revealed 36 proteins which reliably distinguished newborns with respiratory disorders of infectious genesis from those with non-infectious pathologies, including: proteins involved in cell adhesion (CDH-2,-5,-11, NCAM1, TRY1, DSG2), metabolism (LAMP1, AGRN, TPP1, GPX3, APOD, CUBN, IDH1), regulation of enzymatic activity (SERPINA4, VASN, GAPDH), inflammatory and stress response (CD55, CD 93, NGAL, HP, TNFR, LCN2, AGT, S100P, SERPINA1/C1/B1/F1).

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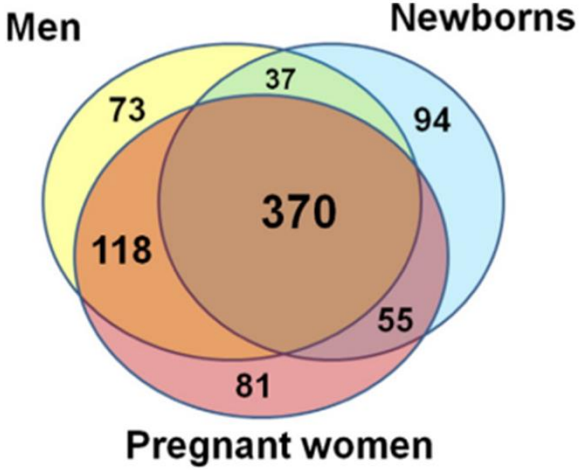

Fig. 1. Venn diagram for three control groups: healthy adults (11 men and 11 pregnant women) and healthy neonates (10 patients).

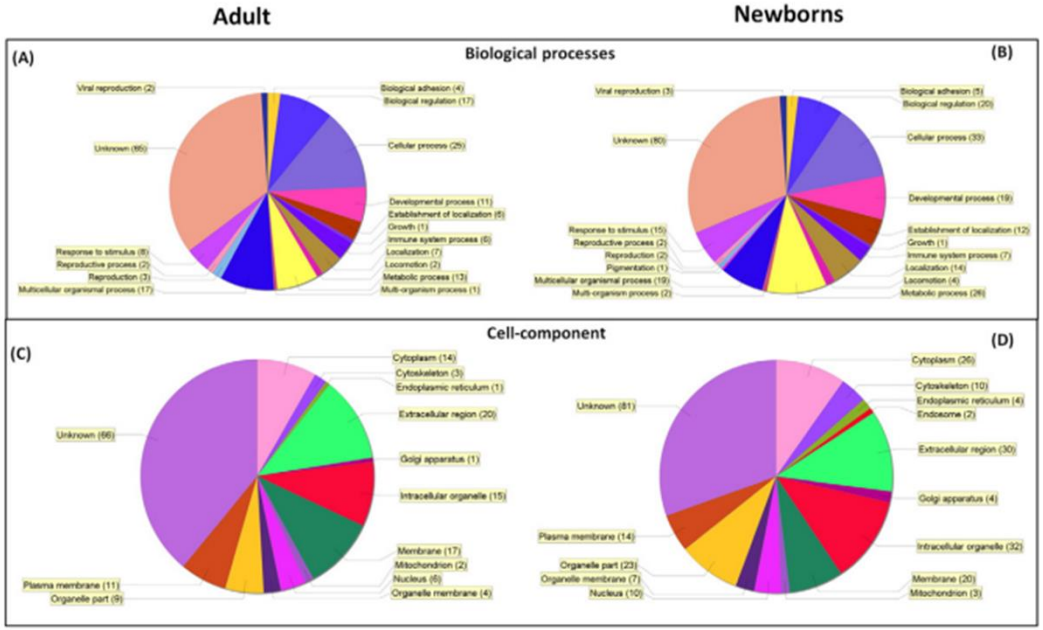

Fig. 2. Gene ontology (GO) enrichment diagrams for adults (11 men and 11 pregnant women) and healthy neonates (10 patients): biological processes – (A), (B); cell-component – (C), (D).

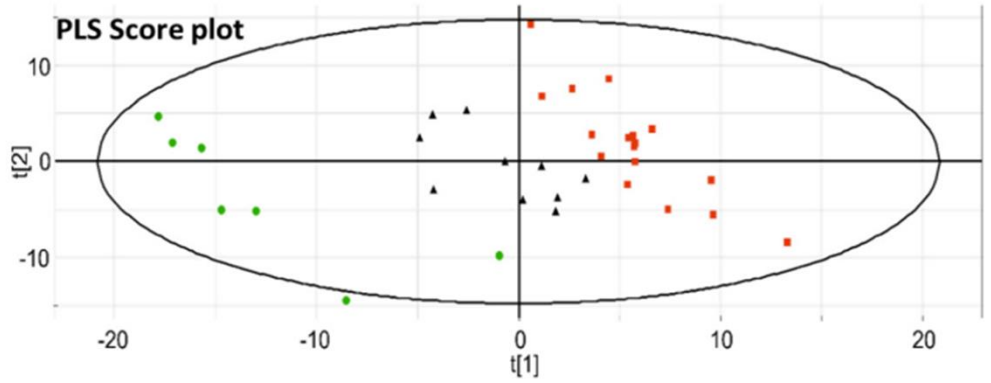

Fig. 3. Score plot of projections to latent structures (PLS) multivariate data analysis of protein intensities: ● – control group of term healthy neonates (green); ▲ – preterm neonates with respiratory disorders of a noninfectious origin (black); ■ – preterm neonates with congenital pneumonia (red).

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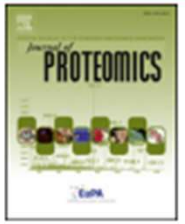

Table 1

Urine proteins that distinguish newborns with respiratory tract disorders according to the Mann-Whitney *U* test ($p < 0.05$). Average protein intensities for each group (group 1 – control healthy full-term newborns, group 2 – preterm newborns with respiratory disorders of non-infectious origin, group 3 – preterm newborns with congenital pneumonia) are given in columns Control, Non-inf, and Pneum for comparison.

Protein ID	Protein names	p-value	Control	Non-inf	Pneum
P30740	Leukocyte elastase inhibitor	0.001	1E+05	30,294	2E+06
P01009	Alpha-1-antitrypsin	0.002	9E+07	7E+07	1E+08
P01019	Angiotensinogen	0.002	8E+06	2E+07	1E+08
P33151	Cadherin-5	0.003	39,536	74,607	1E+06
P08138	Tumor necrosis factor receptor superfamily member 16	0.004	2E+06	3E+06	1E+06
Q96FE7	Phosphoinositide-3-kinase-interacting protein 1	0.005	4E+07	2E+07	1E+07
P80188	Neutrophil gelatinase-associated lipocalin	0.009	5E+05	5E+06	1E+07
P19022	Cadherin-2	0.010	2E+06	1E+06	5E+05
P36955	Pigment epithelium-derived factor	0.010	63,547	7E+06	4E+07
P07911	Uromodulin	0.012	1E+08	2E+08	8E+07
P55287	Cadherin-11	0.012	1E+07	7E+06	2E+07
P11279	Lysosome-associated membrane glycoprotein 1	0.012	0	0	2E+06
O00468	Agrin	0.013	1E+07	1E+07	2E+07
Q6UVK1	Chondroitin sulfate proteoglycan 4	0.013	5E+05	2E+06	7E+05
Q9Y279	V-set and immunoglobulin domain-containing protein 4	0.016	16,276	4E+05	2E+06
O14773	Tripeptidyl-peptidase 1	0.018	2E+06	1E+06	4E+05
P22352	Glutathione peroxidase 3	0.019	1E+06	1E+06	4E+06
P98164	Low-density lipoprotein receptor-related protein 2	0.022	5E+06	8E+06	2E+06
Q9NPY3	Complement component C1q receptor	0.022	0	2E+05	1E+06
P29622	Kallistatin	0.023	0	0	5E+05
P05090	Apolipoprotein D	0.024	3E+07	3E+07	1E+07
P00734	Prothrombin	0.028	1E+07	2E+07	9E+06
P02766	Transthyretin	0.028	2E+06	2E+06	7E+06
P04216	Thy-1 membrane glycoprotein	0.028	2E+05	3E+06	5E+06
O60494	Cubilin	0.029	4E+06	4E+06	2E+06
P08174	Complement decay-accelerating factor	0.033	2E+06	3E+06	1E+06
P25815	Protein S100-P	0.035	0	1E+06	39,998
P05451	Lithostathine-1-alpha	0.037	5E+05	3E+06	3E+06
P13591	Neural cell adhesion molecule 1	0.037	2E+05	6E+05	2E+05
P01008	Antithrombin-III	0.039	7E+06	6E+06	2E+07
Q14126	Desmoglein-2	0.039	0	2E+05	8E+05
P00738	Haptoglobin	0.040	2E+07	2E+06	7E+05
P18428	Lipopolysaccharide-binding protein	0.042	0	5E+05	5E+06
O75874	Isocitrate dehydrogenase [NADP] cytoplasmic	0.043	1E+06	2E+06	3E+05
Q6EMK4	Vasorin	0.045	2E+07	1E+07	6E+06
P04406	Glyceraldehyde-3-phosphate dehydrogenase	0.047	2E+06	2E+06	7E+05

- Cell adhesion
- Metabolism
- Regulation of enzymatic activity
- Inflammation and stress response

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molecules

MDPI

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promise

Article

Shotgun Proteomics of Isolated Urinary Extracellular Vesicles for Investigating Respiratory Impedance in Healthy Preschoolers

Giuliana Ferrante [1,†], Rossana Rossi [2,†], Giovanna Cilluffo [3], Dario Di Silvestre [2], Andrea Brambilla [4], Antonella De Palma [2], Chiara Villa [4], Velia Malizia [3], Rosalia Gagliardo [3], Yvan Torrente [4], Giovanni Corsello [1], Giovanni Viegi [3], Pierluigi Mauri [2,5,*] and Stefania La Grutta [3]

AIM:

LC-MS based proteomics characterization of urinary extracellular vesicles in order to establish whether different patterns of respiratory impedance in healthy preschoolers can be associated to a specific protein fingerprint.

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Workflow

molecules

Sample → nanoChip LC-MS/MS → Data handling → Data Interpretation

URINE

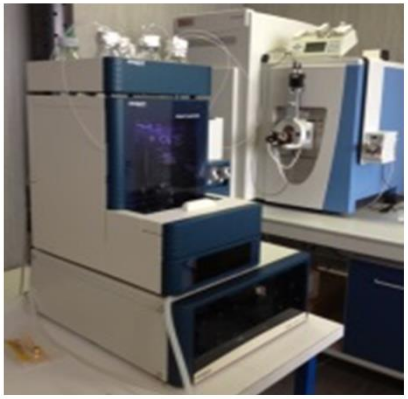
Ultracentrifugation

Extracellular Vesicles

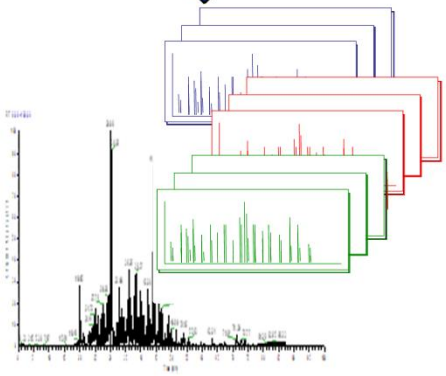
Protein extraction

Protein quantitation by SPN-Protein Assay

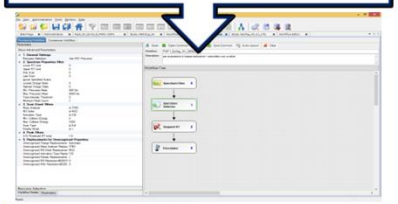
Trypsin Digestion



MS/MS spectra



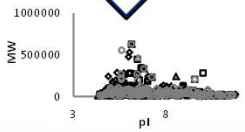
Processing Workflow



Consensus Workflow

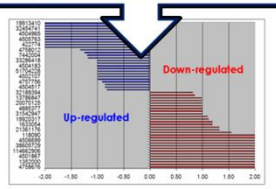
Protein list

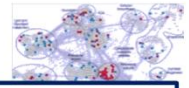
2D Virtual Map



Comparison

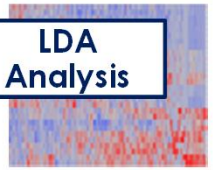
Differentially expressed proteins





Networks

LDA Analysis



Results: EVs characterization

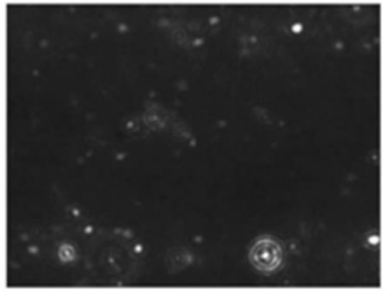
molecules

✓ Isolation of Urinary Extracellular Vesicles

a)

Preterm pregnancy		
Mean ± SD (nm)	Mode ± SD (nm)	Particles/mL
167.1 ± 54.7	96.1 ± 54.7	2.83×10^9
Term pregnancy		
Mean ± SD (nm)	Mode ± SD (nm)	Particles/mL
154.2 ± 54.0	96.8 ± 54.0	5.19×10^8

b)



c)

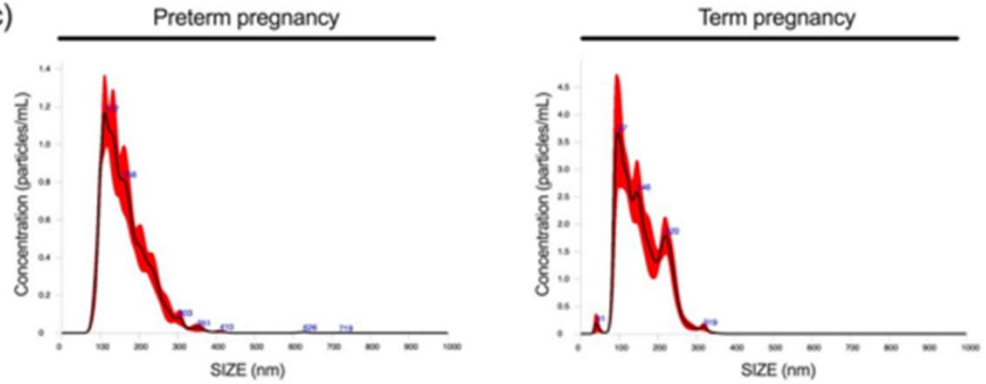


Figure 1. Schematic resume of the extracellular vesicle analysis carried out with nanosight NS300. The tables (**a**) collect the data regarding the measurements of preterm and term pregnancy; the highlighted data are the mean and the mode of the particles recorded by the instrument and the concentration per milliliter of the particles with a diameter lower than 200 nm. The picture (**b**) is a representative image taken during the extracellular vesicle analysis with nanosight. The graphs (**c**) are a simplified representation of the extracellular vesicle absolute size distribution and nanovesicle concentration per ml linked to their sizes; the red shadow is the standard deviation and the black line is the mean of 5 separate and consecutive analyses.

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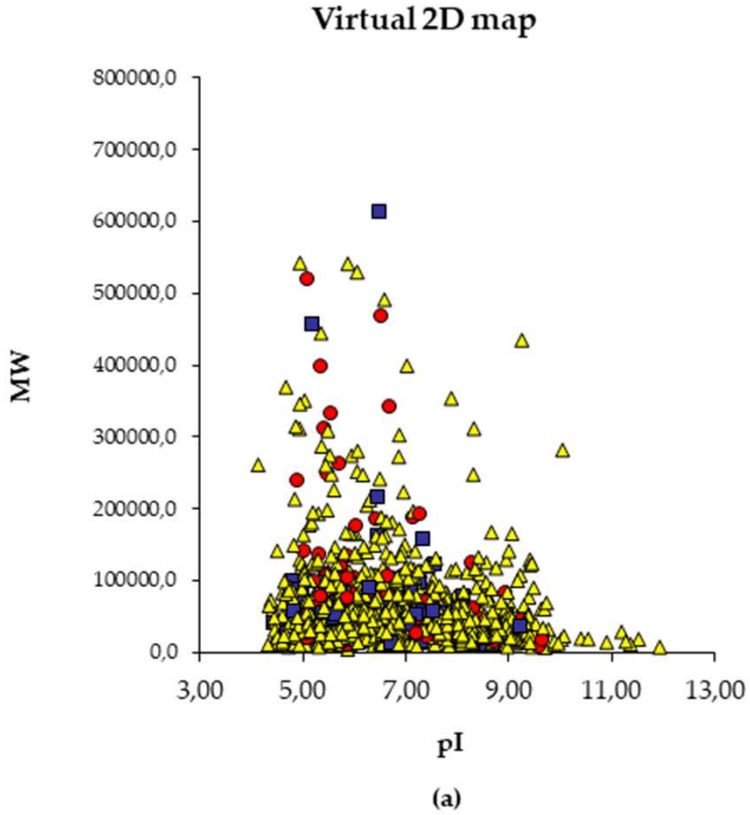
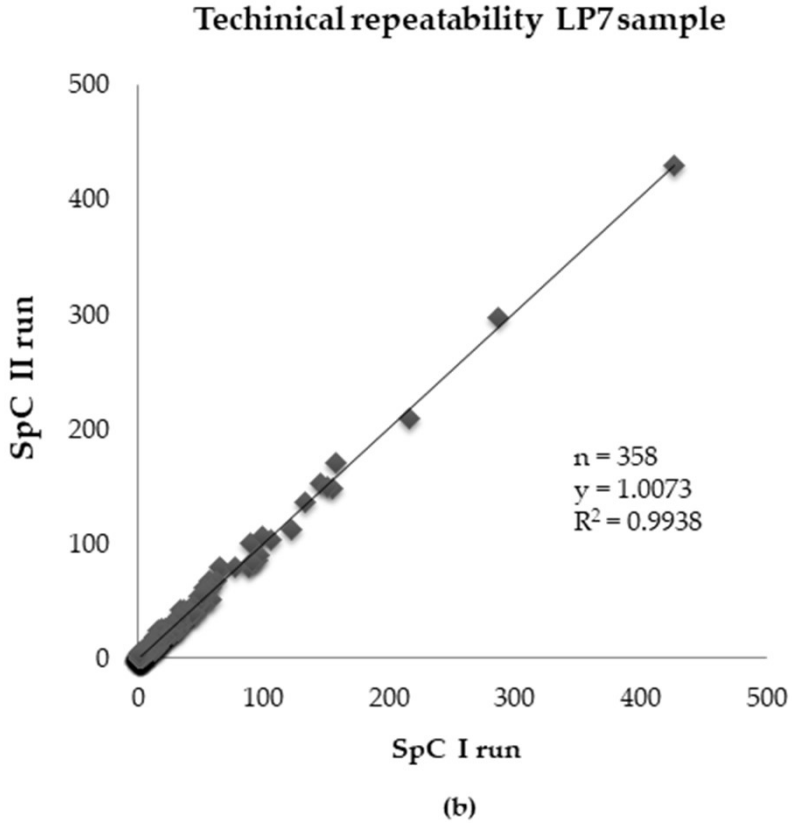
Results: Protein profiles

✓ **Protein profile**
Identification of urinary extracellular vesicles protein content.



✓ **NanoChip LC - MS/MS repeatability**



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Results: Statistical analysis

molecules

✓ **Cluster analysis**

Evaluation of protein profiles.

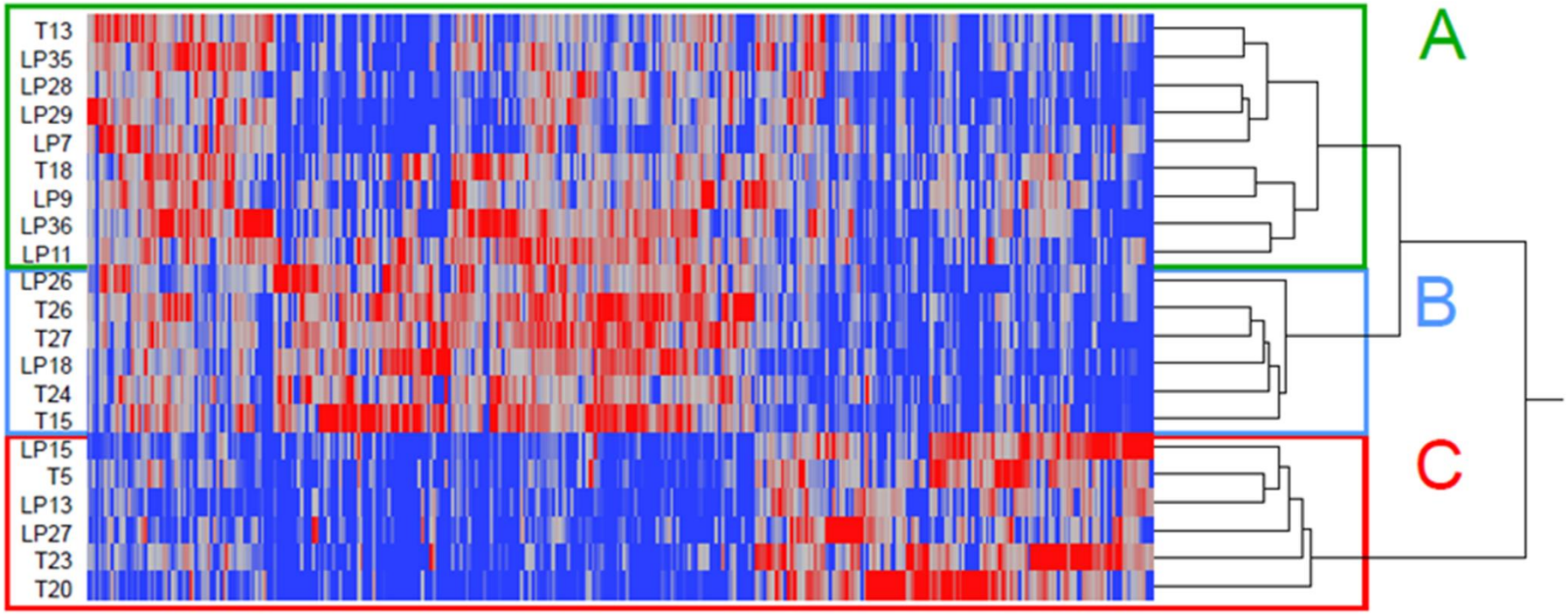

Figure 2. Linear Discriminant Analysis (LDA) placed children in three distinct groups called **A**, **B** and **C**.

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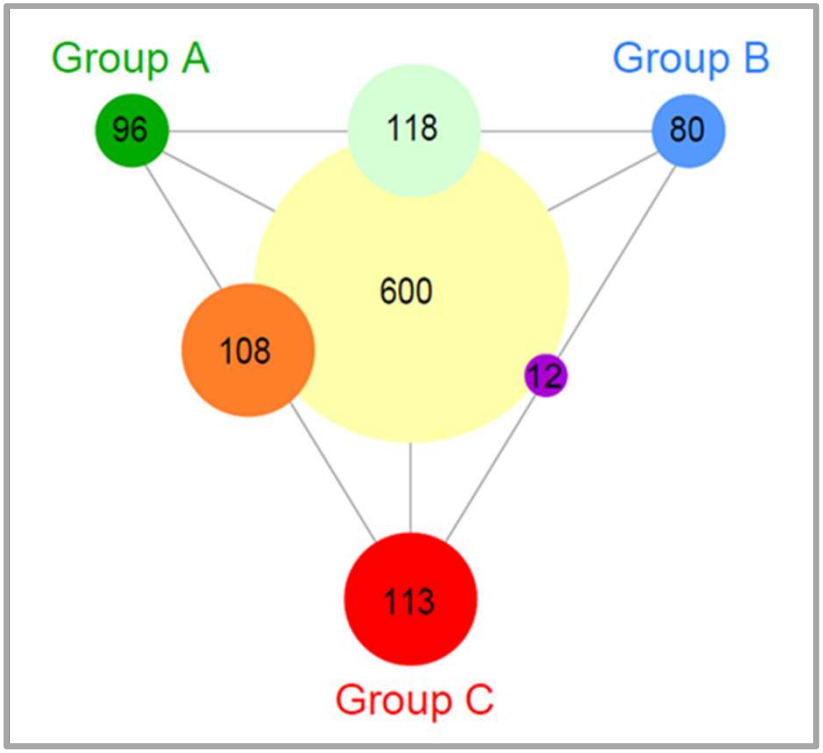
Results: Functional analysis

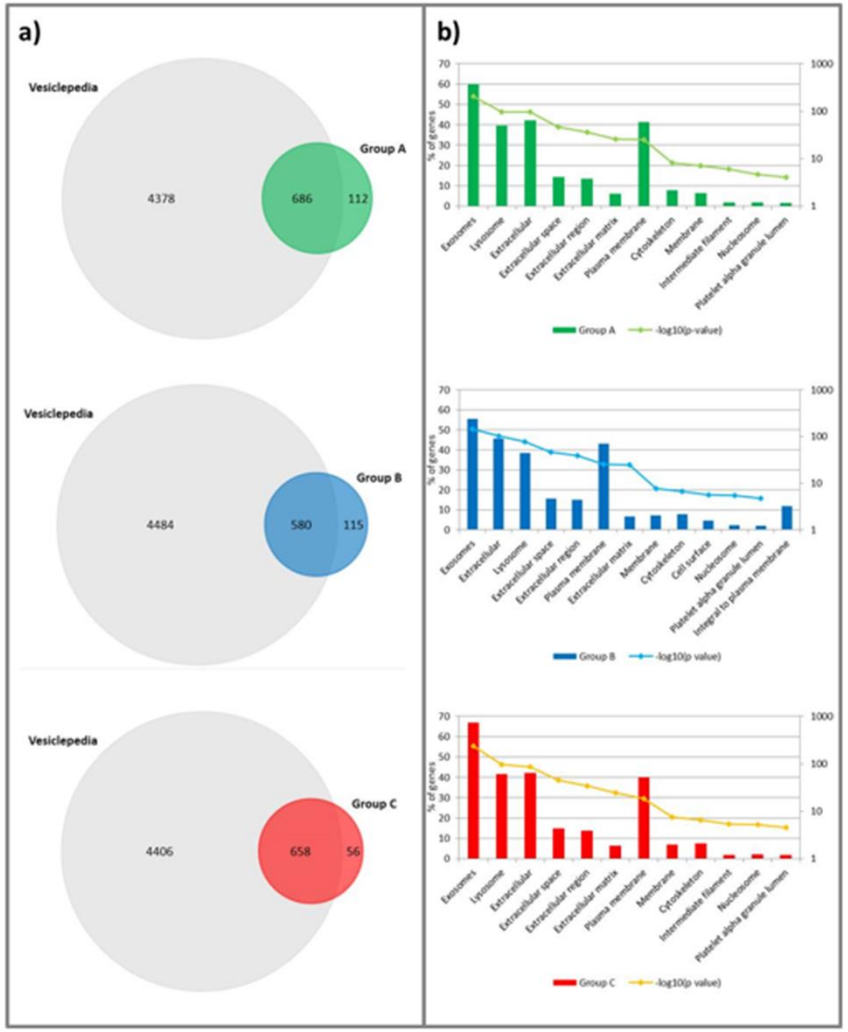
molecules

Venn Diagram

Distribution of proteins among the three stratified groups.



Enrichment analysis of proteins identified in the three groups by nLC-MS/MS.



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Results: Differential analysis

molecules

✓ **Differential expressed proteins (DEP)**

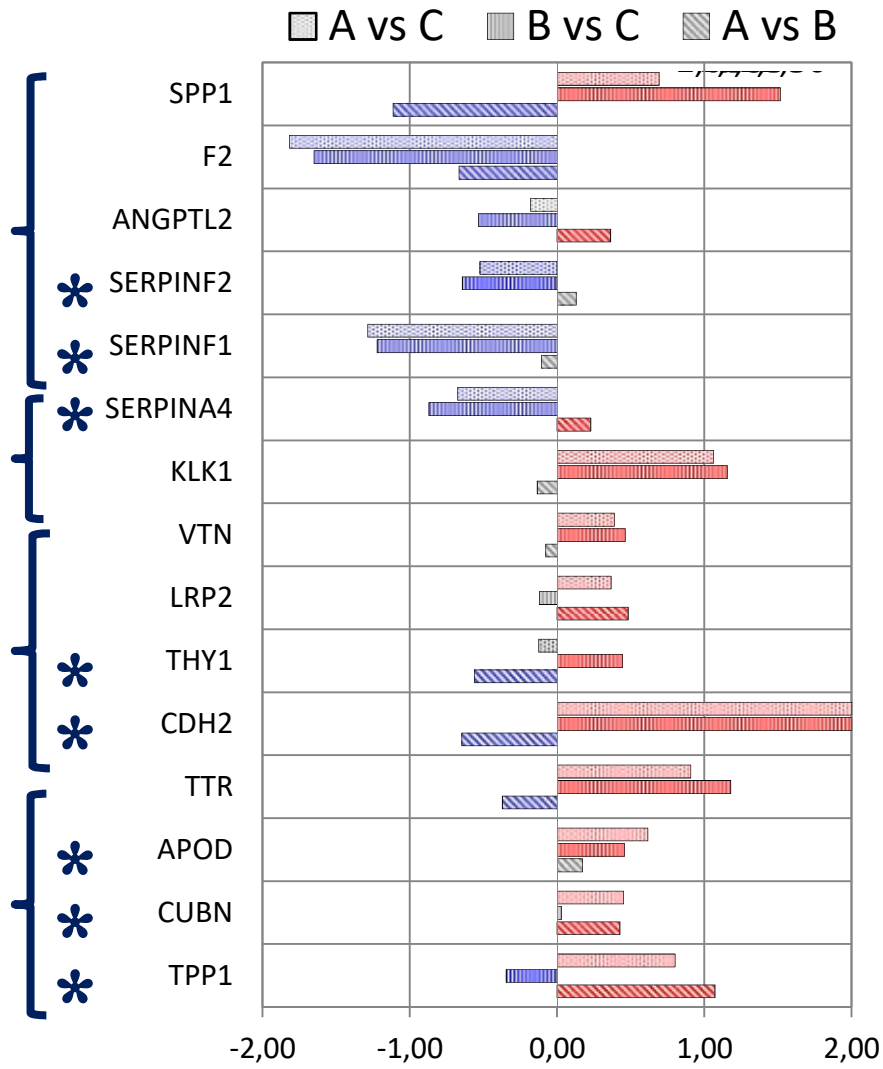
Up-/down-represented proteins into different samples.

Proteins involved in inflammation and stress response

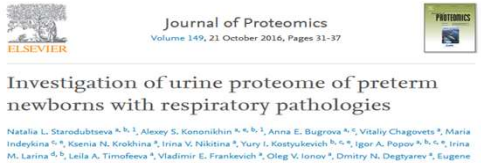
Proteins involved in regulation of enzymatic activity

Proteins involved in cell adhesion

Proteins involved in metabolism



* Proteine già correlate alle malattie respiratorie


Journal of Proteomics
Volume 149, 21 October 2016, Pages 31-37

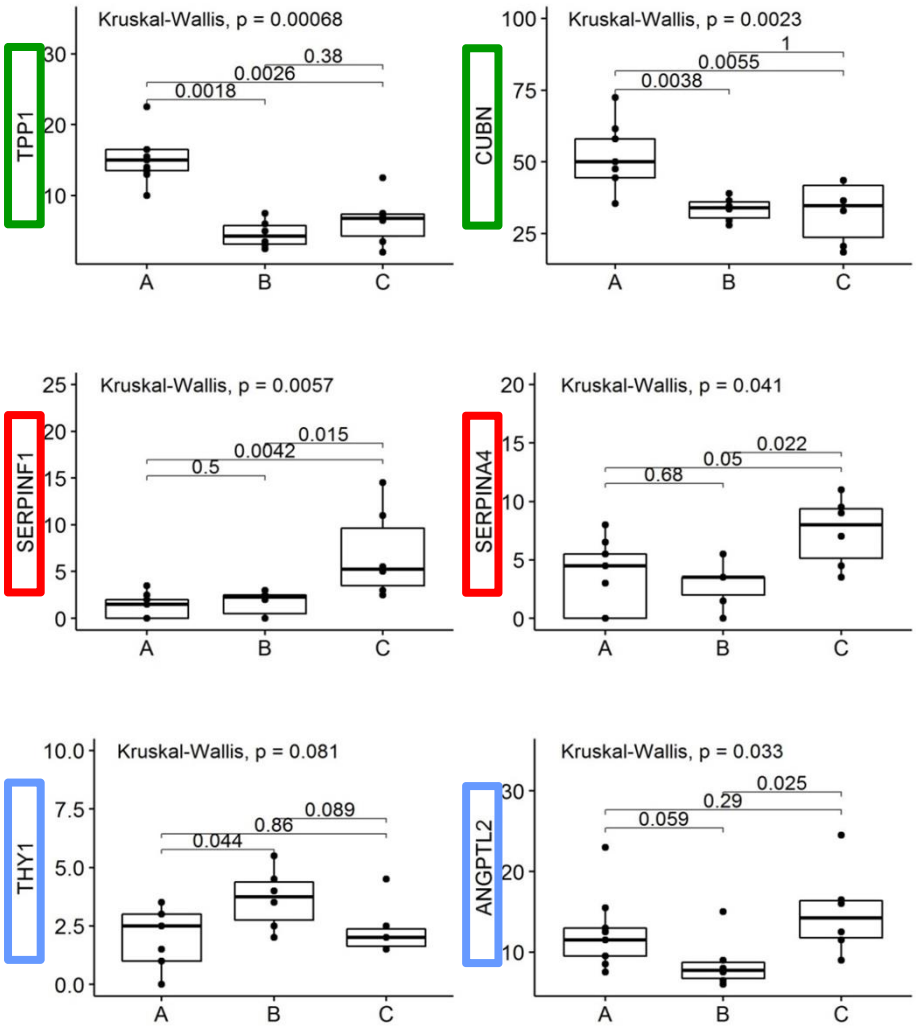
Investigation of urine proteome of preterm newborns with respiratory pathologies

Natalia L. Starodubtseva, Alexey S. Kononikhin, Anna E. Bugrova, Vitaliy Chagovets, Maria Indeykina, Ksenia N. Krokhina, Irina V. Nikitina, Yury I. Kostyukevich, Igor A. Popov, Irina M. Larina, Leila A. Timofeeva, Vladimir E. Frankevich, Oleg V. Ionov, Dmitry N. Degtyarev, Eugene N. Nikolaev, Gennady T. Sukhikh

Results: Differential analysis

molecules

Proteine che tipizzano l'appartenenza dei soggetti ai tre gruppi (A, B e C)



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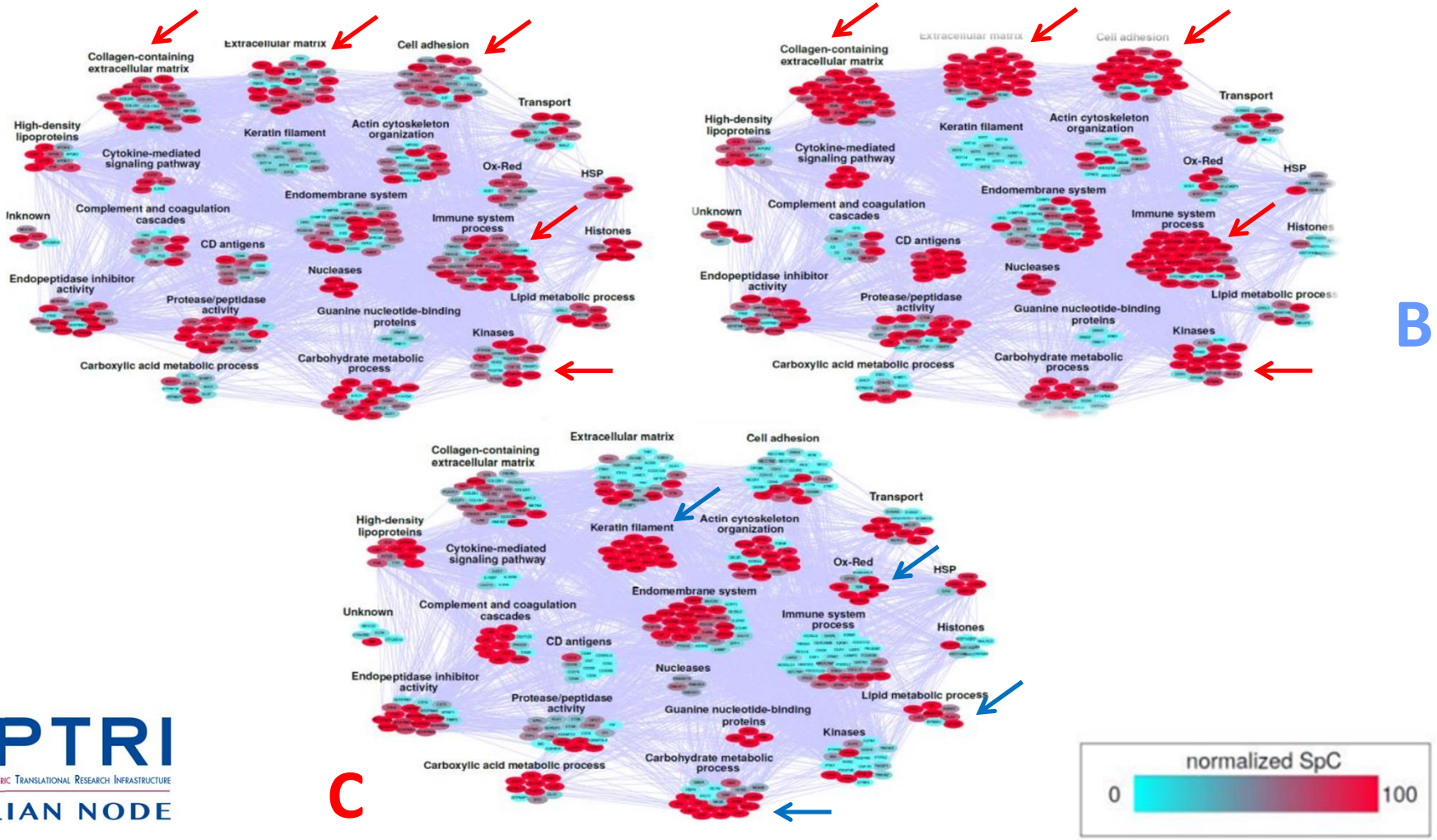
Results: Interactome analysis

molecules

Network Analysis

Investigation of molecular pathways involved in the onset and follow-up of the pathology.



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Results: Clinical parameters

molecules

Valutazione dei parametri di funzione respiratoria considerando la stratificazione ottenuta mediante l'analisi proteomica

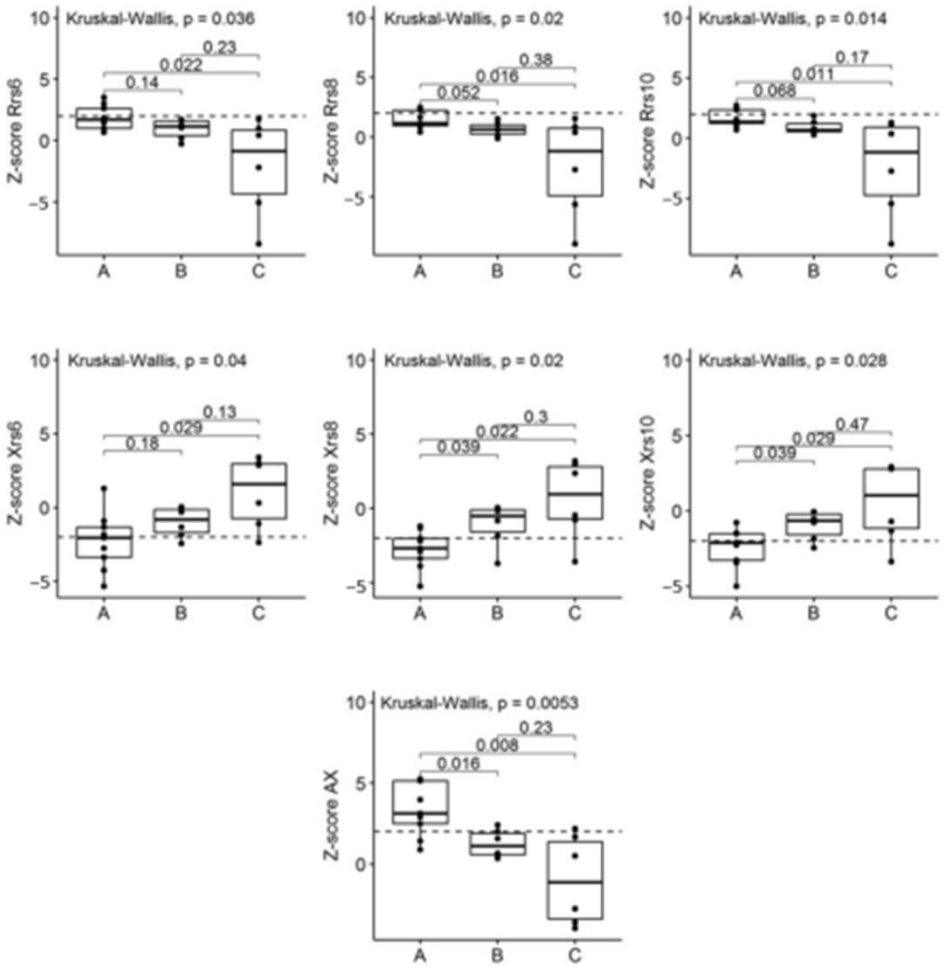

Figure 6. Baseline Z-scores of resistance at Rrs6, Rrs8, and Rrs10 Hz and reactance at Xrs6, Xrs8, and Xrs10 Hz and AX in the three groups A, B and C. *p*-values come from a Kruskal–Wallis test. On the vertical axes, the labels of FOT measures are reported, on the horizontal axes the labels of each group are reported. Dashed lines represent the normal limit.

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Principal findings

molecules

- ✓ Protein fractionation, by the isolation of **urinary extracellular vesicles**, allowed an interesting statistically **confident** proteomic-based **stratification** of **healthy preschool children.**
- ✓ These samples are of **primary importance** to perform investigations involving children, **reducing the invasiveness of collection**; however, the biogenesis of urinary EVs remains unknown, so it will be important to investigate their biogenesis and release.
- ✓ The obtained childhood groups resulted in **good agreement** with the **respiratory impedance** and underlying **biological profiles** in the corresponding healthy preschool children.
- ✓ **Future studies are needed** to **increase** the **number of analyzed subjects** and to **understand any roles** of the selected **proteins** in the evaluation of physiological changes in lung function throughout childhood.

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Take home messages

- ✓ **Urine** is an **excellent source** of **potential biomarkers** due to safety of the collection procedure.
- ✓ The recent **improvements** of **proteomics methodologies**, including sample preparation, instrumentations and computational tools, allow a system oriented **investigation of human diseases**.
- ✓ The strict cooperation among scientists from different areas and the comparison between **molecular** and **clinical data** are permitting the **discovery** of a great number of proteins as useful **biomarkers for diagnostic** and **therapeutic purposes**.
- ✓ The more suitable reality in **clinical practice of proteomics** is useful for the definition of different panels composed by different **biomarkers** leading to the eligibility of the patients to **specific therapeutic treatments**.
- ✓ The **integration** of **proteomics data** with **network analysis** contributes to the **characterization of perturbed molecular machines** (metabolic clusters) in relation to physio-pathological states, and it increases our knowledge of **molecular mechanisms** (endotypes).

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These high throughput technologies are contributing to realize the **Clinical Proteomics approach**, translating basic research to real-life and transforming medicine from **evidence-based to personalized**

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Grazie per l'attenzione

rossana.rossi@itb.cnr.it

EUROPEAN BIOTECH WEEK
INNOVATION IS IN OUR GENES

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ASSOBIOTEC
Associazione nazionale per lo sviluppo delle biotecnologie

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INCiPiT Italian Network for Paediatric Clinical Trials