"CHANGES IN SERUM PROTEIN EXPRESSION IN SMALL-FOR-GESTATIONAL-AGE NEWBORN INFANTS AT DIFFERENT GESTATIONAL AGES"

54th Annual Meeting 1-3 October ESPE 2015 BARCELONA European Society for Paediatric Endocrinology

R. CAÑETE-ESTRADA (1), Mª D. RUIZ-GONZÁLEZ (1), MªD. CAÑETE-VÁZQUEZ (2), JL. GÓMEZ-CHAPARRO (1), N ABRIL DÍAZ (3), JL. LÓPEZ-BAREA (3).
(1) Pediatric Unit. Hosptal Universitario Reina Sofía, Avda Menéndez Pidal s/n 14004 Córdoba, Spain ; (2) Grupo PAIDI CTS-329. IMIBIC, University of Córdoba, Spain; (3) Departament of Biochemistry and Molecular Biology. Campus Universitario de Rabanales. Edificio Severo Ochoa. Universidad de Córdoba. Spain

Introduction

SMALL FOR GESTACIONA AGE

•Most newborn (NB) classed as small for their gestational age (**SGA**) show subsequent catch-up growth, but many still display long-term **comorbidities**, including a number of **pathologies** that influence growth, metabolism and/or the development of other disorders.

•It is unknown whether there are quantitative/qualitative **differences** serum proteome in normal newborn or adecuate for gestacional age (**AGA**) and **SGA**.

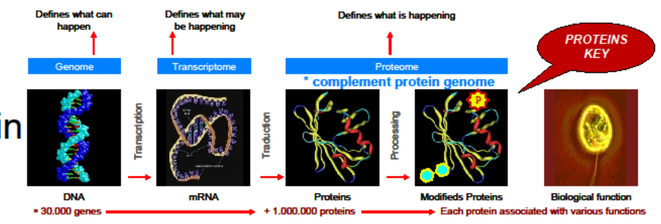
•Therefore we propose a study of proteomics to identify the existence of these differences.

PROTEOMIC

A set of techniques or technologies to obtain information of all proteins

PROTEOME

Complement protein genome



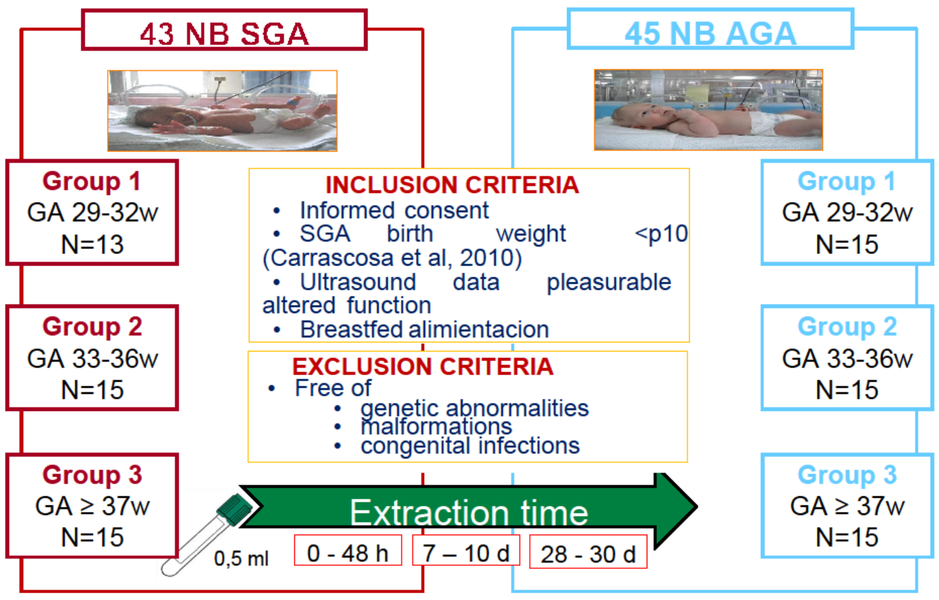
Identification of the protein alterations displayed SGA NNs may help to identify biomarkers for this physiopathology.

Object

"**To detect changes** in the **serum proteome** in **SGA-NB** *vs.* **AGA-NB** during the **first month** of postnatal life."

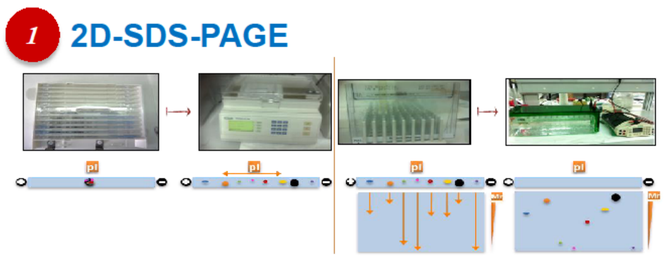
Subjects

88 NBs. In each group longitudinally serum samples were taken: at birth, after 7-10 days, and after 28 to 30 days.



Methods

1 2D-SDS-PAGE



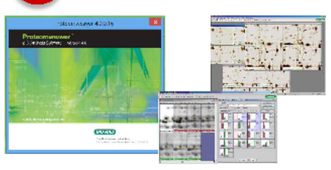
1st dimension (isoelectrofocusing): Separation of protein by isoelectric point (pI)

2nd dimension (electrophoresis): Separation of protein by relative mass (Mr)

2 Laser-scanner

Geles were digitalized in a FX_Imager (Bio-Rad®) laser-scanner

3 Image analysis



The images of the gels were analyzed with the ProteomWeaverTM 2-D Analysis Software v4.0 (Bio-Rad®).

4 Mass spectrometry and identification

Analyzed differences are carried mass spectrometry and compared with databases that will lead to the identification of proteins.

5 Validation purposes

Validation of proteins identified by western bloot

Resultados

Differences were found in the expression of **33** proteins, all identified.

8

1 Present en SGA

- Lysophospholipid acyltransferase 7
- Small ubiquitin-related modifier 3

7 Absent in SGA

- Apolipoprotein L, 1
- Putative beta-defensin 108A
- Ig lambda-6 chain C
- Ig lambda-2 chain C
- Keratin, type I cytoskeletal 9
- Coiled-coil domain-containing protein 51 (fragment)

33 Proteins Identified

20 Up- regulated in SGA

- 5 Sero albúminas
- Gelsolina
- Apolipoprotein E
- Keratin, type I cytoskeletal 10
- Solute carrier fam 41 member 2 (Fragment)
- Anaphase-promoting Complex subunit 2
- 2 Serotransferrin
- Apolipoprotein A-I
- UTP-glucose-1-phosphate uridylyltransferase
- 2 Alpha-1-antitrypsin
- Dynein heavy chain 6, axonemal
- Beta-2-glycoprotein 1

5 Down-regulated in SGA

- Peroxiredoxin-2
- Fibrinogen beta chain
- Ficolina
- 2 vitronectinas
- Ras proteína Rab 35
- beta-2-glicoproteína 1 fragmento

Figure 1. Representative 2-DE gel of AGA infants. The red triangle marks a protein detected in SGA but absent in AGA. Red circles indicate seven other proteins detected only in AGA but absent in SGA.

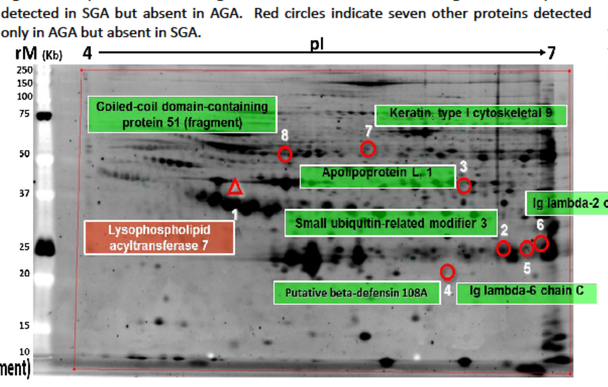


Figure 3. Up. Zoomed-in region of representative 2-DE gel images from SGA of «Very Preterm» (A.1-3), «Moderate Preterm» (B.1-3), and «Term» (C.1-3) groups in venous blood samples drawn during the first 48 h, after 7-10 d, and 28-30 d. The triangle indicate **protein 1**, present only in SGA. **Bottom.** Threedimensional view of protein 1 peak cut from representative 2-DE gels of SGA.

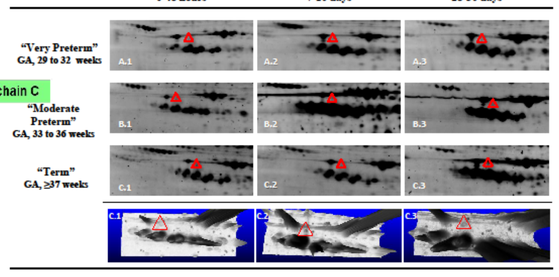


Figure 2. Representative 2-DE gel of one IUGR group. The red circles indicate the twenty upregulated proteins and the green circles indicate five downregulated proteins.

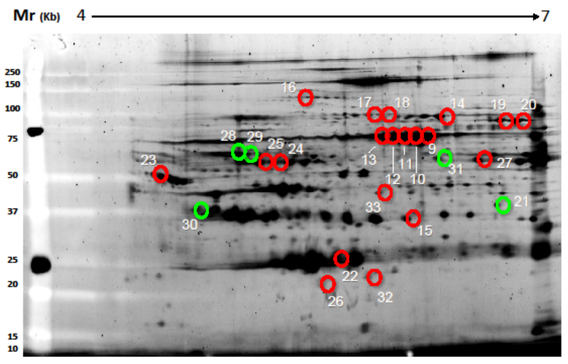


Figure 4. Representative 2-DE gel images from SGA infants of all gestational age groups in samples taken during the first 48 hours, and after 7-10 days and 28-30 days. The yelow circles mark indicate **serotransferrin** proteins was detected in all groups and extraction times

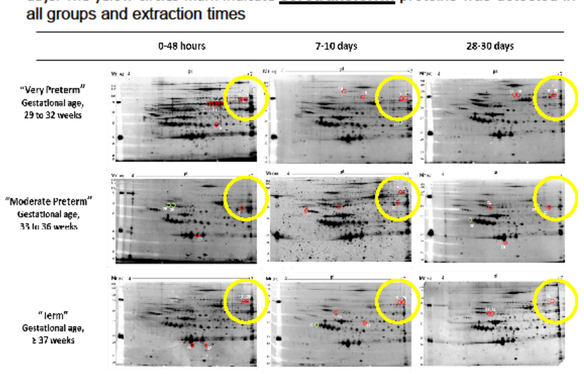


Table 1: Demographic data of AGA and SGA groups according to their gestational age.

	Goup 1 (GA from 29 to 32 w) N: 13/15			Goup 2 (GA from 33 to 36 w) N: 15/15			Group 3 (GA ≥ 37 w) N: 15/15		
	AGA	SGA	*p*	AGA	SGA	*p*	AGA	SGA	*p*
Gender (M/F)	6/9	5/8	NS	6/9	7/8	NS	7/8	6/9	NS
GA (weeks)	30.86±0.9	30.83±1.06	0.953	33.53±0.16	34.57±0.31	0.07	40.3±0.18	38.1±0.31	0.08
Birth weight (g)	1572±48	1132±48	<0.001	2067±98	1740±65	<0.001	3498±124	2056±42	<0.001
Height birth (cm)	41.87±0.45	38.46±0.59	<0.001	43.92±0.66	42.96±0.70	0.255	51.5±0.60	45.9±0.43	<0.001
Head circunference (cm)	28.8±0.41	26.69±0.28	<0.001	30.93±0.34	30.73±0.50	0.743	34.7±0.36	32.3±0.27	<0.001
Apgar score (1 min - 5 min)	7.6±0.49 9.2±0.42	7.3±0.41 8.6±0.49	0.746 0.074	7.9±0.20 9.3±0.15	7.6±0.30 9.0±0.21	0.374 0.228	7.1±0.41 8.7±0.28	7.7±0.52 9.4±0.25	0.384 0.084
Delivery mode (V/C)	6/9	4/9	0.052 0.289	6/9	3/12	<0.001 0.289	6/9	9/6	0.289 0.289

Table 2: Identified proteins found to be present or absent in serum of SGA and AGA infants

	Protein name	Gene name	Mr (kDa) (exp/theor)	pI (exp/theor)	MASCOT score	Cov (%)	Pep. Count	Access	Molecular function GO term	Biological process GO term	Patient type
1	Lysophospholipid acyltransferase 7	MBOAT7	40/52	4.99/9.15	49	35	9	sp|Q96N66|MBOA7	Transferase activity , SUMO ligase activity	Glycerophospholipid biosynthesis process	SGA
2	Small ubiquitin-related modifier 3	SUMO3	25/17.1	6.67/9.71	49	36	7	tr|A8MUZ7|A8MUZ		Positive regulation of protein ubiquitination involved in ubiquitin-dependent protein catabolic process; Protein localization to nucleus; Protein sumoylation	AGA
3	Apolipoprotein L, 1 (Fragment)	APOL1	40/41.1	6.43/5.49	44	52	6	tr|B1AH95|B1AH9	Chloride channel activity Lipid binding	Cholesterol metabolic process; Cytolysis; Innate immune response; Killing of cells of other organism; Lipid transport; Lipoprotein metabolic process	AGA
4	Putative beta-defensin 108A	DEFB108P1	25/ 8.39	6.31/ 8.9	50	78	5	sp|A8MXU8|D108A	Antimicrobial Antibiotic Defensin	Defense response to bacterium	AGA
5	Ig lambda-6 chain C	IGLC6	27/11.4	6.85/6.92	96	46	4	sp|P0CF74|LAC6_	Antigen binding	Fc-γ receptor signaling pathway; Fc-γ receptor signaling pathway involved in phagocytosis; Complement activation,classical pathway; Innate immune response.	AGA
6	Ig lambda-2 chain C	IGLC2	27/11.4	6.90/6.92	91	46	5	sp|P0CG05|LAC2_	Antigen binding	Fc-γ receptor signaling pathway; Fc-γ receptor signaling pathway involved in phagocytosis; Complement activation,classical pathway; Innate immune response.	AGA
7	Keratin, type I cytoskeletal 9	KRT9	60/62	5.8/5.4	160	28	20	sp|P35527|K1C9_	Structural constituent of cytoskeleton	Epidermis development; Intermediate filament organization; Spermatogenesis	AGA
8	Coiled-coil domain-containing protein 51 (Fragment)	CCDC51	58/68.4	5.53/11.25	51	82		tr|C9JPZ8|C9JPZ		required for motile cilia function in animals	AGA

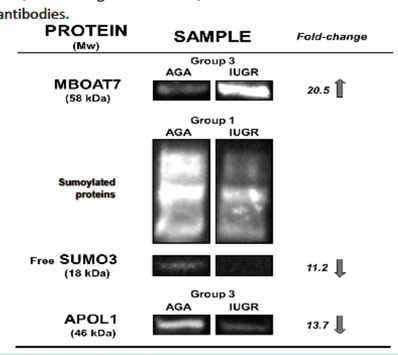
Table 3: Identified proteins that are differentially expressed in SGA infants.

	Name	Gene Name	Mr (kDa) (exp/theor)	pI (exp/theor)	MASCOT score	Cov (%)	Pep. Count	Access	Molecular function GO term	Biological process GO term
9	Serum albumin	ALBU	62.5/71.3	6.24/5.92	1000	64	36	sp|P02768|ALBU_	DNA binding; antioxid activity; chaperone binding; Cu, drug, fatty acid, pyridoxal-P, toxic substance and Zn ion binding	Bile acid transport; blood coagulation; starvation resp; hemolysis; platelet degranuls; circadian cycle; non-REM sleep; response to Hg, nutrient, org subst and Pt; small molecule metabolc; org anion transport; transmemb transport
10	Serum albumin	ALBU	62.5/71.3	6.15/5.92	957	63	27	sp|P02768|ALBU_		
11	Serum albumin	ALBU	62.5/ 71.3	6.1/5.92	745	48	27	sp|P02768|ALBU_		
12	Serum albumin	ALBU	62.5/ 71.3	6.2/5.92	589	41	23	sp|P02768|ALBU_		
13	Serum albumin	ALBU	62.5/ 71.3	5.98/5.92	315	31	19	sp|P02768|ALBU_		
14	Gelsolin	GSN	81/86	6.35/5.9	450	34	17	sp|P06396|GELS	Calcium ion binding	Cilium biogenesis/degradation
15	Apolipoprotein E	APOE	33.5/36.2	6.12/5.65	469	46	15	sp|P02649|APOE_	Antioxidant; beta-amiloid and heparin binding; cholesterol and lipid transport	Cholesterol/sterol/sterol metabolism; lipid metabolism/transport
16	Keratin, type I cytoskeletal 10	KRT10	125/59	5.38/5.13	274	25	18	sp|P13645|K1C10		
17	Solute carrier fam 41 member 2 (Fragment)	SLC41A2	81/83.8	6.24/9.6	54	80	5	tr|C9JIL4|C9JIL	Cation transmembrane transporter activity	Transmembrane transport
18	Anaphase-promoting Complex subunit 2	ANAPC2	81/94.6	6.35/5.12	62	19	13	sp|Q9UJX6|ANC2	Ubiquitin-protein ligase activity	Anaphase-promoting complex-dependent proteasomal Ub-dependent protein catabolism; cell differentation and division; mitosis; nervous system development
19	Serotransferrin	TF	75/79.2	6.75/6.81	198	33	19	sp|P02787|TRFE_	Ferric iron binding	Blood coagul; Fe homeost; platelet activation degranul, transferrin transp; transmemb transport
20	Serotransferrin	TF	75/79.2	6.85/6.81	738	38	28	sp|P02787|TRFE_		
21	Ficolin-2	FCN2	36/34.4	6.8/6.31	105	30	7	sp|Q15485|FCN2_	Antigen binding; calcium ion binding; carbohidrate binding	Complement activation; lectin pathway; opsonization
22	Apolipoprotein A-I	APOA1	23/30.7	4.59/5.56	319	51	13	sp|P02647|APOA1_	β-amiloid binding; colesterol binding/transport; HDL binding; lipase inhibitor; phospholipid binding; phosphatydilcholine-sterole O-acyltransferase activator	Cholesterol metabolism; Lipid metabolism/transport Steroid/sterol metabolism;Transport development
23	UTP-glucose-1-phosphate uridylyltransferase	UGP2	50/57	4.35/8.16	50	25	10	sp|Q16851|UGPA_	UTP:glucose-1-P uridyltransferase activity; glucose binding; metal ion binding; pyrimidine ribonucl binding	UDP-glucose/glucuronate metabolism; carbohydrate metabolc; glucuronidation; glucose 1-P metabolism; glycogen biosynthesis; small molecule metabolic; xenobiotic metabolism
24	Alpha-1-antitrypsin	SERPIN1	50/46.87	5.16/5.37	192	40	12	sp|P01009|A1AT_	Identical protein binding; protease binding; Ser-type endopept inhibr	Acute phase; blood coagulation; hemostasis
25	Alpha-1-antitrypsin	SERPIN1	50/46.87	5.16/5.37	1090	58	22	sp|P01009|A1AT_		
26	Dynein heavy chain 6, axonemal	DNAH6	20/47.96	5.62/5.72	48	10	27	sp|Q9C0G6|DYH6	ATP binding; ATP-ase; microtubule motor activity	ATP catabolism; microtubule based-movement
27	Beta-2-glycoprotein 1	APOH	50/39.5	6.7/8.34	230	36	9	sp|P02749|APOH_	Heparin binding; lipoprotein lipase activator activity; phospholipid binding	Blood coagul intrinsic pathway; negative regulation of angiogenesis; plasminogen activation, negat regul of endothelial cell migration; triglyceride metabol
28	Vitronectin	VTN	60/55	4.86/5.55	226	16	7	sp|P04004|VTNC	Extracellular matrix binding; heparin/polisaccharide binding; scavenger receptor activity.	Cell adhesion
29	Vitronectin	VTN	59/55	4.98/5.55	350	25	10	sp|P04004|VTNC		
30	Ras-related protein Rab-35	RAB35	20/23.29	5.4/6.92	91	43	3	sp|Q15286|RAB35	GDP/GTP binding; protein binding; GTPase activity; phosphatidylinositol-4,5-biphosphate binding	Antigen processing/presentation; cytokinesis; GTP catabolism, protein localization and transport; celular response to nerve growth factor stimulus; endosomal transport, neuron projection development; protein localization to endosome; small GTPase mediated signal transduction
31	Beta-2-glycoprotein 1 (Fragment)	APOH	50/20.5	6.4/7.98	52	27	3	tr|J3QRN2|J3QRN		
32	Peroxiredoxin-2	PRDX2	22/22	5.6/5.66	515	36	9	sp|P32119|PRDX2	Thioredoxin peroxidase activity	H_2O_2 catabolism; negative regulation of apoptosis and of neuronal apoptosis; removal of superoxide radicals
33	Fibrinogen beta chain	FGB	42/31.6	5.96/6.88	51	23	4	tr|E9ER5|E9ER5L	Platelet agregation	

Conclusiones

1. **Significant differences between SGA-NBs and AGA-NBs at different ages were found for the expression of proteins involved in phospholipid synthesis, protein ubiquitination, lipid transport, antimicrobial activity and the innate immune response.**
2. **In SGA-NBs, expression of LPIAT1 and SERPIN1 may be an adaptive response to protect the brain in an adverse fetal environment, recalling Barker's fetal programming theory.**
3. **This is the first study to evaluate changes in the serum proteome of SGA-NBS as a function of gestational age: 1, 7 and 30 days after birth.**

Figure 5. Western Blots obtained with sera from AGA and SGA, after using anti-MBOAT7, anti-SUMO3 and anti-APOL1 antibodies.



Work funded by Pfizer, PAIDI group CTS-329 and SEN.

DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: ESPE 2015 BARCELONA
PosterSessionOnline.com