Journal of Inborn Errors of Metabolism & Screening
1: 1-84
© The Author(s) 2013
DOI: 10.1177/2326409813511871
iem.sagepub.com
SAGE

Abstracts of Free Communications Accepted for Presentation at the IX Latin American Congress of Inborn Errors of Metabolism and Newborn Screening, Medellin, Colombia, December 1 to 4, 2013

001 - A Multicenter, Open-Label, Extension Study to Evaluate the Long-term Efficacy and Safety of BMN 110 in Patients With Mucopolysaccharidosis IVA (MPS IVA, Morquio A Syndrome)

R. Giugliani[1], C. J. Hendriksz[2], A. Vellodi[3], S. Jones[4], T. Hiwot[5], S. Santra[6], P. Slasor[7], and C. Decker[7]

[1]*Department of Genetics/UFRGS and INAGEMP, Medical Genetics Service/HCPA, Porto Alegre, Brazil*
[2]*Birmingham Children's Hospital NHS Foundation Trust, Birmingham, United Kingdom*
[3]*Great Ormond Street Children's Hospital, London, United Kingdom*
[4]*St Mary's Hospital, Manchester Academic Health Sciences Centre, CMFT, University of Manchester, Manchester, United Kingdom*
[5]*New Queen Elizabeth Hospital Birmingham, Birmingham, United Kingdom*
[6]*Birmingham Children's Hospital, Birmingham, United Kingdom*
[7]*BioMarin Pharmaceutical Inc, Novato, California, USA*

Introduction, Objectives, and Patients: Twenty patients with mucopolysaccharidosis IVA (MPS IVA) were enrolled in a phase 1/2, 36-week dose-escalation (0.1, 1.0, 2.0 mg/kg/wk) safety and efficacy study of BMN110 enzyme replacement therapy, followed by a 36- to 48-week continuation phase at 1.0 mg/kg/wk. Seventeen patients were enrolled in the ongoing extension study at 2.0 mg/kg/wk evaluating long-term outcomes. **Results, Discussion, and Conclusion**: Interim results (as of July 2012) indicate BMN110 was well tolerated, with <1% of infusions interrupted or discontinued because of adverse events requiring medical intervention. Means trend to increase from baseline in the 6-minute walk test and 3-minute stair climb by 13.8 (+63.25) m and 7.8 (± 13.96) stairs/min after 36 weeks and 27.2 (+62.51) m and 9.6 (+19.63) stair/min after approximately 120 weeks. Means trend to decrease by 52.7 (+133.78) m and 3.3 (+21.97) stairs/min after approximately 156 weeks due to 4 patients with recent orthopedic surgery who had large decreases in endurance measures, which reversed at the following visit. Respiratory function improved by 16.1% (± 21.96%) in forced vital capacity and 10.1% (± 27.83%) in *maximal voluntary ventilation* after approximately 156 weeks. Urinary keratin sulfate levels decreased and remained low over time, with lowest levels occurring during 2.0 mg/kg/wk dosing in both the dose-escalation phase and the extension study, demonstrating the drug's pharmacodynamic effects.

002 - A New Heterozygous Compound Mutation of the GALNS Gene as a Cause of Mucopolysaccharidosis Type IV in a Patient of Southwest Colombia

H. Pachajoa[1], and F. Ruiz-Botero[1]

[1]*Centro de Investigaciones en Anomalías Congénitas y Enfermedades Raras, Cali, Colombia*

Introduction: Mucopolysaccharidosis type IVA (MPS IVA), or Morquio syndrome type A, is an autosomal recessive disorder caused by deficiency of the lysosomal enzyme N-acetylgalactosamine-6-sulfatase (GALNS). **Objective:** Clinical and molecular characterization of a patient with clinical and biochemical manifestations of MPS IVA. **Methodology:** Clinical evaluation of a patient with decreased enzyme activity to GALNS, DNA extraction, GALNS, and gene sequencing. **Result:** A 6-year-old patient with clinical features of the disease. In the molecular study, we found a mutation in the exon 3 (c.280C> T p.R94C) and a new nonreported mutation in the exon 9 (c.998G> A p.G333D). Results of parents and brother pending. Software analyses by polyphen 2 (2.2.2) predict this variant is probably damaging. **Conclusion:** Two different heterozygous mutations were found to cause MPS IVA in the presented patient, a new mutation was found, and another had been previously described by Ogawa in 1995.

003 - A Novel CLN8 Mutation Underlies a Late Infantile Variant of Neuronal Ceroid Lipofuscinosis in Latin America

F. Pesaola[1], I. A. Cismondi[2], N. Guelbert[1], R. Kohan[1], M. N. Carabelos[1], G. Alonso[1], P. Pons[3], A. M. Oller-Ramirez[1], and I. Noher de Halac[4]

[1]*Centro de Estudio de las Metabolopatías Congénitas (CEMECO), Córdoba, Argentina*
[2]*Facultad de Odontología, Universidad Nacional de Córdoba, Córdoba, Argentina*
[3]*Centro de Microscopía Electrónica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina*
[4]*Consejo Nacional de Investigaciones Científicas y Tecnológicas (CONICET), Córdoba, Argentina*

Introduction: Neuronal ceroid lipofuscinosis (NCL), inherited neurodegenerative diseases of all ages, presents with storage of lipofuscin-like lipopigments in cerebral neurons and peripheral tissues. Mutations in CLN8 gene causing epilepsy progressive with mental retardation (EPMR) of Scandinavia and late infantile variant (vLI) phenotype in other countries had not yet been described in Latin America. The change p.Pro229Ala, found in the DNA of 2 individuals from Argentina and Mexico, was not validated as a mutation. **Aim:** To analyze and to validate changes in CLN8 gene in individuals suspected of vLI NCL. **Participants:** Fifteen individuals with normal *palmitoyl protein thioesterase 1* (*PPT1) and tripeptidyl peptidase 1* (*TPP1*) enzymes, positive electronic microscopy, and lack of mutations in other NCL genes. **Method:** Polymerase chain reaction, sequencing, and bioinformatics analyses were performed on the coding region of CLN8 gene, and validation of mutations was carried out on 200 control alleles. **Result:** The novel mutation c.1A>G, p.Met1Val, was validated for an Argentinean child with clinical suspicion of vLI who presented at the age of 3 years with onset of seizures, psychomotor retardation, myoclonus, cortical and cerebellar atrophy, and electronic microscopy with fingerprint and curvilinear profiles. Ocular disorders have not been studied. She died at 12 years of age. The changes p.Pro229Ala and p.Pro3Pro were validated as polymorphisms of the local population, which have been found, respectively, in 10 of 100 (1 in homozygous state) and 1 of 100 controls. **Conclusion:** The girl with vLI phenotype is the first confirmed CLN8 (vLI) case in Latin America. In the future, CLN8 should be considered in the search of possible mutations in individuals with vLI in the region.

004 - A Novel Familial Case of Diffuse Leukodystrophy Related to NDUFV1 Compound Heterozygous Mutations

C. Rivera-Nieto[1], H. Mateus[2], O. Ortega-Recalde[2], D. Fonseca[2,3], L. Patiño[2], C. Restrepo[2,3], M. Van der Knaap[4], and P. Laissue[2,3]

[1]*Fundación CardioInfantil, Instituto de Cardiologia, Bogota, Colombia*
[2]*Unidad de Genética, Escuela de Medicina y Ciencias de la Salud, Universidad del Rosario, Bogotá, Colombia*
[3] *Departamento de Genética Molecular, Genética Molecular de Colombia, Bogotá, Colombia*
[4]*Departament of Child Neurology, VU University Medical Center, Amsterdam, the Netherlands*

Introduction: Mitochondrial leukodystrophy due to complex I deficiency is an entity with high genetic heterogeneity, the mutations of the gene *NDUFV1* being one of the causes of this disease. It is an autosomal recessive entity that causes a variable phenotype, from a fatal neonatal onset to neurodegenerative disorders in adulthood. **Problem Studied:** We report 2 siblings, 5 and 2 years of age, with nystagmus, ataxia, impaired consciousness, hemiparesis, hyporeflexia, and psychomotor regression. In brain magnetic resonance imaging, signal abnormalities in large regions of the cerebral white matter were observed, suggesting a demyelinating disease. **Materials and Methods:** Genomic DNA was obtained from whole blood. The complete coding region of *NDUFV1* was amplified in patients and their parents. Each amplicon was purified. Direct sequencing was performed. Intron 6 and exon 7 amplicons from patient 1 were cloned. A skin biopsy was performed in the mother. RNA was isolated. The *NDUFV1* complementary DNA (cDNA) was amplified and directly sequenced. In silico analysis was performed. Following diagnosis, treatment with ubiquinol and riboflavin was started. **Results:** Both patients have the missense mutation c.1156C> T and the 42-base pair deletion in the gene *NDUFV1*. Bioinformatics analysis indicates that this deletion leads to messenger RNA (mRNA) synthesis with a premature stop codon. Probably, mutant mRNAs were recognized and degraded by the nonsense-mediated mRNA machinery. Analysis of the maternal *NDUFV1* cDNA supports this hypothesis. **Conclusion:** Our results add information on the molecular basis and the phenotypic features of mitochondrial disease caused by *NDUFV1* mutations. We can affirm that the mutations are causative of the phenotype. The patients are having a good therapeutic response to the treatment.

005 - Adherence to Treatment in a Group of Teenagers and Adults With Classic Phenylketonuria in Cuba

G. M. Zayas[1], H. N. Chinfantsavez[1], D. Dominguez[1], J. M. Torriente[1], U. Cabrera[2], L. Martinez[3], S. Cardenas[1], B. Suarez[3], and S. C. Gonzalez[1]

[1]*Instituto de Nutrición e Higiene de los Alimentos, La Habana, Cuba*
[2]*Hospital Pediátrico de Centro Habana, La Habana, Cuba*
[3]*Centro Nacional de Genética Médica,* ANLIS, Buenos Aires, *Argentina*

Introduction: In Cuba, the National Program of Precocious Detection of Hyperphenylalaninemia began during the year 1983 for newly born infants in Havana and was generalized to the whole country since the year 1986. All patients who were

diagnosed with hyperphenylalaninemia received alimentary/nutritional treatment and special formulas free of phenylalanine. **Objective:** To verify the percentage of adolescents and adults that follow the protocol for the phenylalanine determination and to analyze whether the phenylalanine values are those recommended according to age. **Methodology:** Longitudinal descriptive study. The study population was composed of 20 patients between 10 and 34 years with classic phenylketonuria (PKU) who received treatment. The analyzed data were gathered from January 2012 to June 2013. The protocol for realization of phenylalanine was monthly (18 in the period), and the kit UMTEST-PKU (Tecnosuma, Havana, Cuba) was used. The recommended values were between 4 and 10 mg/dL. The average of phenylalanine values was obtained in the study period. **Results:** It was found that 10% (n = 2) went through monthly examination and 55% (n = 11) was evaluated less than 9 times during this period. Of them, 1.8% (n = 1) made it less than 5 times in the period. In relation to the phenylalanine levels, 70% (n = 14) was above the recommended level of phenylalanine. Of them 20% (n = 4) was above 15 mg/dL. **Conclusion:** This study highlights the difficulties that exist in achieving good adherence to the alimentary/nutritional treatment in adults that do not value the consequences.

006 - Advances in Expression Systems of Recombinant Human Iduronate Sulfate Sulfatase for Use in Enzymatic Therapy Replacement

C. M. Rivera-Hoyos[1,2], R. A. Poutou-Piñales[2], and P. Landazuri[1]

[1]Grupo de Investigación en Enfermedades Cardiovasculares y Metabólicas (GECAVYME), Facultad de Ciencias de la Salud, Universidad del Quindío, Armenia-Quindío, Colombia
[2]Laboratorio de Biotecnología Molecular, Grupo de Biotecnología Ambiental e Industrial (GBAI), Departamento de Microbiología, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá, D.C. Colombia

Introduction: In an attempt to develop less expensive techniques of enzyme replacement therapy (ERT) for Hunter syndrome, our group has demonstrated in several works the iduronate sulfate sulfatase (IDSh) in models such as *Escherichia coli* and *Pichia pastoris*, with low expression and activity in both the cases, possibly because of a conflict between the native signal peptide at the IDSh and the recognition and processing by *E coli*. **Objective:** To improve IDSh expression system in *E coli*. **Methods:** We cloned complementary DNA in the vector pLEX to express in *E coli*, removing the sequence encoding the native signal peptide by polymerase chain reaction amplification. **Results:** Iduronate sulfate sulfatase sequencing obtained without signal peptide (IDSsps) showed variations in 2 codons, so by bioinformatics analysis we determined the 3-dimensional structure of the cloned protein. The comparison of the model obtained for IDSspn versus IDS native showed that it is feasible that in future experiments of the protein expression in pLEX-IDSsps system, the active enzyme could be obtained as the differences between the 2 molecules were not significant. **Conclusion:** Three-dimensional structure of IDShr expressed in the new cloning system indicates no difference with native IDS that could have conflict with the expression in *E coli*.

007 - Alkaptonuria: Report of 1 Case in Venezuela

J. L. Alvarez[1], V. Landa[1], I. Bolivar[1], D. Rodriguez[1], T. Rodriguez[1], A. Mahfoud[1], and C. L. Dominguez[1]

[1]Unidad de Errores Innatos del Metabolismo (UDEIM), Fundación Instituto de Estudios Avanzados-IDEA, Caracas, Venezuela

Introduction: Alkaptonuria is a rare autosomal recessive disease that is caused by deficiency of homogentisic acid (HA) dioxygenase that catalyzes the third step in the catabolism of tyrosine. This deficiency leads to the accumulation of HA, and it is excreted in large amounts in the urine of patients affected causing it to darken, which guides the suspected diagnosis. The HA and its polymerized oxidation products are deposited in connective tissue causing pigmentation (ochronosis), accumulated in large joints causing arthritis, and produces impairment of heart valves. The diagnosis is made by determining the levels of HA in urine. **Objective:** The purpose of this article is to describe the first case of alkaptonuria diagnosed in Unidad de Errores Innatos del Metabolismo, Fundación Instituto de Estudios Avanzados from a total of 19 960 samples analyzed until this patient was diagnosed in February 2013. **Methods:** We determined the presence of organic acid by gas chromatography/mass spectrometry (GC/MS) in the urine of a female patient, 19 months old, who was referred to our center for urine and small red lesions on the skin that darkened and became hyperpigmented as days passed. **Results:** The determination of organic acid showed high levels of HA. **Conclusion:** The discovery of dark urine and the organic acid analysis by GC/MS established the diagnosis of the first case of alkaptonuria in our center.

008 - Alternative Nutritional Management of Pancreatitis in a Patient With Maple Syrup Urine Disease, Without Using Branched-Chain Free Amino Acid Solution on Total Parenteral Nutrition

S. Guillen-Lopez[1], A. Pinzon-Navarro[1], M. Bautista-Silva[1], W. Villegas-Hernandez[2], S. Monroy-Santoyo[1], L. Belmont-Martinez[1], A. Hernandez-Montiel[1], and M. Vela-Amieva[1]

[1]Instituto Nacional de Pediatría; Federal District, México
[2]División de Ciencias Biológicas y de la Salud Universidad Autónoma Metropolitana

Introduction: Acute pancreatitis (AP) is a serious complication of maple syrup urine disease (MSUD) that makes dietary management more difficult, especially in countries where no branched chain amino acid (BCAA)-free intravenous solutions are available. **Objective:** To describe the management of a patient with MSUD having AP. **Results:** A 2-year-old boy was diagnosed with MSUD at his sixth day of life. At 35 months of age, he presented with symptoms such as irritability, sore throat, and fever; antipyretic and antibiotic medications were administered with no improvement. He also showed diarrhea and abdominal pain. Elevated amylase and lipase were found, and ultrasonography confirmed AP. Oral intake was contraindicated, so total parenteral nutrition (TPN) was maintained for 17 days, energy intake varied from 30 to 86.5 kcal/kg per day. Available parenteral amino acid solution low in BCAA, Levamin at 8.5%, was used. Leucine requirement was calculated, resulting in a contribution of total protein intake of 0.57 to 1.3 g/kg per day. Clinical and biochemical improvement allowed starting enteral metabolic formula free of BCAA (1 mL/kg/h), gradually increasing volume until BCAA met requirements. Protein and BCAA supplementation were calculated depending on amino acid levels in blood. Despite AP, the patient maintained a relative constant weight and height. The AP resolved after a month. **Conclusion:** Adequate use of TPN, along with constant measurement of levels in blood, can help solve serious complications such as AP in patients with MSUD, even in the absence of formulas lacking BCAA. However, the real incidence in México is still unknown. This study supports the need to implement this newborn screening in all health institutions in the country.

009 - Animal Model for the Study of Acquired α-Manosidosis

L. Cancelarich[1], A. Martinez[2,3], D. Gardner[4], C. A. Robles[2], E. J. Gimeno[3], and P. Rozenfeld[1]

[1]Laboratorio de Investigación del Sistema Inmune (LISIN) FCE, UNLP, La Plata, Argentina
[2]Grupo Salud Animal INTA, Bariloche, Río Negro, Argentina
[3]Laboratorio de Patología "Dr. Bernardo Epstein" FCV, UNLP, La Plata, Argentina
[4]USDA Poisonous Plant Research Laboratory, Logan, Utah, USA

Acquired α-manosidosis is caused by feeding of swainsonine-containing plants. Swainsonine is an alkaloid that inhibits lysosomal α-manosidasa. Swainsonine has been detected in plants of genus Astragalus and causes intoxication. Different domestic animals are susceptible to intoxication. Moreover, different animals, such as mouse, rat, and guinea pig, have been used as animal models. Guinea pig model has been shown to be the most suitable because of higher sensitivity to the toxin.[1] The aim of this work is to characterize the pathology generated by intoxication with plants from genus Astragalus, using guinea pigs as the animal model. Guinea pigs were fed with *Astragalus pehuenches*-containing food (GP) or control food (GC). Clinical, biochemical, and histopathologic evaluation were performed. Clinically, activity of animals fed GP reduced when compared with those fed GC. Excretion of oligosaccharide was observed in the GP group but not in GC animals in the first week and during whole course of the experiment. Changes in lysosomal α-manosidasa activity were not detected. Histologically, GP animals showed vaculation and cellular degeneration in central nervous system cells. These results suggest neuronal injury in central nervous system caused by oligosaccharide deposits, affecting the functionality.

Reference

1. Cholich LA, Gimeno EJ, Teibler PG, Jorge NL, Acosta de Pérez OC. The guinea pig as an animal model for Ipomoea carnea induced α-mannosidosis. *Toxicon*. 2009; 54:276-282.

010 - Assessment of Energy Homeostasis in Patients With Type I Gaucher Disease in Enzyme Replacement Therapy

D. Doneda[1], A. L. Lopes[2], B. C. Teixeira[2], and I. V. D. Schwartz[3,4]

[1]LTD Laboratory, Nutrition Program, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[2]School of Physical Education, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[3]Genetics Department, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[4]Post-Graduate Program in Medicine: Medical Science, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil

Introduction: Gaucher disease (GD) is an inborn error of metabolism caused by the deficient activity of glucocerebrosidase. The GD is characterized by clinical heterogeneity and clinical manifestations of varied intensity, such as hepatosplenomegaly, hematologic dysfunction, and bone pain; energy homeostasis dysfunction is also present. **Objective:** To assess the energy homeostasis in patients with GD using enzyme replacement therapy (ERT). **Methods:** Assessment, by means of controlled transversal study, of ghrelin, leptin, and adiponectin levels in patients with GD-I of 18 years old and receiving ERT for at least 6 months (n = 15); the patients were pair-matched with healthy controls for sex, age, and body mass index (BMI). **Results:** The median of ghrelin, leptin, and adiponectin levels of patients did not differ from that of the controls. Ghrelin and adiponectin levels presented positive correlation between themselves, with HDL cholesterol, and inverse correlation with BMI, waist circumference, and triglycerides. Leptin levels presented inverse correlation with LDL cholesterol and direct correlation with BMI, waist circumference, enzyme dose, triglycerides, insulin, and homeostasis model assessment-estimated insulin resistance (HOMA-IR). A total of 8 patients (n = 15) met the criteria for metabolic syndrome, 4 of which had insulin resistance as measured by the HOMA-IR index. **Discussion and Conclusion:** Leptin

presented a high association with insulin and with the HOMA-IR index and may eventually become a biomarker to evaluate early evidence of insulin resistance in patients with GD. Metabolic syndrome and insulin resistance seem to be frequent in patients with GD type I. Further research is necessary to investigate the findings obtained in this study.

011 - Atypical Histidinemia: Case Report

G. M. Zayas[1], H. N. Chavez[1], D. Dominguez[1], J. M. Torriente[1], U. Cabrera[2], L. Martinez[3], S. Cardenas[1], B. Suarez[3], and S. C. Gonzalez[1]

[1]Instituto de Nutrición e Higiene de los Alimentos, La Habana, Cuba
[2]Hospital Pediátrico de Centro Habana, La Habana, Cuba
[3]Centro Nacional de Genética Médica, ANLIS, Buenos Aires, *Argentina*

Introduction: Histidinemia is a rare inherited metabolic disorder characterized by histidase enzyme deficiency, which results in elevated histidine levels in blood, urine, and cerebrospinal fluid. **Objective:** To do the clinical, anthropometric, and biochemical characterization in a patient with histidinemia. **Method:** A 7-year-old boy with nonconsanguineous parents, gestational age of 40.3 weeks, Apgar score of 9/9, and birth weight of 3520 g. At 6 months, he was referred for psychomotor developmental delay. Physical examination: Hypotonic, joint hyperlaxity, tongue protrusion, abundant sialorrhea, big and prominent ears, long filtrum, wide forehead, long and thin fingers, convergent bilateral strabismus, and feet valgus. Genetic examination: normal karyotype. Indication: physiotherapy. He presented with speech disorders, and learning difficulties were noted after scholarization. Complementary: electroencephalogram within normal limits, TAC: slight hemispheres asymmetry. Neuropsychologic profile near to average but with disability for executive abilities. Behavior: hyperactivity and aggressiveness. Histidine test in urine and serum was positive. Serum histidine quantification by UMTEST: 6.94 mg/dL (reference value < 3.76 mg/dL), urocanic acid in sweat: present. Anthropometric assessment: normal. **Discussion:** Patient with impaired verbal intelligence, academic abilities (reading, writing, and sustained visual attention), global difficulties in the learning, and high values of serum histidine. **Results:** Diagnosis: atypical histidinemia. Treatment: nutritional, dietary protein restriction. Currently, the patient is attending in a specialized institution in second grade. **Conclusion:** Histidinemia should be considered in patients with learning and language disabilities.

012 - B4GALNT1 Deficiency as a Cause of Hereditary Spastic Paraplegia: A New Inborn Error of Metabolism Affecting Glycosphingolipid Biosynthesis

C. Marques Lourenço[1], G. Stevanin[2], S. Zuchner[3], and W. Marques Jr.[1]

[1]Department of Neuroscience and Behaviour Sciences, School of Medicine of Ribeirão Preto, University of Sao Paulo, Brazil
[2]Université Pierre-et-Marie-Curie, Universite Paris VI, Centre de Recherche de l'Institut du Cerveau et de la Moelle epiniere, EPHE, and Centre national de la recherche scientifique, Unité mixte de recherche 7225, Groupe Hospitalier Pitie-Salpêtriere, Paris, France
[3]John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida, USA

Hereditary spastic paraplegias (HSPs) comprise a complex and heterogeneous group of neurologic disorders. Although the majority of the cases of HSPs are due to genes involved in axonal growth or vesicular trafficking, there is an overlooked group of HSPs that can be caused by inborn errors of metabolism (IEMs). Adrenomyeloneuropathy, late-onset biotinidase deficiency, and cerebrotendineous xanthomatosis are among the relatively known metabolic causes of HSPs. Here, we present a new hereditary metabolic cause of HSP in a Brazilian family caused by a deficiency of enzyme β-1,4-N-acetylgalactosaminyl transferase 1 (B4GALNT1), involved in ganglioside biosynthesis. Patients affected by this disease have early-onset spastic paresis, mild intellectual disability, cerebellar ataxia, and strabismus, and some can develop psychiatric disturbance. Male hypogonadism was also noticed. Brain magnetic resonance imaging showed nonspecific white matter changes in older patients. Although there are many IEMs involved in ganglioside catabolism presenting as neurodegenerative disorders, this enzyme deficiency is the second human disorder identified in the pathway of ganglioside biosynthesis, suggesting that other human diseases can be caused by metabolic errors in this biochemical pathway.

013 - Bile Acid Synthesis Defect Misdiagnosed as Autoimmune Hepatitis

G. P. Duran[1] and C. Gana[1]

[1]División de Pediatría, Escuela de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile

Introduction: Inborn errors of bile acid synthesis are rare hereditary defects presented as progressive cholestatic liver disease and fat-soluble vitamin malabsorption secondary to failure to produce normal bile acids and accumulation of unusual bile acids. Deficiency of 3β-hydroxy-Δ5-C27-steroid dehydrogenase (3β-HSD) is the most frequent defect. Autoimmune hepatitis is an inflammatory disease associated with hypergammaglobulinemia, presence of autoantibodies, and response to immunosuppressants. Hyperbilirubinemia, elevation of transaminases, normal γ-glutamyl transpeptidase (GGT), and nonspecific liver biopsy are common to both disorders. **Objective:** To Report defect of bile acid misdiagnosed as an autoimmune hepatitis. **Case Report:** A previous healthy 4-year-old girl, first child of nonconsanguineous parents, who at 30 months presented with coluria, jaundice, progressive increase in transaminases (AST 378 IU/L, ALT 262 IU/L), normal GGT, presence of antismooth muscle antibodies, and

unspecific liver biopsy diagnosed as autoimmune hepatitis, initially responsive to prednisone and azathioprine. After 6 months, clinical worsening was evident and bile acid synthesis defect was suspected. The urine bile acid profile was consistent with 3β-HSD deficiency. Cholic acid was initiated followed by normalization of liver function. **Conclusion:** Defects of bile acid synthesis must be included in the differential diagnosis of cholestatic liver disease beyond neonatal period, because it is a good prognosis treatable defect.

014 - Biotinidase Deficiency: Assessment of Clinical and Molecular Aspects in a Sample of Brazilian Patients

T. Borsatto[1,2], F. Sperb-Ludwig[3,4], L. L. C. Pinto[5], G. R. De Luca[5], L. F. Carvalho[5], C. F. M Souza[6], P. F. V. De Medeiros[7], C. M. Lourenço[8], S. Leistner-Segal[3,6], and I. V. Schwartz[1,2,6,9]

[1]*Programa de Pós-graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, Brazil*
[2]*Laboratório BRAIN, Centro de Pesquisa Experimental (CPE), Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil*
[3]*Programa de Pós-graduação em Medicina: Ciências Médicas, UFRGS, Brazil*
[4]*Centro de Terapia Gênica, CPE, HCPA, Porto Alegre, Brazil*
[5]*Hospital Infantil Joana de Gusmão, Florianópolis, SC, Porto Alegre, Brazil*
[6]*Serviço de Genética Médica, HCPA, Porto Alegre, Brazil*
[7]*Universidade Federal de Campina Grande, PB, Porto Alegre, Brazil*
[8]*Serviço de Genética Médica, Hospital das Clínicas de Ribeirão Preto, Universidade de São Paulo, Porto Alegre, Brazil*
[9]*Departamento de Genética, UFRGS, Porto Alegre, Brazil*

Introduction: Confirmation of biotinidase deficiency (BD), an autosomal recessive inborn error of metabolism, depends on enzyme activity measurement in plasma. Enzyme lability affects the identification of its true activity level (normal, heterozygous, partial deficiency, or total deficiency), making the decision about starting therapy difficult. **Objective:** To evaluate the clinical history and BTD gene sequence in Brazilian patients with BD. **Methods:** This is a cross-sectional multicenter study with convenience sampling. Clinical data and blood samples were obtained from 30 unrelated individuals with BD (15 male; 1 case of consanguineous parents) aged between 1 month and 18 years. Exons 2, 3, and 4 of BTD gene will be sequenced; exon 4 was the first. **Results:** The BD was identified by neonatal screening in 26 patients (23 are currently using biotin, and none shows symptoms) and based on clinical suspicion in 4 (most common manifestations: optic atrophy, motor regression, spastic paresis; onset of symptoms: 2 months-10 years of age; diagnosis: 7 months-18 years of age). Among the 19 patients whose exon 4 was analyzed, 6 different mutations and 1 synonymous substitution were found, all previously described in the literature. In 1 of 19 patients, no alteration was detected in the region analyzed. Mutation p.D444H (c.1330G>C) was the most frequent and was present in at least 1 allele in 15 of 19 patients. **Conclusion:** Our preliminary results suggest that there is a high prevalence of p.D444H in Brazil, which, according to the international literature, is the variant that is most frequently associated with partial BD.

015 - Nutritional Treatment for β-Ketothiolase Deficiency

Sanchez Peña Alejandra[1], Elizondo Cardenas Gabriela[2], Ibarra Ramirez Marisol[1], Torres Sepulveda Rosario[1], Lopez Uriarte Areli[1], and Martinez de Villarreal Laura E[1]

[1]*Departamento de Genética, Facultad de Medicina, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México*

Background: β-ketothiolase deficiency is a mitochondrial defect in acetoacetyl-CoA thiolase in isoleucine catabolism. **Objective:** To present a patient with β-ketothiolase deficiency under nutritional management. **Case:** A 1-year-5-month-old boy who presented with jaundice at birth. At consultation he had diarrheal stools, vomiting, tachypnea, and anaphylactic breathing. When he started enteral feeding, new deterioration signs such as loss of sucking and swallowing, leading to gastrostomy were evident. In organic acids determination by GC/MS, 2 methylglutaconic, 3 hydroxyvaleric acid, 2-methyl 3-hydroxybutyrate, and triglilcarnitine were detected. Amino acid quantitation (high-performance liquid chromatography) showed high amino acids levels for alanine, methionine, serine, glycine, isoleucine, and valine, establishing the diagnosis of β-ketothiolase deficiency. Weight: 6.9 kg (3rd percentile), height: 79 cm (50th percentile), body mass index: 12.9 kg/m^2 (<3rd percentile), WBC: 12 cm. (<5th percentile) PCT: 6 mm. (5th percentile), C. Thigh: 19 cm, C. Cephalic: 47 cm. (25th percentile), P/T: −3SD, T/E: −1SD, P/E: −3SD. Nutritional management consisted of isoleucine-restricted diet and carnitine-formula supplement Ketonex providing 120 kcal/kg. **Results:** One year after treatment onset, the patient was 12 kg (50 percentile) in weight and height was 87 cm. Gastrostomy was removed, and amino acid levels were in the normal range 2 months later. **Conclusion:** Rapid diagnosis and monitoring of ongoing nutritional treatment is important for growth and proper development of patients with inborn errors of metabolism.

016 - Bone Disease as the Only Clinical Manifestation of Type I Gaucher Disease

N. Guelbert[1], H. Robledo[2], A. B. Becerra[1], C. J. Angaroni[1], A. N. Giner-Ayala[1], A. Ramirez-Oller[1], and R. Dodelson de Kremer[1]

[1]*Centro de Estudio de las Metabolopatías Congénitas (CEMECO), Cátedra de Clínica Pediátrica, Facultad de Cs Médicas, UNC, Hospital de Niños de Córdoba, Argentina*

[2]Instituto de Diagnóstico por Imágenes, Conci-Carpinella, Córdoba, Argentina

Introduction: Gaucher disease (GD) is a lysosomal disease, due to the deficiency of Î²-glucosidase, characterized by involvement of hematopoietic organs such as the spleen, liver, bone marrow, lung, and bone. Bone is the second most commonly affected structure, presenting bone infiltration and macrophage interleukins that determine osteoblast/osteoclast imbalance and a deleterious effect on bone. This impacts a patient's quality of life, causing pain, fracture, and orthopedic surgery requirements. We present a patient without hematologic or visceral involvement but with skeletal manifestations as the only clinical finding. **Objective:** To highlight bone disease as the main clinical manifestation in a patient with GD. To consider total body magnetic resonance imaging (MRI) scan as the most sensitive diagnostic tool in the assessment of skeletal involvement. **Patient:** C.K., a woman, 40 years old, was diagnosed with GD during a family study. Her sister was affected since she was 3 years, splenectomized at 18 years, and was under enzyme replacement therapy (ERT) for 14 years. After 10 years of follow-up without ERT, C.K. did not present visceromegaly, hematologic compromise, or crisis. Radiologic studies and bone density were assessed because of bone pain, showing normal results. Total body MRI showed severe skeletal involvement, with infiltration and osteolytic images in vertebrae, hip bones, and femurs. These findings associated with very high chitotriosidase lead to immediate ERT implementation. **Conclusion:** As bone is frequently affected in GD, it is important to perform skeletal studies using sensitive methods such as MRI to demonstrate affections and to determine the most appropriate therapy.

017 - Brain Macroangiopathy in Fabry Disease: Evidence by Magnetic Resonance Imaging

J. Politei[1], M. Szlago[1], A. B. Schenone[1], and S. Lescano[2]

[1]FESEN-Laboratorio de Neuroquímica "Dr. N.A. Chamoles," Buenos Aires, Argentina
[2]Servicio de Neuroimágenes, Hospital Juan Fernandez, Buenos Aires, Argentina

Introduction: Fabry disease (FD) is a rare hereditary lysosomal storage disease that has been recognized as a possible etiology of stroke at a young age. Typical MRI findings are white matter lesions, microbleedings, vascular tortuosity (basilar dolichoectasia [BD]), Arnold-chiari type 1, and pulvinar sign. **Aim:** To present magnetic resonance imaging (MRI) findings in patients with FD from Argentina and relationship with the presence of cardiovascular risk factors (CVRFs). **Patients and methods:** A total of 70 patients with FD (27 men) were enrolled. We measured the presence of acroparesthesias, renal compromise, cardiac compromise, and corneal involvement. Enzyme replacement therapy (ERT) status was evaluated as well as the presence of cardiovascular risks factors. For BD, smoker's criteria were used. **Results:** Global mean age was 32.6 $\pm$ 1.8 years; 48% and 96.3% of women and men were under ERT, respectively. Renal compromise was reported in 60% of the population, cardiac compromise in 30%, corneal involvement in 91.4%, and acroparesthesias were present in 87.1%. Silent ischemias were found in 26% of the men and 30.2% of the women. The BD was the most frequent finding in men (63%) and women (39.5%). **Conclusion:** Our results show that cerebrovascular involvement may occur before the third decade of life. Presence of white matter lesions in MRI in young population is suggestive of FD. Given the high frequency of BD in young patients, this sign should increase FD suspicion by neuroradiologist.

018 - Broadening of Neonatal Screening in the Federal District of Brazil Through the Implementation of Tandem Mass Spectrometry

M. T. O Cardoso[1], R. Pogue[2], R. N. Oliveira[1], I. O. Silva[1], R. S. Freitas[1], M. S. Viegas[1], L. M. C. Avelino[1], H. Pogue[2], J. V. Thomas[1], E. Ranieri[3], and R. D. Teixeira[1]

[1]Núcleo de Genética/HAB/SES-DF, Brasília, Brazil
[2]Universidade Católica de Brasília, Brasília, Brazil
[3]South Australian Neonatal Screening Centre, Adelaide, Australia

Until 2008, the Brazilian Federal District neonatal screening program included phenylketonuria, congenital hypothyroidism, and hemoglobinopathies. In compliance with the Regional Law 4190/2008 that instituted a broadened neonatal screening protocol in the Federal District public network, tandem mass spectrometry was incorporated for screening of aminoacidopathies, beta oxidation defects, carnitine capture and transport defects, and organic acidemias. The objective of the current work is to describe mass spectrometry implementation in Federal District screening protocols. Cutoff establishment for diseases included in the broadened program for this population was carried out by a 3-step sampling process: first including 3500 samples (provided through collaboration with Dr Enzo Ranieri), then adding 9700 samples 5 months later, and finally 23 500 samples analyzed after another 7 months. Cutoff values were defined by calculation of 99th, 99.5th, and 99.9th percentiles for upper limits, and 0.1th and 0.5th percentiles for lower limits. Confirmatory tests for children with positive results were performed in partner laboratories using gold standard techniques. With the paradigm change in the data collection and analysis methods, after 2 years of experience and 90 000 samples analyzed, we have achieved 95.6% population coverage including children up to 7 years of age. Currently, we are the only Brazilian public service that performs neonatal screening for 22 diseases, together with the National Neonatal Screening Program.

019 - Characterization of the A359D Acid Sphingomyelinase Mutation Causing Niemann-Pick B in Chilean Patients

M. Acuña[1], P. Martinez[1], S. Faundez[1], M. Latorre[2], V. Castro[3], M. Lagos[4], V. Guiche[3], M. Gonzalez[3], J. F. Miquel[1], J. L. Santos[5], E. Schuchman[6], P. Mabe[7], and S. Zanlungo[1]

[1]*Departamento de Gastroenterología, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile*
[2]*INTA, Universidad de Chile, Santiago, Chile*
[3]*Facultad de Ciencias, Universidad de Chile, Santiago, Chile*
[4]*Laboratorio Clínico, Pontificia Universidad Católica de Chile, Santiago, Chile*
[5]*Departamento de Nutrición, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile*
[6]*Mount Sinai School of Medicine, New York, USA*
[7]*Hospital Exequiel González Cortés, Santiago, Chile*

Introduction: Acid sphingomyelinase (ASM) catalyzes hydrolysis of sphingomyelin. Mutations in the ASM gene are responsible for Niemann-Pick A and B (NPB). The A359D mutation is described only in Chilean patients with NPB, and it was found in 39 (93%) of 42 of the sequenced alleles. Considering this, it is likely that this mutation comes from a common ancestor. **Aim:** To determine whether A359D mutation comes from a common ancestor and to study its effect on ASM's 3-dimensional structure, expression, localization, and activity. **Methods:** To determine a common ancestor origin for the A359D mutation, DNAs from controls and homozygous patients were analyzed using genomic markers. Three-dimensional ASM modeling was carried out using homology modeling to predict the A359D mutation effect. Fibroblasts from controls and A359D homozygous patients were used for analyses of ASM activity, expression, and localization. The *ASM* expression levels were also analyzed by Western blot in hepatic biopsies from A359D homozygous patients. **Results:** Patients have identical genetic markers near the A359D mutation suggesting a common ancestor. Three-dimensional model of *ASM* suggests that A359D destabilizes a beta-sheet structure near the catalytic site. The ASM activity was decreased in fibroblasts from A359D homozygous patients; although quantitative polymerase chain reaction analysis, Western blotting, and fluorescent microscopy showed that the mutant ASM was expressed at normal levels and trafficked to lysosomes. Normal ASM expression levels were also found in hepatocytes from A359D homozygous patients. **Discussion:** A359D mutation derives from a common genetic ancestor and causes a decrease in *ASM* activity but doesn't change the protein expression and localization. The study was supported by FONDAP 15090007.

020 - Cholesteryl Ester Storage Disease With Early Hepatic Complications in Mexican Siblings With New Mutations in Lysosomal Acid Lipase Gene

R. E. Piña-Aguilar[1], E. Almanza-Miranda[1], A. Vera-Loaiza[1], M. E. Gorraez de la Mora[1], and Y. Santillan-Hernandez[1]

[1]*Centro Médico Nacional, Federal District, México*

Cholesteryl ester storage disease (CESD, *Online Mendelian Inheritance in Man* [OMIM] 78000) is poorly reported but has an estimated incidence of 1 in 40 000. It is caused by a partial deficiency of the enzymatic activity of lysosomal acid lipase (LAL). Clinical presentation includes hepatosplenomegaly, dyslipidemia type IIb, and early atherosclerosis; however, mild forms can be asymptomatic until adult age. In this report, we describe 2 siblings with very early presentation and complications. **Case 1:** Symptomatology began at 2 months of age with alternating diarrhea and constipation; at 6 months she presented with hepatomegaly, transaminasemia, high cholesterol and triglicerides, and low HDL; bone marrow aspirate showed foamy histiocytes; at 4 years portal hypertension and esophageal varices grade III were detected. Hepatic biopsy showed microvesicular steatosis, lipofuscin deposits in Kupffer cells and portal macrophages, disseminated fibrosis, and cirrhosis. Cholesterol esterification in fibroblasts was 2% of the control with normal filipin stain. **Case 2:** Symptomatology began at 6 months of age with hepatomegaly; at 2 years, she presented with transaminasemia and dyslipidemia. Currently, Doppler shows an increased hepatic flux and she has normal endoscopy. Activity of LAL in both the patients was <0.02 nmol/L/punch × h (normal: 79.9-378.6 nmol/L/punch × h). Sequencing of LAL gene in both cases demonstrates 2 heterozygous mutations in exon 4, c.253C>A (p.Gln85Lys) y and c.294C>G (p.Asn98Lys). This is the first report of mutations in Mexican patients, which have not been described previously and which correspond to the absence of LAL activity. These explain an initial symptomatology similar to Wolman disease, including the rapid progression and early hepatic damage.

021 - Classic Ketogenic Diet Versus Modified Atkins Diet: Effectiveness Evaluation

H. Fain[1], S. Galicchio[1], V. Blanco[1], A. Cabrera[1], V. Bonetto[1], V. Buiras[1], J. Muniategui[1], N. Villanova[1], and C. Fain[1]

[1]*Hospital de Niños Víctor J. Vilela, Rosario, Argentina*

Objective: To assess the effectiveness for reducing seizures of a classic ketogenic diet (CKD) compared to with modified

Atkins Diet (MAD) in patients with refractory epilepsy (RE). **Methods:** Twenty-three patients under treatment with ketogenic diet, between April 2008 and July 2013, were evaluated. In all, 15 patients received CKD and 11 received MAD. **Results:** Classic ketogenic diet was implemented in 15 patients. The average age was 8.2 years. It was observed that 66.62% had a good, very good, or excellent reaction to the treatment. In 33.3% with partial answer, we saw an improvement in attention level. All patients lost weight or remained stable. Diet had to be stopped in 3 of them because of significant loss of weight, metabolic disturbances, or alterations in platelet adhesiveness. From March 2012 to July 2013, MAD was implemented in 11 patients. Average age was 11.3 years. It was observed that 72.58% had a good, very good, or excellent reaction to the treatment. Weight progress was acceptable in all patients, and the diet did not have to be stopped because of disturbances. **Conclusion:** Improvement in seizure' control was observed in both the groups, with a reduction in crisis in 72.72% in MAD and 66.62% in CKD groups. Weight loss was reported and complications such as metabolic disturbances were observed with CKD. Weight progress was acceptable, and no complications were recorded with MAD. We consider that MAD is a good alternative for RE treatment.

022 - Clinical and Biochemical Aspects of Gaucher Disease Type 2

L. Miranda[1], A. Bracho[1], A. Morales[1], K. Mendez[1], J. Chacin[1], W. Delgado[1], Y. Sanchez[1], C. Zara[2], C. J. Chavez[3], and F. Cammarata-Scalisi[1]

[1]*Instituto de Investigaciones Genéticas, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela*
[2]*Fundación Hospital de Especialidades Pediátricas, Maracaibo, Venezuela*
[3]*Instituto de Investigaciones Biológicas, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela*

Introduction: Gaucher disease type 2 or acute neuropathic form starts in the first months of life with central nervous system involvement and causes death before 2 years of age. Its incidence is less than 1:100 000 newborns. Affected children present neck retroflexion, developmental delay, protuberant abdomen (hepatosplenomegaly and hypersplenism), and signs of brain stem involvement (convergent squint, ocular paresis, and dysphagia). **Objective:** To present the clinical and biochemical findings of Gaucher disease type 2. **Patients and Methods:** A 14-month-old female infant born from first pregnancy of nonconsanguineous parents. Since 6 months of age, she presented with swallowing difficulty and is unable to stay seated. She was hospitalized at 7 and 10 months because of seizure syndrome, pneumonia, and anemia. Physical examination showed poor clinical conditions, stare, constant drooling, indrawing chest, and bilateral prominent abdomen with hepatosplenomegaly. **Neurologic Examination:** neck retroflexion, ophthalmoplegia, and generalized hypertonia. **Results:** Hematologic studies showed moderate anemia with thrombocytopenia and moderate hepatosplenomegaly. β-glucosidase enzyme activity was determined, showing decreased values (0.1 mmol/L/h normal value: 1.69-3.47 mmol/L/h) and chitotriosidase activity with elevated values (1622.27 mmol/L/h, normal value: 1.0-74 mmol/L/h). **Discussion:** The clinical and biochemical findings were consistent with Gaucher disease type 2. Diagnosis establishment indicates that through diagnosis it is possible to offer available therapeutic options and appropriate family counseling.

023 - Clinical and Biochemical Profile of Brazilian Patients With Classical Homocystinuria

S. Poloni[1], C. F. M. Souza[1], P. Bernardi[2], C. M. Lourenço[3], M. J. R. Doriqui[4], E. Valadares[5], M. B. P. Toralles[6], and I. V. D. Schwartz[1]

[1]*Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil*
[2]*Universidade Federal de Santa Catarina, Santa Catarina, Brazil*
[3]*Hospital das Clínicas de Ribeirão Preto, São Paulo, Brazil*
[4]*Complexo Hospitalar Materno-Infantil do Maranhão, Brazil*
[5]*Departamento de Genética, Universidade Federal de Minas Gerais, Minas Gerais, Brazil*
[6]*Universidade Federal da Bahia, Salvador, Brazil*

Introduction: Classic homocystinuria is an inborn error of metabolism caused by cystathionine β-synthase deficiency and characterized by high levels of homocysteine. Four systems are mainly affected in this disease: ocular, vascular, skeletal, and central nervous. **Methods:** A questionnaire containing diagnostic data, current clinical status, metabolic control, and treatment strategies was sent to referral centers for treatment of genetic diseases, in Brazil. Ten centers participated in the study. **Results:** A total of 18 patients from 15 families was included. The median age was 17 years (8-36 years). Age at diagnosis ranged from 2 to 25 years (median = 7 years). The main reasons for homocystinuria investigation were ectopia lentis (13 of 18 patients) and intellectual disabilities (7 of 18 patients). Of the 15 patients with pyridoxine responsiveness, 12 were nonresponsive, 2 partially responsive, and 1 fully responsive. At the evaluation, all patients had ocular complications, 11 had intellectual disabilities, 4 had already presented a thromboembolic event, and 11 had bone abnormalities. Median homocysteine level was 211 μmol/L (14-454 μmol/L). Only 3 patients had homocysteine <60 μmol/L (therapeutic target). Treatment: Of 15 patients, 13 were using pyridoxine, 11 were using betaine, 10 were using folate, and 4 were using metabolic formula. Three patients were considered nonadherent, 8 partially adherent, and 5 fully adherent. **Conclusion:** The characteristics of this sample suggest that classic homocystinuria is underdiagnosed in Brazil. Furthermore, it is observed that there is great difficulty in metabolic control. This may be related to

poor adherence to treatment and lack of access to adequate treatment, especially with regard to metabolic formula.

024 - Clinical, Biochemical, and Epidemiologic Diagnosis of Lysosomal Storage Disorders in Cuba

D. Hernandez[1], M. Monaga[1], M. C. Menendez[2], and E. C. Gonzalez[3]

[1]*Centro Nacional de Genética Médica, Habana, Cuba*
[2]*Instituto de Neurología y Neurocirugía, Habana, Cuba*
[3]*Centro de Inmunoensayo, Habana, Cuba*

Objective: To characterize the diagnosis of lysosomal storage disorders (LSDs) in Cuba for the past 7 years, taking into account clinical and biochemical elements. **Methodology:** All postnatal studies under LSD suspicion during January 2006 to June 2013 were included: 172 patients, 256 parents, and 174 healthy controls. Information regarding clinical symptoms, sex, age, place of origin, and medical specialist referring each patient was obtained. Six prenatal studies on pregnant women with genetic risk were included. Enzyme activity analysis was used to confirm each particular LSD. **Results:** Neurologic and facial–skeletal abnormalities, weight–height retardation, and hepatosplenomegaly were the most common clinical signs. Of the studies, 54% and 38% were referred by clinical geneticists and neurologists, respectively. A total of 26 patients were confirmed as having LSD, most of them were children. Metachromatic leukodystrophy (n = 8) and fucosidosis (n = 6) were the higher incidence diseases. In all cases, the enzyme activity in patients was less than 20% of controls. **Conclusion:** The positivity in the confirmation of LSD significantly increased in relation to previous studies (15.1% vs 8.2%). A specific province of our country has the highest incidence of fucosidosis worldwide.

025 - Cognitive Functioning and Health-Related Quality of Life of Early-Treated Brazilian Patients With Phenylketonuria

E. Vieira Neto[1,2], H. S. Maia Filho[3], C. B. Monteiro[4], M. A. Mendes[5,6], L. M. Carvalho[7], V. N. Paiva[8], and M. G. Ribeiro[1]

[1]*Serviço de Genética Médica, Instituto de Puericultura e Pediatria Martagão Gesteira- IPPMG, UFRJ, Rio de Janeiro, Brazil*
[2]*Agência Nacional de Saúde Suplementar, Rio de Janeiro, Brazil*
[3]*Departamento Materno-Infantil, Faculdade de Medicina, UFF, Rio de Janeiro, Brazil*
[4]*Núcleo de Estudos da Saúde do Adolescente-NESA, UERJ, Rio de Janeiro, Brazil*
[5]*Instituto de Puericultura e Pediatria Martagão Gesteira-IPPMG, UFRJ, Rio de Janeiro, Brazil*
[6]*Centro Universitário Celso Lisboa*
[7]*Serviço de Metabologia, Instituto Estadual de Diabetes e Endocrinologia Luiz Capriglione-IEDE, Rio de Janeiro, Brazil*
[8]*Núcleo de Atendimento Neonatal-IEDE, Rio de Janeiro, Brazil*

Introduction: Early-treated patients with phenylketonuria (PKU) can achieve normal intellectual development. However, living with a chronic disease that requires treatment throughout life may have consequences on patients' health-related quality of life (HRQoL). Moreover, cognitive functioning may influence HRQoL. **Objectives:** To investigate, in children and adolescents with PKU, the implications of intellectual development, assessed by Raven Colored and Standard Progressive Matrices Test and locale of residence on HRQoL assessed by the Pediatric Quality of Life Inventory– PedsQL 4.0 Generic Core Scales. **Methodology:** Raven Test and PedsQL were applied to children/adolescents with early and continuously treated patient with PKU attending a pediatric metabolic clinic at Rio de Janeiro. PedsQL parent proxy-reports were also used. **Results:** Raven test, PedsQL child self-reports, and parent proxy reports were administered, respectively, to 35 and 34 children and adolescents, aged 6 to 17 years, and to 31 parents. PedsQL scores for the child self-reports were significantly lower than that for the parent proxy-reports ($P = .0430$). Patients with intellectual disability, by Raven test results, and from nonmetropolitan areas had lower PedsQL scores for the child self-reports than their counterparts, without intellectual disability and from metropolitan areas ($P = .0171$ and .018). In contrast, PedsQL scores for the parent proxy reports showed no significant differences in relation to the presence of intellectual disability ($P = .1889$) and locale of residence ($P = .2437$). **Conclusion:** The detection of intellectually disabled early-treated PKU in children and adolescents may reflect poor dietary compliance. We demonstrated the negative impact of intellectual disability and locale of residence disparities in HRQoL scores in patients with PKU.

026 - Compendium of 18 Years of High-Risk Screening for Lysosomal Disorders in Colombia (1995-2012)

Uribe Alfredo[1], Adis Ayala[2], España Monica[1], Pacheco Natalia[1], Jay Lina[1], Arevalo Isidro[1], and Benavides Jacqueline[1]

[1]*Centro de Investigaciones en Bioquímica, Dpto Ciencias Biológicas, Universidad de los Andes, Colombia*
[2]*Universidad Distrital Francisco José de Caldas, Bogotá, Colombia*

Introduction: The diagnostic approach of lysosomal disorders (LDs) in Colombia is made through clinical suspicion and biochemical screening protocols that involve different methodologies and samples. Some of the greatest difficulties along this process are low patient access to research centers, sample handling and stability, shipping costs, and misdiagnosis of several pathologies. **Objective:** In order to document strategies that help solve some of the above-mentioned difficulties, we present the results of 18 years of high-risk screening for LDs, in

Colombia. This information includes developed protocols, test evolution, frequencies and implemented alternatives, and enzymatic evaluation in dried blood spots (DBS). **Methods:** We analyzed 9112 patients with features suggesting a lysosomal disease. They were referred between 1995 and 2012. From them, 2469 were studied with traditional protocols that involved liquid samples (urine, serum, or total blood) and 6643 were analyzed through DBS samples (since 2005). **Results/Discussion:** Among the analyzed patients, we found 351 affected by a LD (90% of them, detected with the DBS protocol). Lysosomal disorders found include Fabry (n = 24), fucosidosis (n = 2), Gaucher (n = 117), gangliosidosis-GM1 (n = 13), Krabbe (n = 1), metachromatic leukodystrophy (n = 12), mucolipidosis (n = 6), mucopolysaccharidosis (type-I, n = 15; type-II, n = 43; type-III, n = 11; type-IVA, n = 42; type-IVB, n = 1; type-VI, n = 38; type-VII, n = 1), Pompe (n = 19), Sandoff (n = 2), sialidosis (n = 1), and Tay Sachs (n = 3). These results are evidence that DBS studies facilitate detection of patients with LDs, in Colombia.

027 - Congenital Disorder of Glycosylation: A Putative Role of Human Platelets NCX1 and NCKX1 CA+2 Exchangers in Thrombus-Hemorrhagic Events Associated With CDG

M. B. Bistue Millon[1], M. Syravegna[2], N. B. Specola[3], S. Chacon[4], R. Dodelson de Kremer[1], G. Elso de Berberian[2,5], and C. G. Asteggiano[1,5,6]

[1]*Centro de Estudio de las Metabolopatías Congénitas (CEMECO), Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba Argentina*
[2]*Laboratorio Biofísica, Instituto Mercedes y Martín Ferreyra (IMMF-CONICET), Córdoba, Argentina*
[3]*Unidad Metabolismo, Hospital de Niños de La Plata, Buenos Aires, Argentina*
[4]*Servicio Neurología Infantil, Hospital Centenario de Gualeguaychu, Entre Rios, Argentina*
[5]*Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina*
[6]*Facultad de Medicina, Universidad Católica de Córdoba, Córdoba Argentina*

Congenital disorders of glycosylation (CDG) are genetic diseases due to defects in glycoproteins or glycolipids synthesis. The phenotype is multisystemic and thrombus–hemorrhagic events are frequently observed in these patients. In platelets, Ca^{2+} signaling is necessary to prevent inappropriate thrombus formation. The Na^{+}/Ca^{2+} (NCX) and Na^{+}/K^{+}-Ca^{2+} (NCKX) exchangers play a crucial role in controlling cytosolic Ca^{2+}. **Methods:** Blood from healthy and patients with PMM2-CDG was obtained, and subcellular fractions were analyzed by Western blot to determine NCX1 and NCKX1 expression and 45 Ca^{2+} activity with or without 150 mmol/L K^{+}(e). Glycomic characterization was performed by lectin affinity chromatography, immunoprecipitation, and enzymatic deglycosilation followed by Western blot. **Aim:** To biologically characterize these exchangers in human platelets, with special emphasis on glycosylation status and function in patients with CDG. **Results:** We demonstrate for the first time (1) NCX and NCKX protein expressions in human platelets and NCX1 and NCKX1 45 Ca^{2+} uptake, (2) heavy N- and O-glycosylation of both the proteins, (3) significant decrease in Na^{+}-dependent 45 Ca^{2+} capture in P1 (PMM2-CDG) with abnormal platelet aggregation (*t* test, $P < .05$), and (4) lower levels of NCKX1 (n = 3; $P < .05$) and reduced expression of NCX1 in a patient with PMM2-CDG (n = 3; $P < .0001$) and low 45 Ca^{2+} exchange levels by NCKX1 (n = 3, $P < .05$) and reduced NCX1 expression (n = 3, $P < .0001$) in P2 (PMM2-CDG). **Discussion:** We demonstrated that both exchangers are glycosylated and that the expression and activity of NCX1 are more dramatically reduced than NCKX1, suggesting a possible role for the abnormal platelet aggregation in patients with CDG.

028 - Correlation Between Quantification of Glycosaminoglycan in Urine and Urine-Impregnated Filter Paper

Ana Carolina Breier[1], C. E. Jaqueline[1], and Janice Carneiro Coelho[1]

[1]*Universidade Federal do Rio Grande do Sul, Porto Alegre/RS, Brazil*

Mucopolysaccharidoses (MPS) are a group of lysosomal storage disorders caused by deficiency of enzymes responsible for the degradation of glycosaminoglycans (GAGs). The MPS are multisystemic because of accumulation of GAGs. Diagnosis is generated by enzymatic activity determination; however, the initial screening can be performed by measuring GAGs, which are disease markers in urine. Our aim was to establish correlation of results between creatinine and GAGs techniques as well as electrophoresis, which were applied in both the urine sample and the urine-impregnated filter paper (UFP). We collected healthy samples from different ages (n = 17) and from patients with MPS-I and MPS-VI (n = 7). For UFP sample preparation, we cut Whatman 903 filter paper into rectangles, which were impregnated directly into flasks with approximately 10 mL of urine. The techniques used were based on Jong et al, 1992 and Uribe et al, 2008 studies. Creatinine and GAG results were very close in both the sample types. Pearson correlation for creatinine was $r = .97$ and $r = .92$, and for GAGs was $r = .96$ and $r = .93$, between patients and controls, respectively. In addition to that the electrophoretic pattern remained the same. Our results have shown a strong correlation between urine and UFP for quantification of creatinine and GAGs. Therefore, this type of sample collection presents reliable results and enables safer transportation, besides favoring the test availability among high-risk population with MPS.

029 - Creatine Metabolism and Hyperammonemia in Argentinian Patients With Ornithine Transcarbamilase Deficiency

L. D. Martinez[1,2], L. E. Larovere[1], M. Bezard[1], S. Silvera[1], and R. Dodelson de Kremer[1]

[1]Centro de Estudio de las Metabolopatías Congénitas, CEMECO, Cátedra de Clínica Pediátrica, Facultad de Ciencias Médicas, UNC, Hospital de Niños Córdoba, Argentina
[2]Biología Celular-Cátedra "B", Facultad de Odontología, UNC, Argentina

Introduction: Creatine (Cr) biosynthesis requires 2 enzymes, arginine–glycine amidinotransferase and guanidinoacetate methyltransferase, and it can be taken up by cells using transporters. Recent studies demonstrated the impact of toxic ammonia ($NH4^+$) in ornithine transcarbamilase deficiency (OTCD), a urea cycle defect (UCD), on Cr metabolism. The Cr secondary deficiency has been found in mice experimental models and in brain cells' primary culture but are yet to be tested in humans. **Objective:** To evaluate relationships between $NH4^+$ and Cr synthesis by guanidine compounds analysis in patients with OTCD. **Methodology:** A total of 6 patients with OTCD, 3 hemizygotes and 3 symptomatic carriers. The studies were performed in samples during crisis conditions. Measurement of guanidinoacetate (GAA) and Cr in urine were performed by gas chromatography; creatinine (Crn) in urine and $NH4^+$ in plasma by spectrophotometric methods. **Results:** In hemizygote patients with OCTD, GAA range was 0 to 11 mmol/mol Crn (controls: 2-220), with Cr in normal range, 28 to 1070 mmol/mol Crn (controls: 6-1208); all patients had severe hyperammonemia, 451 to 2182 µmol/L (controls: 10-47). In symptomatic carriers, the GAA range was 0 to 39 mmol/mol Crn with Cr concentration at lower limits of normal range, 44 to 70 mmol/mol Crn; patients had mild and severe hyperammonemia, 140 to 1093 µmol/L. **Conclusion:** According to this report, Cr metabolism is shown to be altered and may participate in central nervous system dysfunction in patients with UCD. Therefore, Cr supplementation should be a neuroprotective tool of toxic effect on brain exposed to ammonium in patients with UCD.

030 - Current Picture of Peroxisomal Diseases in Colombia From a Diagnostic Reference Center

Pulido Ninna Fernanda[1], Y. A. Ardila[1], Sanchez Yasmin[2], Cabarcas Lisseth[1], Echeverri Olga Yaneth[3], Guevara Johana Maria[3], Espinosa Eugenia[4], and L. A. Barrera[1,3]

[1]Hospital Universitario San Ignacio, Bogotá, Colombia
[2]Hospital San Rafael, Tunja, Colombia
[3]Instituto de Errores Innatos del Metabolismo, Pontificia Universidad Javeriana, Bogotá, Colombia
[4]Hospital Militar Central, Universidad Nueva Granada, Bogotá, Colombia

Introduction: The peroxisomal disorders are inherited diseases, resulting from alterations in the peroxisome biogenesis. Its incidence is estimated at 1:50,000 newborns alive, being the most common the X-linked adrenoleukodystrophy (X-ALD) with an incidence of 1/17.000, followed by Zellweger Syndrome (ZWS) and rhizomelic chondrodysplasia punctata (RCDP). **Objective:** To present the experience of the Institute of Inborn Errors of Metabolism at the Hospital Universitario San Ignacio in collaboration with the Kennedy Krieger Institute in the diagnosis of peroxisomal disorders between 2010-2013. **Methods:** Retrospective analysis of peroxisomal diseases diagnosis in Colombia. **Results:** 167 samples were evaluated and there was an increase in the number of ordering of this test (increase of 10.5 % related to the number in 2010). During this period, 7.2 % of the samples were positive (9 X-ALD, one ZWS, one RCDP, one elevation of triene/tetraene acids and 2 heterozygous X-ALD). Among the positive samples for X-ALD we identified two asymptomatic patients. The age of the onset of symptomatic patients was between 7-9 years and the main clinical signs were seizures and demyelination. **Conclusions:** There is no effective treatment for symptomatic patients with X-ALD but the use of Lorenzo's oil could lower very long chain fatty acids levels. The timely diagnosis also contributes to an adequate genetic counseling to families suffering from these diseases.

031 - Delayed Diagnosis of Nephrophatic Cystinosis in Mexico

L. Belmont-Martinez[1], M. Vela-Amieva[1], C. Fernandez-Lainez[1], S. Guillen-Lopez[1], A. Hernandez-Montiel[1], I. Ibarra-Gonzalez[1], and S. Monroy-Santoyo[1]

[1]Instituto Nacional de Pediatria, Federal District, Mexico

Background: Cystinosis is an autosomal recessive lysosomal disorder that may represent a challenging diagnosis because of low prevalence and symptoms similar to other diseases. **Objective:** To estimate the time elapsed between age of onset of clinical manifestations and age of diagnosis in Mexican patients with nephropathic cystinosis. **Methods:** Longitudinal observations of 36 patients with documented nephrophatic cystinosis. **Results:** A total of 36 patients (14 girls and 23 boys) from 25 families were diagnosed with nephrophatic cystinosis. In all, 34 patients were diagnosed with infantile cystinosis, and in 31 patients symptoms began before 2 years of age (15 before 6 months). All patients had Fanconi syndrome and failure to thrive, 33 had corneal crystals, and 30 rickets. A total of 24 patients were diagnosed after 7 years (1 month to 16 years) since the first clinical manifestation occurred. Four patients were diagnosed after kidney transplant. **Conclusion:** Cystinosis is a difficult disease to suspect, symptom onset is on average at 6 months, and diagnosis is delayed until 16 years in some

cases. Time elapse from symptom onset to diagnosis is very long. Early diagnosis and opportune treatment can change the course of the disease. Further studies should be conducted to detect patients in early stages of the disease.

032 - Demographic and Socioeconomic Conditions of Patients With Hunter Disease in Argentina

L. D'Annunzio[1], M. Bonanno[1], O. Riemersma[1], and H. Amartino[2]

[1]Shire de Argentina, Buenos Aires, Argentina
[2]Hospital Universitario Austral, Pilar, Argentina

Hunter syndrome (MPS II) is an X-linked lysosomal storage disease (LSD) due to deficiency of iduronate-2-sulfatase. Its estimated incidence is 1 of 155 000 males. **Aim:** To describe socioeconomic characteristics of Argentinian patients with Hunter disease, in order to determine its influence on disease evolution. **Method:** Observational study on 53 patients with Hunter disease, in Argentina. Information about diagnosis, neurologic involvement, source of family income, education level, health coverage, and access to enzyme replacement therapy (ERT) was obtained by interview. Socioeconomic status was determined by the Graffar scale. **Results:** Age distribution: 68% < 5 years old; 26%, 6 to 15 years; and 6% > 16 years. Average age at diagnosis was 5 years, and the median was 3.5 years (range, 0-37 years). Residence was 40% in rural areas and 60% in urban and suburban areas. Only 4% belong to upper class, 36% middle class (high and low), 41% are in poverty range, and 19% in extreme poverty. Age at diagnosis: 79% under 5 years; 15%, 6 to 15 years; 5% between 16 and 25 years; and 2% > 26 years. Schooling: regular 26%, special 34%, and no schooling 36%. Health Coverage: 32% belong to Federal Health Program, 55% to Union-based programs, and 13% to private health systems. In all, 100% of patients in upper and middle classes are on ERT against a 57% and 71% of patients in poverty and extreme poverty, respectively. **Conclusion:** The urbanization conditions and higher socioeconomic level show positive correlation with ERT treatment access, whereas no significant correlation between these variables and earlier diagnosis was found. Further study of socioeconomic markers in LSD health care should be done in Latin America.

033 - Determination of Palmitoyl Protein Thioesterase Activities in Venezuela for Diagnosis of Infantile Neuronal Ceroid Lipofuscinoses

N. Zerpa[1], L. Miranda[2], J. Chacin[2], C. Chavez[3], C. L. Dominguez[1], A. Mahfoud[1], and C. Zara[4]

[1]Fundación Instituto de Estudios Avanzados, Unidad de Pesquisa Neonatal, Caracas, Venezuela
[2]Universidad de Zulia, Facultad de Medicina, Instituto de Investigaciones Genéticas Médica, Maracaibo, Venezuela
[3]Instituto de Investigaciones Biológicas, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela
[4]Fundación Hospital de Especialidades Pediátricas, Maracaibo

Background: The neuronal ceroid-lipofuscinoses (NCLs) is the most common group of inherited, neurodegenerative, lysosomal progressive diseases of the infancy, with an incident of 1:12500 born alive, characterized for accumulation of autofluorescent lipopigment in the neurons and tissues such as, muscle, skin, conjunctive, and the rectal mucous membrane. The clinical manifestation begins with progressive intellectual and motor deterioration and early death. Ten genetically different forms of the disease are currently known, among them the infantile classic form (CLN1) has been described, which is caused by a deficiency of the lysosomal enzyme palmitoyl protein thioesterase 1 (PPT-1). **Objectives:** To determine values of reference of the activity of the PPT1 in dried blood spots in Venezuelan infants. **Methodology:** We carried out the determination of the enzymatic activities of PPT1, based on the use of a fluorescent substrate and dried blood spots (3 mm), in healthy infants (age 6 m to 2 years) in order to establish normal values for the Venezuelan population. **Results:** We found that PPT-1 activity values in healthy individuals ($n = 20$) were between 0.30 and 0.7355 nmol/spot with a mean value of 0.406 nmol/spot. Enzymatic activities on DBS from 3 of the 5 patients included in this study showed values below the normal range. **Conclusion:** This method allows a rapid, easier, and accurate determination of the enzymatic activities of PPT-1 on dried samples for CLN1 diagnosis.

034 - Diagnosis and Molecular Study of Mucolipidosis Type II in Colombia: Two Cases

H. Velasco[1], T. Vinasco[1], A. Uribe[3], J. Acosta-Guio[4], and D. Ramirez[2]

[1]Maestría en Genética Humana, Departamento de Morfología, Universidad Nacional, Bogotá, Colombia
[2]Internado especial, Maestría en Genética Humana, Departamento de Morfología, Universidad Nacional-Universidad del Valle, Bogotá, Colombia
[3]Centro de Investigaciones en Bioquímica, Departamento de Ciencias Biológicas Universidad de los Andes. Bogotá, Colombia
[4]Genetista, Instituto de Ortopedia Infantil Roosevelt, Bogotá, Colombia

Introduction: Type II mucolipidosis is a lysosomal disorder with AR inheritance, caused by *GNPTAB* gene mutations, which codify for α–β subunits of N-acetilglucosamine-1-phosphootransferase. It is characterized by an early clinical onset (6-12 months) pseudohurler phenotype, severe multiplex dysostosis, and death during early childhood. **Cases:** We report 2 unrelated patients. The first is a male neonate from nonconsanguineous parents of the same geographical origin. At physical examination, microcephaly, gingival hyperplasia, coarse facies, bowed tubular bones, and developmental delay

were observed. He showed neonatal autoregulated thrombocytopenia, cholestasis, and hyperparathyroidism. Oligosaccharide excretion analyzed by thin-layer chromatography showed an abnormal pattern. Serum arylsulfatase A was elevated (1899 U/mL with VR 25-100 U/mL). The second case is a 27-month-old woman from nonconsanguineous parents from the same geographical origin. They consulted at 5 months of age due to neurodevelopmental delay, coarse facies, and joint limitation. Her brother died at 2 months of life with similar symptoms. Physical examination showed short stature, multiplex dysostosis, coarse facies, gingival hypertrophy, hepatomegaly, and kyphosis. Biochemical studies showed nonspecific pattern of chondroitin sulfate in electrophoresis and increased activity of multiple sulfatase in serum (> 4 times the reference value). Both patients with the same homozygous mutation in *GNPTAB* gene was found by loss of 2 pairs (3503_4delTC) causing Frameshift-type mutation that produces a premature stop codon (p.L1168QfsX5). **Conclusion and Discussion:** Functional studies previously showed that homozygous mutation 3503_04delTC predicted no activity of GlcNAc-phosphotrasferase. This is the most common mutation in patients with type II mucolipidosis around the world.

035 - Diet and Nutritional Status Evolution in a Group of Children With Mild Hyperphenylalaninemia During the First 4 Years of Life

P. Bravo[1], A. De La Parra[1], C. Arias[1], P. Peredo[1], J. F. Cabello[1], G. Castro[1], V. Hamilton[1], E. Raimann[1], and V. Cornejo[1]

[1]Laboratorio de Genética y Enfermedades Metabólicas (LabGEM), Instituto de Nutrición y Tecnología de los Alimentos (INTA), Universidad de Chile, Santiago, Chile

Introduction: Mild hyperphenylaninemias (HPA) are defined by phenylalanine (Phe) levels higher than 2 and less than 10 mg/dL. Usually, they are managed with a vegetarian diet. Most of them begin diet release according to their Phe tolerance, eventually acquiring a general population diet. This fact is important, given the increasing overweight and obesity prevalence in preschool Chilean children, being 22.5% and 9.9%, respectively, in 2010. **Objective:** To establish the nutritional status evolution of patients with mild HPA belonging to phenylketonuria (PKU) follow-up program at Instituto de Nutrición y Tecnología de los Alimentos, from Chile University, from their diagnosis until 4 years old and to correlate this with their diet. **Method:** A convenience sample was used. Clinical charts from patients with follow-up at 12, 18, 24, and 48 months of age were included. Statistics were made using Excel. **Results:** A total of 40 patients were reviewed, 50% were girls. At 18 months of age, 20% presented overweight and 7.5% obesity, while at 4 years of age such conditions were present in 32.5% and 10% of the cases. At 12 months of age 88% had a vegetarian diet, decreasing to 75% and 53% at 18 months and 4 years of age, respectively. **Conclusion:** Overweight prevalence in our group of patients with mild HPA is higher than that in the Chilean preschool population. This fact can be related to progressive diet liberation at this stage. However, a multivariate analysis with a bigger sample should be performed.

036 - DNA and RNA Studies of the GNPTG Gene in Brazilian Patients With Mucolipidosis II/III: Report of 4 Novel Mutations and Description of Intriguing Cases

R. V. Velho[1,2], T. Alegra[2], F. Sperb[1,3], O. A. Artigalas,[1,2] G. K. Cury[1], U. Matte[1,2], S. Pohl[4], T. Braulke[4], and I. V. D. Schwartz[2,3,5]

[1]Gene Therapy Center, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil
[2]Post-Graduation Program on Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[3]Post-Graduation Program in Health Sciences, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[4]University Medical Center Hamburg-Eppendorf, Department of Biochemistry, Children's Hospital Research, Hamburg, Germany
[5]Department of Genetics and Molecular Biology, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil

Mucolipidosis (ML) α/β and γ are autosomal recessive lysosomal diseases that are due to the deficiency of GlcNAc-phosphotransferase encoded by GNPTAB and GNPTG genes. Mutations in GNPTAB cause ML II/III α/β, and mutations in GNPTG cause ML III γ. **Objective:** To describe 3 Brazilian patients with ML III γ (A, B, C; B and C are siblings) and to report the GNPTG mutations found. **Methodology:** The GNPTG gene was sequenced from genomic DNA (gDNA) in 3 patients with ML III γ and their parents, and it was also analyzed in 12 patients with ML α/β. RNA studies were performed through reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative RT-PCR (qRT-PCR). **Results:** The following novel mutations were found: c.328G>T, c.244_247 dupGAGT, c.-112C>G, and c.233+7G>T as well as the following genotypes: patient A: c.[244_247dupGAGT]+[-112C>G, 328G>T] and patients B and C: c.[-112C>G,328G>T]+[-112C >G,328G>T]. In patient A, c.244_247dupGAGT was found to be a de novo mutation. The qRT-PCR indicated that mutations in the GNPTG gene are associated with a significant decrease in the levels of GNPTG and increase in GNPTAB messenger RNA (mRNA). In patient B, RT-PCR analysis failed to detect mutation c.328G>T (p.E110X). **Discussion/Conclusion:** This is the first report of a patient with ML III γ resulting from a de novo mutation. The decreased level of mRNA found could be the result of the nonsense-mediated decay of mRNA or the presence of homozygous mutation in the 5′untranslated region. Moreover, the difference between gDNA and complementary DNA analysis in this patient may be explained by RNA editing. GNPTG analysis should be performed on gDNA due to the instability of the mRNA-containing premature stop codons.

037 - Duarte Galactosemia: Clinical Case Report

L. Sua[1], M. Ramirez[1], P. Reyes[1], E. Crespo[1], M. De Lucca[1], C. Dominguez[1], K. Araujo[1], Y. Guevara[1], A. Mahfoud[1], L. Casique[1], and I. Arias[1]

[1]Fundación Instituto de Estudios Avanzados IDEA, Caracas, Venezuela

Introduction: Galactosemia is an inherited disease caused by the total or partial deficiency of one of the enzymes involved in the metabolism of galactose to produce glucose, which are galactokinase, galactose-1-phosphate uridil transferase (GalPUT), and uridine diphosphate galactose 4 epimerase. Lack of any of these 3 enzymes disturbs the metabolism of galactose accumulating galactose and/or its metabolites. Classic galactosemia is caused by a deficiency of the enzyme GalPUT, producing accumulation of Gal-1-P, galactose, and galactitol, responsible for hepatic, renal, and cerebral impairment. There are 2 types of enzyme activity levels, classic level, where enzyme activity is completely absent and the Duarte variant with a partial deficiency of it. In Duarte variant, the newborns are asymptomatic and detected only by newborn screening test. **Objective:** To report a patient with Duarte galactosemia. **Description:** A 38-day-old patient, who presented with vomiting and physiologic neonatal jaundice, was referred to this institute to be evaluated for galactosemia. Confirmatory tests were requested: total galactose (15 mg/dL) and GalPUT activity determination, which was absent. After these results, galactose had been restricted from his diet, and there was clinical improvement. Molecular studies were performed and p.Q188R/p.N314D mutations were detected, both of them heterozygous. **Conclusion:** Currently the most common mutations including N314D mutation causing Duarte variant form are measured by molecular biology techniques, obtaining diagnostic efficiency up to 96%.

038 - Enzymatic Studies of Mucopolysaccharidosis Type IV in High-Risk Colombian Population: Reference Values

Alfredo Uribe[1] and Adis Ayala[2]

[1]Centro de Investigaciones en Bioquímica, Universidad de los Andes, Bogotá, Colombia
[2]Grupo Bioquímica y Biología Molecular, Universidad Distrital, Bogotá, Colombia

Introduction: Mucopolysaccharidosis (MPS) type IV is an alteration in glycosaminoglycan metabolism that is inherited in an autosomic recessive manner. It is characterized by impaired keratan sulfate degradation and has been classified in 2 subtypes, A and B, which are related to the deficiency of galactosamine-(N-acetyl)-6-sulfate sulfatasae (Gal-NAc-6-S-sulfatase) and β-galactosidase (BG), respectively. Clinical symptoms are varied, including spondyloepiphyseal dysplasia, sternum protrusion, short neck, short stature, and odontoid hypoplasia, among others. Definition and diagnostic discrimination is oriented by enzymatic analysis in leukocytes or fibroblasts. **Objective/Methodology:** We evaluated the enzymatic activity of BG and Gal-NAc-6-S-sulfatase in leukocytes of control participants and high-risk population for MPS IV between 2005 and 2013. **Results/Discussion:** We analyzed 784 controls (age: 0.6-72 years) who showed activity ranges of 82.5 to 448 nmol/mg protein/h for BG and 2.3 to 21.14 nmol/mg protein/h for Gal-NAc-6-S sulfatase. Also, we evaluated 285 patients with phenotypic features suggesting MPS IV, finding 1 deficient patient for BG (26.1 nmol/mg protein/h) and 61 for NAc-Gal-6-S-sulfatase (activity range:0.0-0.14 nmol/mg protein/h). The age range of affected patients was 0.4 to 55.1 years old. This analysis showed a marked difference between controls' and patients' activities, allowing a fast and specific diagnostic definition. These results also reaffirm the high frequency of MPS IVA in Colombia.

039 - Evaluation of Anthropometric Patients With Inborn Errors of Metabolism

V. C. Kanufre[1,2], A. F. Cruz[1], C. D. N. Rocha[1], E. R. Valadares[1], L. M. Almeida[3], R. D. L. Soares[1,2], and M. R. A. Alves[2]

[1]Federal University of Minas Gerais, Hospital das Clínicas, Belo Horizonte, Brazil
[2]Federal University of Minas Gerais, Center for Research and Support Diagnostic Medical School, Belo Horizonte, Brazil
[3]Federal University of Minas Gerais, Nutrition Course, Brazil

Introduction: Patients with inborn errors of metabolism (IEN) are treated with diet restrictions and are susceptible to changes in nutritional status (NS). **Objective:** To evaluate the NS in patients with IEN. **Methods:** The indices of 26 patients were compared with the World Health Organization standards regarding weight/age, height/weight, body mass index (BMI)/age for children below 5 years old and height/age, BMI/age between 5 and 9 years old. **Results and Discussion:** Patients with glycogen storage disease, galactosemia, fructosemia, methylmalonic acidemia and methylglutaric, maple syrup urine disease, homocystinuria, HMG-CoA lyase, very long-chain acyl-CoA dehydrogenase, and Medium-chain acyl-CoA dehydrogenase deficiencies, Chanarin-Dorfman Syndrome, and citrullinemia were included. According to the height/age, 31.25% had stunting, probably because of late diagnosis or the disease itself. In relation to weight/height, the deficit was smaller (12.5%), indicating nutritional recovery. For children more than 5 years old, though, the deficit was the same with 30% in the indices height/age and BMI/age. The weight deficit is probably related to metabolic decompensation and catabolism or insecurity of the caregiver and believing that increasing the quantity of food causes the symptoms. The risk of overweight in children more than 5 years old was 12.5% (weight/

height) and 18.75% (BMI/age) **Conclusion:** Although no patient presented with overweight, the data show the necessity of diet control in order to avoid excessive ingestion. The majority of patients are eutrophic, indicating successful nutritional treatment.

040 - Evaluation of Bone Manifestations of Gaucher Disease

Bertholdo Debora[1], Vairo Filipp[1,2,3], Vanz Ana Paula[1], Salvi Leticia[4], Netto Cristina[1], Wilke Matheus[1], Camargo Matheus[1], Vedolin Leonardo[2], and Schwartz Ida[1,4,5]

[1]*Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, RS, Brazil*
[2]*Laboratório BRAIN, Centro de Pesquisa Experimental (CPE), Hospital de Clínicas de Porto Alegre, Brazil*
[3]*Programa de Pós-Graduação em Genética e Biologia Molecular, UFRGS, Brazil*
[4]*Serviço de Radiologia, Hospital de Clínicas de Porto Alegre, RS, Brazil*
[5]*Departamento de Genética, Universidade Federal do Rio Grande do Sul (UFRGS), Brazil*

Introduction: Bone manifestations of Gaucher disease (GD) result from bone marrow infiltration by Gaucher cells and consequent mechanical obstruction and impairment of vascular supply. Magnetic resonance imaging (MRI) is a semiquantitative method to evaluate bone marrow infiltration. Several scores were created to quantify bone damage, among which is Bone Marrow Burden (BMB). **Objective:** To describe bone disease identified by MRI and apply the BMB score in patients with Gaucher disease seen at the Reference Center of Rio Grande do Sul, Brazil. **Methods:** Nineteen patients were evaluated. Coronal images of the femur were obtained, and sagittal T1- and T2-weighted images of the spine were analyzed for the presence of complications and the degree of bone marrow infiltration. The BMB 0-2 values were used to indicate the absence of bone disease; 3 to 7, mild; 8 to 12, moderate, and 13 to 16, severe disease. **Results:** The BMB scores obtained ranged from 0 to 13 (median = 8). Two patients had a score between 0 and 2; 7 patients had mild infiltration; 8 patients had moderate infiltration, and 2 patients had severe infiltration. The median pain domain score (evaluated by SF36 questionnaire) was 73.75 (from 0 = worst to 100 = best) showing no correlation with BMB score. **Discussion/Conclusion:** Bone changes and bone infiltration are better evaluated with MRI than x-rays. Only longitudinal assessment of patients and larger sample sizes will allow the establishment of associations between clinical picture, enzyme replacement therapy dosage, and severity of bone marrow infiltration.

041 - Evaluation of the Intelligence Quotient Children <15 Years With Congenital Hypothyroidism IMSS Users of The Mexican Institute of Social Insurance Mexico

L. Valdes Ramirez[1] and L. Santillan Arreygue[1]

[1]*Institute Mexico Seguro Social*

Introduction: A neurodevelopmental disorder, or disorder of neural development, is an alteration of brain or central nervous system growth and development. **Objective:** To know the IQ of children with a diagnosis of congenital hypothyroidism (HC) <15 years. **Methods:** Children <15 years diagnosed with HC detected through the program of early detection of congenital metabolic disease were classified into those who were under treatment and control. All were cited for their evaluation through WPPSI-III and WISC-IV, through counseling service written authorization requested to parents. **Results and Discussion:** We analyzed 27 children with HC diagnosis, which represents 27.8% of the cases, 53.5% were girls and 46.5% boys with an average age of 7 years. IQ in WPPSI-III children from 3 to 6 years was average in 97.36%, above normal in 21.4%, superior in 7.1%, and average in 71.4%, and in WISC-IV children >6 years was on average 93.6%, below normal standard in 35.5%, and normal in 62.5%. **Conclusion:** The Programs of Timely Detection of Congenital Metabolic Illness help in diagnostic and pertinent treatment in boys with HC. They have shown to be effective in the prevention of mental delay.

042 - Evidence That Hyperphenylalaninemia Alters Neurotrophic Factor Pathway in the Brain of Rats

P. F. Schuck[1], T. P. Macan[1], M. P. Reis[1], T. Heitich[1], G. Scaini[1], S. O. Marques[1], C. T. Souza[1], G. C. Ferreira[1], and E. L. Streck[1]

[1]*Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil*

Introduction: Phenylalanine (Phe) accumulation in tissue and body fluids of patients is the hallmark of phenylketonuria (PKU), a disease that affects the central nervous system. The aim of this study was to measure brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) levels as well as extracellular signal-regulated kinase (ERK) 1/2 and Akt signaling pathways in brain of rats submitted to an experimental model of hyperphenylalaninemia. **Materials and Methods:** Male 30-day-old Wistar rats received a single subcutaneous injection of Phe (5.2 μmol/g) and/or p-chlorophenylalanine (p-Cl-Phe; 0.9 μmol/g), an inhibitor of phenylalanine hydroxylase. Control group received saline solution in the same volume. One hour after the injection, cerebral cortex, striatum,

and hippocampus were isolated and BDNF and NGF levels were determined. **Results and Discussion:** It was observed that NGF levels were not altered in any structure tested. On the other hand, the simultaneous administration of Phe and p-Cl-Phe diminished BDNF levels in cerebral cortex and striatum. Furthermore, acute administration of Phe plus p-Cl-Phe altered ERK 1/2 signaling pathway in cerebral cortex, without altering Akt pathway. Herein, it was shown that high levels of Phe decreased BDNF levels and expression, probably due to alterations of ERK signaling activity. **Conclusion:** Considering that alterations of BDNF levels may impair memory, learning, plasticity, and neural survival. It is tempted to speculate that our results might be related to brain damage found in patients with PKU.

043 - Evolution and Adherence to Treatment in a Group of Children With Hyperpenylalaninemia

G. M. Zayas[1], J. M. Torriente[1], H. N. Chavez[1], D. Dominguez[1], U. Cabrera[2], L. Martinez[3], S. Cardenas[1], B. Suarez[3], and S. C. Gonzalez[1]

[1]Instituto de Nutrición e Higiene de los Alimentos, La Habana, Cuba
[2]Hospital Pediátrico de Centro Habana,
[3]Centro Nacional de Genética Médica,

Introduction: Phenylketonuria is an inherited metabolic disorder characterized by deficiency of phenylalanine hydroxylase enzyme, which results in elevated phenylalanine levels in blood and urine. It causes severe neurologic impairment with mental retardation and behavioral disorders. Early diagnosis allows effective treatment establishment preventing neurologic sequelae. **Objective:** To describe the evolution and adherence to treatment of a group of children with hyperphenilalaninemia. **Methods:** Longitudinal descriptive study. A total of 26 children among 1 and 9 years assisted in Pediatric Hospital of Center Havana were included. Of them, 16 had classic phenylketonuria and 10 hyperphenilalaninemia. Diagnosis was performed by neonatal screening in 25 cases. All assisted to clinical control received monthly (analysis of weight, height, and calculation of weight/height, weight/age and height/age). Nutritional management included dietary phenylalanine restriction and special formulas, using the nutritional recommendations for Cuban. It was considered controlled if phenylalanine values were between 4 and 6 mg/dL in <5 years and between 4 to 8 mg/dL among 5 to 9 years old. **Results:** Global evaluation of patients: 80.7% (n = 21) present an appropriate nutrition state, 3.8% (n = 1) thin, 15.3% (n = 4) overweight/obese. In all, 77% (n = 20) attended for phenylalanine testing monthly. In relation to the phenylalanine levels, 57.7% (n = 15) had at least 1 value above acceptable limits. Dietary formula was used by 89% (n = 23) but not all carry out a suitable diet. **Conclusion:** The pursuit of the patients is adapted. The necessity of a better execution of the diet exists on the part of the parents.

044 - Experience in Tetrahydrobiopterin Treatment for Patients With Phenylketonuria

M. Nuñez Miñana[1], L. Muschietti[1], M. Salerno[1], M. Collini[2], and N. B. Specola[1]

[1]Laboratorio de Metabolismo, Hospital de Niños SM Ludovica de La Plata, Argentina

Introduction: Tetrahydrobiopterin (BH4) increases phenylalanine (Phe) tolerance in some patients, improving their quality of life. A decrease >30% in 48-hour test is considered a positive response. This response does not assure to cover recommendations with natural proteins. **Objective:** To present an initial experience of the BH4 treatment in a group of selected BH4-sensitive patients with phenylketonuria (PKU), with different magnitude of responses. R1 indicates does not respond (decrease <30%), R2 decrease from 30% to 40%, R3 decrease >40%. **Methodology:** Of 158 patients with PKU in follow-up, 19 older than 4 years were selected with moderate and mild PKU (Phe tolerance of 500 to 900 mg) and social coverage. A BH4 48-hour test was selected. Depending on the response to the test, treatment with BH4 was prescribed to 10 patients. School performance evaluations, nutrition, and metabolic parameters were followed during a period from 2 months to 2 years. **Results:** All the patients were able to increase their Phe tolerance. Seven patients (R3) did not receive supplement and cover the recommendations. Three patients (R2) are under combined treatment. **Conclusion:** A positive response of 30% does not ensure a free diet. Only those responding >40% can stop the protein supplement without evidencing shortages. No collateral effects were produced and quality of life was improved in both the groups of responders. The combined treatment is difficult to cover in our country

045 - Fabry Disease: An Advance in the Diagnostic Laboratory for Recognition of Female Carriers

A. N. Giner-Ayala[1], C. J. Angaroni[1], A. Delgado[1], N. Gomez[1], L. Peralta[1], and R. Dodelson de Kremer[1]

[1]Centro de Estudio de las Metabolopatías Congénitas-CEMECO, Cátedra de Clínica Pediátrica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Hospital de Niños, Córdoba, Argentina

Fabry Disease (FD) is an X-linked lysosomal disorder caused by deficiency of α-galactosidase A (α-galA), codified by GLA gene. Diagnosis of patients with compatible phenotype begins with α-galA activity assay. In male patients, enzyme deficiency is confirmatory; in female patients molecular analysis is required. **Objective:** To validate in our laboratory, α-galA–β-glucuronidase ratio determination in dried blood spots (DBS) to guide biochemical diagnosis of FD, especially in heterozygotes. **Patients:** Index case was a man with classical FD. We studied 11 male and 11 female patients belonging to an Argentinean

family, and 24 healthy controls. **Methods:** Enzymatic determinations: α-galA (DBS and leukocytes) and β-glucuronidase (DBS) by fluorometric assays. Analysis of GLA gene was done by polymerase chain reaction/sequencing. **Results:** The missense mutation p.Ala292Thr was identified in the index case. In the family member studied, 9 hemizygous patients and 8 heterozygous patients were diagnosed. In DBS α-galA determination results, 9 hemizygotes (0-0,11) and 4/8 heterozygotes (0,23-0,49) were detected (normal range: 0.58-3.38 nmol/h/mL). The α-galA determination in leukocytes was deficient in the 9 hemizygotes (0-2.9) and 7 of 8 heterozygotes (4.1-17.8; normal range: 21.2-41.1 nmol/h/mg). For α-GalA–β-glucuronidase ratio in DBS normal controls, cutoff value was established over mean less 1 standard deviation (0.029). Ratio lead to identification of 4 of 4 analyzed hemizygotes (0-0.002) and 8 of 8 heterozygotes (0.007-0.023). Although in carriers sensitivity was 100%, specificity in normal controls declined to 85.7% (4 false positives). The α-GalA–β-Glucuronidase ratio proved to be a useful and accessible tool to guide the biochemical FD diagnosis of female carriers, prior to molecular analysis.

046 - Fabry Disease: Molecular Analysis of Affected Colombian Families, Preliminary Report

Paez Paola[1], Garcia Reggie[1,2], Lopez Andrea[3], and Ayala Paola[3]

[1]*Instituto de Nutrición, Genética y Metabolismo, Facultad de Medicina, Universidad el Bosque*
[2]*Laboratorio Genzyme de Colombia*
[3]*Instituto de Genética Humana, Pontificia Universidad Javeriana, Bogotá, Colombia*

Introduction: Genotypic characterization of families affected by Fabry Disease (FD) in Colombia has not been completed; therefore, it is necessary to update family trees and study the patients at high risk of being affected or carriers. **Methods:** Genomic DNA was isolated from affected/possible men or obligate/possible carrier women and the entire α-galactosidase A (α-galA) coding region and flanking sequences were amplified by polymerase chain reaction and analyzed by automated sequencing. **Results:** In all, 3 families and 12 individuals were analyzed. Three private mutations were found in 3 different families, 1: p.342X c1024c>T rs104894843 (previously reported); 2: Del298_300AGG (not reported); and 3: c1072G>A Glu358Lys (previously reported). Of the 12 patients studied, 6 had a mutation, 3 were female carriers, and 3 were affected males. Carrier status was ruled out in 4 women. **Conclusion:** Mutations found in these families are private which is a common finding in FD. One of these mutations (Del298_300AGG) has not been previously reported. One of the most important contributions of this work was that we could rule out carrier status in 4 high-risk women. Since α-galA activity measurement methods fail to detect one-third of female patients with FD, it is necessary to develop a molecular panel to detect these high-risk individuals, and this work is an advance in this topic. Finally, we will have to complete molecular and phenotype characterization in order to establish genotype–phenotype correlations.

047 - Fabry Registry Annual Report 2012: Comparison of Argentina With the Rest of the World

G. Cabrera[1], J. Politei[2], H. Amartino[3], R. Valdez[4], F. Masllorens[5], D. Ripeau[6], N. Antongiovanni[7], P. Luna[8], M. Cedrolla[9], S. Fernandez[10], A. B. Schenone[2], M. Szlago[2], and A. Fainboim[11]

[1]*Servicio de Cardiología, Del Viso Medical Center, Buenos Aires, Argentina*
[2]*FESEN-Laboratorio "Dr. N.A. Chamoles", Buenos Aires, Argentina*
[3]*Servicio de Neuropediatría, Hospital Austral, Buenos Aires, Argentina*
[4]*Servicio de Genética, Hospital Central Militar, Buenos Aires, Argentina*
[5]*Servicio de Genética, Hospital Posadas, Buenos Aires, Argentina*
[6]*Servicio de Nefrología, Hospital Posadas, Buenos Aires, Argentina*
[7]*Servicio de Nefrología, Clínica Pergamino, Buenos Aires, Argentina*
[8]*Servicio de Dermatología, Hospital Alemán, Buenos Aires, Argentina*
[9]*Servicio de Neurología, Hospital de Alta Complejidad, Formosa, Argentina*
[10]*Servicio de Nefrología, Ciperca srl, Catamarca, Argentina*
[11]*Servicio de Pediatría, Hospital Ricardo Gutiérrez, Buenos Aires, Argentina*

Introduction: Fabry Registry (FR) is a global, observational, and voluntary program that collects data from patients with Fabry disease, regardless of whether they are receiving treatment, sponsored by Genzyme, a Sanofi company. **Objective:** This report summarizes enrollment, demographic, and certain clinical data of patients included in the FR in Argentina until October 5, 2012. Eleven physicians had recruited a total of 87 patients (40 men and 47 women) in the FR within Argentina, which represents a 7.4% increase in patient recruitment compared with final 2011 through October 5, 2012, period. **Results:** The median current age of Argentinean men was 34 years versus 42 years for men in the ROW, and the median current age of Argentinean women was 37 years versus 45 years for women in the ROW. Men in Argentina were diagnosed at a median age of 22 years, compared with 27 years for men in the ROW. The median age at which women were diagnosed was 34 years in Argentina, compared with 33 years in the ROW. A higher percentage of men in Argentina have received enzyme replacement therapy compared with women (95% vs 45%). A higher percentage of Argentinean men and women in the FR had been treated with agalsidase β compared with the ROW. **Conclusion:** Most Argentinean patients have been enrolled in the FR for 4 years or more. Argentinean patients are substantially younger than patients enrolled in the ROW. Men

in Argentina were diagnosed earlier than in the ROW. Higher percentage of Argentinean man and women in the FR were treated with agalsidase β compared with the ROW.

048 – Follow-Up of 12 Tyrosinemia Type 1 Cases in Chile (1998-2013)

C. Arias[1], E. Raimann[1], J. F. Cabello[1], G. Castro[1], V. Hamilton[1], A. Valiente[1], K. Betta[1], P. Bravo[1], P. Peredo[1], and V. Cornejo[1]

[1]Laboratorio de Genética y Enfermedades Metabólicas, INTA, Universidad de Chile, El Líbano 5524, Santiago, Chile

Introduction: Tyrosinemia type 1 (Tyr 1) is autosomal recessive and is due to the deficiency of fumarylacetoacetate hydrolase (FAH). Symptoms include acute liver failure, cirrhosis, hepatocellular carcinoma, Fanconi Syndrom, and crisis of peripheral neuropathy. Diagnosis is confirmed finding hypertyrosinemia and high succinylacetone (SA). The prognosis with dietary treatment based on restriction of phenylalanine (Phe) and tyrosine (Tyr) is poor. Liver transplantation cures the disease. At present, the drug (2(2-nitro-4- Trifluoromethylbenzoyl)-1,3-cyclohexanedione), NTBC, improves the hepatic and renal functions. **Objective:** To present 12 patients with Tyr 1 followed up between 1998 and 2013 in Chile. **Method:** Twelve patients with Tyr 1 treated with Phe and Tyr restriction and NTBC are presented. Of 12 patients, 7 had hepatic failure, 4 before 2 months of age, 1 at 7 months of age, 1 at 11 months, and another at 17 months of age. Of 12 patients, 5 had rickets and Fanconi Syndrome. **Results:** The diagnosis was confirmed with average SA 523 μmol/L (normal value <0.1) and Tyr 299 μmol/L (normal value 12-108). Treatment consists in Phe- and Tyr-restricted diet (1000 mg/day), protein 1.8 g/kg/d, and NTBC 0.6-1.2 mg/kg/d. Of 12 patients, 11 were evaluated with psychometric tests, 4 have normal development. Follow-up has been on average 63 months. Ten patients have normal hepatic function. Two patients were transplanted with good outcome. **Conclusion:** Treatment with Phe and Tyr restriction and NTBC is effective if initiated early.

049 - Free Intake of Vegetables and Fruits Containing Less Than 75 Mg Phenylalanine/ 100 G in Phenylketonuria

V. Hamilton[1], G. Benedetti[1], G. Castro[1], C. Arias[1], E. Raimann[1], J. F. Cabello[1], A. Valiente[1], K. Betta[1], M. Colombo[1], and V. Cornejo[1]

[1]Genetics and Metabolic Diseases Laboratory, Institute of Nutrition and Food Technology, (INTA), University of Chile,

Introduction: Phenylketonuria (PKU) treatment consists of a restricted phenylalanine (Phe) intake, mainly based on special formula, vegetables, fruits, and few amounts of cereals. The phe levels must be maintained below 8 mg/dL (480 μmol/L). **Objective:** To evaluate whether free intake of some vegetables and fruits modifies Phe level. **Methods:** Eight patients with PKU 3 to 15 years old were included in a longitudinal study. The Phe intake was maintained and children could consume as free food all fruits and vegetables with less than 75 mg Phe/100 g. The Phe intake records were done daily and plasma Phe concentrations were measured once a week (fluorometric method). **Results:** Four boys (6.5 $\pm$ 2.4 years old) and 4 girls (7.6 $\pm$ 4.6 years old). Before the study, the Phe intake was 453.6 $\pm$ 104.6 mg/d and post intervention increased to 555.7 $\pm$ 134.8 mg/d ($P < .05$). They ate 23% additional Phe intake from cereals, vegetables, and fruits with more than 75 mg Phe/100 g ($P < .05$). They had 102 mg extra Phe intake from vegetables and fruits with less than 75 mg Phe/100 g. The Phe levels before the study were 4.1 $\pm$ 0.8 mg/dL (246 $\pm$ 48 μmol/L) and increased to 5.3 $\pm$ 0.9 mg/dL (318 $\pm$ 54 μmol/L) during the study ($P < .05$). **Conclusion:** This study suggests that liberation of vegetables and fruits containing less than 75 mg/100 g Phe does not increase Phe level above the accepted range in patients older than 10 years.

050 - Gaucher Disease: Report of 15 Patients From Central America and the Caribbean

C. Perez de Ferran[1], B. Honchong[2], C. Paulino[3], R. Nieves Paulino[1], M. Saborio Rocafort[4], J. Quesada Alvarado[4], G. Cossio de Gurrola[5], R. Aparicio[6], A. Samudio de Correa[6], L. Sotillo[6], J. Ortiz[7], J. Cabrera[8], and G. Drelichman[9]

[1]Hospital Infantil Dr. Robert Reid Cabral, Dominican Republic
[2]Ciudad Sanitaria Dr. Luis E. Aybar, Dominican Republic
[3]Hospital Infantil Dr. Arturo Grullón, Dominican Republic
[4]Hospital Nacional de Niños Dr. Carlos Sáenz Herrera, Costa Rica
[5]Hospital del Niño de Panama
[6]Complejo Hospitalario Metropolitano Dr. A.A.M.CSS, Panama
[7]Hospital General de Enfermedades. Instituto Guatemalteco de Seguridad Social, Guatemala
[8]Hospital General San Juan de Dios and Hospital Roosevelt, Guatemala
[9]Hospital de Niños Ricardo Gutiérrez, Argentina

Summary: Experts on Gaucher Disease from Central America and the Caribbean evaluated 15 regional diagnosed cases, as part of a consensus for treatment of this disease, reporting the main findings and conclusions. **Introduction and objective:** Considering that frequency and characteristics of patients with Gaucher disease (GD) in the region are largely unknown, the consensus group for the diagnosis and treatment of this disease chose to evaluate epidemiologic, clinical, laboratory, and response to enzyme replacement therapy (ERT) with imiglucerase. **Methodology:** A total of 15 cases were evaluated from patients diagnosed in Costa Rica, Guatemala, Panama, and Dominican Republic. **Results:** From March 1986 to March 2013 (27 years), 15 patients with GD were diagnosed, 90% <18 years old, 66% female, and 34% male. Type I: 60%; type II and III: 40%. Mean age at diagnosis: 13.3 years. Molecular studies:

73%. Clinical and laboratory diagnosis: 100% splenomegaly, 91,6% hepatomegaly, 90% thrombocytopenia, 70% anemia, 37% leukopenia, 27% bone involvement. Enzyme replacement therapy with Imiglucerase in 5 patients and follow-up with ERT after 3.7 years. All patients achieved therapeutic targets (TTs). **Conclusion:** The achievement of TT at 3.7 years is high, demonstrating the efficiency of the ERT with Imiglucerase. The main difficulties are subdiagnoses as well as late diagnoses and a low percentage of patients receiving ERT.

051 - Gene Therapy for Morquio: Advances in the Development of Viral Vectors

C. J. Almeciga-Diaz[1], R. Cuaspa[1], J. Herrera[1], H. Barbosa[1], and L. A. Barrera[1]

[1]Instituto de Errores Innatos del Metabolismo, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá DC, Colombia

Introduction: Morquio A disease is produced by the deficiency of the lysosomal enzyme N-acetylgalactosamine-6-sulfate sulfatase (GALNS). Previously, we reported the use of adeno-associated virus (AAV) vectors as tools to mediate GALNS gene delivery. In addition, the cotransduction with SUMF1, using separated vectors, significantly increased GALNS activity. **Objective:** In this study, we evaluated a new set of AAV vectors carrying both GALNS and SUMF1 complementary DNA (cDNA) into the same vector. **Methodology:** The AAV vectors expressing GALNS and SUMF1 were built using a viral (encephalomyocarditis virus) or human (nuclear respiratory factor) internal ribosome entry site (IRES) element. Furthermore, for the first time, lentiviral vectors carrying GALNS or SUMF1 cDNA were also evaluated. All vectors were assessed using HEK293 cells and Morquio A human skin fibroblasts. **Results:** The AAV vectors with SUMF1-IRES-GALNS configuration induced higher enzyme activity compared with cells cotransduced with AAV-GALNS and AAV-SUMF1 vectors. On the other hand, the use of lentiviral vectors increased up to 100 times the enzyme activity in comparison with the levels observed with AAV vectors. Possible therapeutic effect was evaluated in Morquio A fibroblast by measuring total Î²-hexosaminidase levels that were reduced after transduction with AAV-IRES and lentiviral vectors. **Conclusion:** These results show the potential use of this new set of vectors, especially lentiviral vectors, for the development of a gene therapy for Morquio A.

052 - Genetic Defects in the Liver Phosphorylase System in Argentine Patients: Nosological Definition Through a Strategy of Enzymatic and Molecular Analysis

C. J. Angaroni[1], A. N. Giner-Ayala[1], A. L. Castillo[1], V. Lanza[1], A. E. Paschini-Capra[1], and R. Dodelson de Kremer[1]

[1]Centro de Estudios de las Metabolopatías Congénitas (CEMECO), Hospital de Niños de la Santísima Trinidad, Facultad de Ciencias Médicas, UNC, Córdoba, Argentina

Phosphorylase and phosphorylase-b kinase (PHK) deficiencies in liver constitute the phosphorylase system defects (PSD), leading to glycogenosis Type VI (GSD-VI) and Type IX (GSD-IX), respectively. Hepatic phosphorylase is encoded by PYGL gene. The GSD-IX is caused by a genetic defect in one of the hepatic PHK subunits encoded by PHKA2, PHKG2, and PHKB genes, respectively. X-linked PHK deficiency (PHKA2 gene) presents 2 enzymatic variants, XLG1 (reduced in liver and erythrocytes) and XLG2 (only decreased in liver). The aim of this work is to nosologically define PSD taking into account patients' gender, PHK activity in erythrocytes, and molecular analysis of PYGL gene. In all, 2 women and 16 men (14 unrelated families) were studied. The PHK activity in erythrocytes was deficient in 14 male patients and was normal in 2 male probands (still without diagnostic definition) and in 2 women. In the latter, molecular analysis of PYGL gene identified 2 novel missense mutations: p.Gly233Ser and p.Gly686Arg and IVS15-2delA polymorphism. Allele frequency of P.Gly686Arg was 75%. In silico studies predict that both new mutations would affect enzyme functionality. This study allowed accurate diagnosis of GSD-VI (2/2) and GSD-IX (14/16). Molecular analysis of PHKA2 gene, responsible for X-linked EAG-IX, is currently in progress in our center. This research represents a continuation of the project for exact definition of PSD, a largely unknown area in our country.

053 - Geographic Distribution of Mucopolysaccharidosis Type VI in the Center of the Department of Cauca: Is It Possibly a Founder Effect?

H. Pachajoa[1], Y. Ariza[1], V. Villota[1], M. E. Miño[2], and M. A. Acosta-Aragon[3]

[1]Centro de Investigaciones Congénitas y Enfermedades Raras, Universidad Icesi, Cali, Colombia
[2]Hospital Universitario San José, Popayán, Facultad de Ciencias de la Salud, Universidad del Cauca, Cauca, Colombia
[3]Departamento de Pediatría, Facultad de Ciencias de la Salud, Universidad del Cauca, Popayán, Cauca, Colombia

Introduction: Mucopolysaccharidosis VI (MPS VI) is an autosomal recessive genetic disease caused by deficiency of the enzyme arylsulfatase. **Methodology:** Cross-sectional study, based on records review from patients attending Hospital Universitario San José de Popayan (Cauca, Colombia). Informed consent was obtained prior to medical history reviews, and interviews were performed in order to document the current geographical location and ancestors in 3 generations. Period prevalences were estimated for each municipality, and spatial aggregation of cases was analyzed according ancestor origin. Estimated prevalence was compared with hypothetical proposed by the literature, using the statistical program Stata12.

Objective: To describe the geographical distribution of patients with MPS VI diagnosed in Cauca. **Results:** Fourteen cases were identified in an 18-year period, using total population data reported by DANE. The estimated period prevalence was 1 case per 8850 inhabitants. Such analyses were performed for 5 municipalities; prevalence ranged from 1 in 3521 to 1 in 30 300 individuals. All prevalences were higher than expected ($P < .001$). **Conclusion:** In the central region of Cauca, MPS VI prevalence was up to 36 times higher than previously reported in other regions. There is an aggregation of 5 municipalities according to ancestry, suggesting not 1 but 2 founder effects, which must be confirmed by molecular studies. Such prevalence should be considered a public health problem that requires an interdisciplinary and intersectoral management.

054 - Glycogen Storage Diseases: Diagnosis and Follow-up of 6 Colombian Cases

Y. A. Ardila[1], Pulido Ninna Fernanda[1], Echeverri Olga Yaneth[2], Guevara Johana Maria[2], Cediel Monica[3], Espinosa Eugenia[3], Montoya Jorge[4], and L. A. Barrera[1,2]

[1]*Hospital Universitario San Ignacio. Bogotá, Colombia*
[2]*Instituto de Errores Innatos del Metabolismo, Pontificia Universidad Javeriana, Bogotá, Colombia*
[3]*Instituto de Ortopedia Infantil Roosevelt. Bogotá, Colombia*
[4]*Hospital Universitario San Vicente de Paul, Medellín, Colombia*

Introduction: Glycogen storage diseases (GSDs) are genetic disorders characterized by deficiency of enzymes involved in glycogen synthesis or degradation. Clinically, the main organs affected are liver and muscle. Diagnosis of GSD is based on clinical manifestations according to hepatic (developmental delay, doll-like face, hepatomegaly, etc) and/or muscle involvement (exercise intolerance, muscle weakness, muscular atrophy, etc). Confirmation of the diagnosis relies on basic (glycemia, ketone bodies, lipid profile, uric acid, transaminases, CPK, lactic acid) and specialized laboratory tests (microscopic study of hepatic and/or muscular biopsy, enzyme activity assays in tissue). **Objectives:** To show essential aspects of diagnosis and follow-up of hepatic GSD. **Materials and Methods:** Retrospective analysis. **Results:** Here, it is shown 6 patients with GSD diagnosis and follow-up who are assenting to Instituto de Ortopedia Infantil Roosevelt-Bogotá and Hospital Universitario San Vicente de Paul-Medellín. Patients' ages range from 1 to 5 years. Structural and enzymatic analysis allowed the confirmation of 4 GSD type I cases and suggest 1 type III and 1 type IX. In all patients, treatment has improved clinical manifestations. **Conclusion:** In Colombia, there is no availability of all confirmatory tests for GSD, although the biochemical studies at hand can guide diagnosis accurately based on glycogen structure in muscle or hepatic biopsy as well as glucose-6-phosphatase enzymatic activity. Establishing the diagnosis is important for treatment purposes, since the specifications vary among GSDs.

055 - Haplotype Analysis in Brazilian Patients With Mucolipidosis II and III α/β

F. Sperb[1,2], G. K. Cury[1], R. V. Velho[1,3], L. M. Farencena[1,4], A. C. Burlamaque-Neto[1,4], U. Matte[1,3], and I. V. D. Schwartz[1,2,3]

[1]*Gene Therapy Center of Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil*
[2]*Post Graduate Program in Health Sciences of Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[3]*Post Graduate Program in Genetics and Molecular Biology of Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil*
[4]*Centro Universitário Metodista of IPA, Porto Alegre, Brazil*

Introduction: Mucolipidoses II and III α/β (ML II and ML III α/β) are autosomal recessive lysosomal disorders in which the essential mannose 6-phosphate recognition marker is not added to lysosomal hydrolases and other glycoproteins. These disorders are caused by mutations in the GNPTAB gene, which encodes 2 of the 3 subunits of the N-acetylglucosamine-1-phosphotransferase. Most of the mutations have been found to be private or rare; however, the c.3503_3504delTC is the most frequent mutation found worldwide. **Objective:** To investigate whether the spread of the frequent c.3503_3504delTC mutation was due to a unique founder molecular event in Brazil or whether the deletion arose more than once through a recurrent mutational event. **Methodology:** The c.3503_3504delTC mutation was found in 8 Brazilian patients with ML II and III α/β unrelated patients (homozygous: 3; compound heterozygous: 5). Ten intragenic polymorphisms were analyzed (-41_-39delGGC, c.18G>A, c.27G>A, c.323+20delT, 365+96_97 delGT, c.365+145C>T, c.1285-166G>A, c.1932A>G, c.3135 +5T>C, c.3336-25T>C). Haplotypes phase was predicted using PHASE program. Alignment analyses were performed with MEGA software; haplotypes were confirmed with DnaSP software, and phylogenetic analyses were performed with Arlequin software. One hundred control samples were analyzed for the phylogenetic analysis. **Results:** Twelve haplotypes were found. Of these, 3 are unique to Brazilian patients. **Conclusion:** This series is the most extensive in terms of number of polymorphisms analyzed for ML II and III. The conclusion of the analysis will define the origin of the most common mutation found in the Brazilian population.

056 - Hyperferritinemia in Gaucher Disease

T. B. Koppe[1,2], D. Doneda[1,3], F. Vairo[1,2,4], C. B. Netto[4,5], and I. V. Schwartz[1,4,5]

[1]*Universidade Federal do Rio Grande do Sul–UFRGS, Porto Alegre, Brazil*
[2]*Programa de Pós-graduação em Genética e Biologia Molecular, UFRGS, Porto Alegre, Brazil*
[3]*Programa de Pós-graduação em Medicina: Ciências Médicas, UFRGS, Porto Alegre, Brazil*

[4]Hospital de Clínicas de Porto Alegre- HCPA,Porto Alegre, Brazil
[5]Serviço de Genética Médica, HCPA, Porto Alegre, Brazil

Gaucher disease is characterized by decreased β-glucosidase activity, leading to an intracellular accumulation of glycosphingolipids and bone/hematological/visceral disease. Since 1983, there have been reports of hyperferritinemia in patients with Gaucher disease, but the impact of this finding on iron homeostasis is unknown. **Objective:** To evaluate iron metabolism in patients with Gaucher disease seen at the Reference Center of Hospital de Clínicas de Porto Alegre, Brazil. **Methods:** A retrospective chart review study. The following parameters of iron metabolism were evaluated: serum iron, transferrin saturation, total iron-binding capacity, and serum ferritin (SF). Several statistical approaches were performed using SPSS software (IBM, Chicago, Illinois), comparing serum ferritin to different variables. Statistic level was $\alpha = .05$. **Results:** A total of 39 patients were included (mean age $= 33.35 \pm 16.49$ years and mean treatment time $= 7.02 \pm 6.29$ years). Of 19 women and 20 men, 8 women and 15 men presented with hyperferritinemia. Presence or absence of splenectomy, splenomegaly and hepatomegaly did not influence SF, but there as a borderline effect of enzyme replacement therapy (ERT; $n = 19$, $t = 1.745$, $P = .098$). Among Pearson correlations, the Zimran score and the GS3 score did not correlate to SF, as well as to the plasma activity of chitotriosidase and hemoglobinemia, but there was a tendency regarding treatment time and SF ($n = 33$, $r = -.313$, $P = .056$). There was a strong correlation between SF and age ($r = .718$, $P < .001$). Only 2 patients with hiperferritinemia had evidence of iron overload. **Conclusion:** The ERT is likely to influence SF. Serum ferritin cannot be used as a biomarker of severity of Gaucher disease. Total body iron status must be properly quantified to establish a correct relationship with hiperferritinemia in patients with Gaucher disease.

057 - Hyperinsulinism-Hypoglycemia-Hyperammonemia Syndrome: A New GLUD1 Mutation

W. R. Marquez[1], P. I. Rincon[1], S. Chain[1], A. Lema[1], and P. Duran[1]

[1]Fundación hospital de la Misericordia, Bogotá Universidad Nacional de Colombia, Fundación Cardioinfantil, Universidad del Rosario, Colombia

The hyperinsulinism hypoglyemia hyperammonemia syndrome (SHI/HA) is the second most common syndrome of congenital hyperinsulinism. The gain of function mutations of the GLUD-1 gene that encodes for the mitochondrial enzyme glutamate dehydrogenase causes a form of hyperinsulinism associated with hyperammonemia. Inheritance of glutamate dehydrogenase (GDH) hyperinsulinism (HI) is autosomal dominant. Patients usually present with recurrent symptomatic hypoglycemia secondary to HI. We present a case report of a 3-year-old girl who presented at 9 months with tonic clonic movements, neurodevelopmental delay, and hypoglycemia (10 mg/dL) not associated with fever or other triggers, requiring high-metabolic flux. Analytical determinations of amino acids, lactic and pyruvic acid, growth hormone, and cortisol were normal, except for HI and hyperammonemia. Genetic studies confirmed a unique genetic variant glutamate dehydrogenase 1 (GLUD1) in the DNA sequence, with unknown significance (heterozygous GLUD1: C 1493>T), serine was replaced by leucine at position GLUD1 AA498 of the protein. Our patient presented adequate response to diazoxide, although there was no hyperammonemia improvement. The role of chronic hyperammonemia in the brain damage is not well known. N-carbamylglutamate (Carbaglu) treatment was used with protein restriction in the diet to lower plasma ammonium with good response. **Conclusion:** The association of hypoglycemia and hyperammonemia should induce suspicion of SHI/HA, which should be confirmed by molecular studies. The early diagnosis and treatment improves the prognosis.

058 - Identification of a Novel Mutation in the Human ARSB Gene for Patients With Autosomal Recessive Muchopolysacharidosis Type VI in Southwest Colombia

M. A. Acosta-Aragon[1], M. R. Lago-Leston[2], F. Barros-Angueira[2], and A. Carracedo-Alvarez[2,3]

[1]Departamento de Pediatría, Facultad de Ciencias de la Salud, Universidad del Cauca, Popayán, Colombia
[2]Hospital Clínico de Santiago de Compostela, Galicia, España
[3]Departamento de Patología, Facultad de Medicina, Universidad de Santiago de Compostela, Galicia, España

Introduction: Mucopolysaccharidosis type VI (Maroteaux-Lamy syndrome) is a severe rare hereditary disease in humans caused by a deficiency in a single gene encoding the lysosomal hydrolase N-acetylgalactosamine 4-sulfatase or arylsulfatase B. **Problem:** A total of 32 patients with MPS VI are identified in Colombia, 16 in the Department of Cauca, corresponding to 45% of the total cases with Maroteaux-Lamy syndrome registered in the country. Of these individuals, 2 belong to Guambiano Amerindian shelter in Cauca Department. **Methods:** In both index cases and in the 22 related family belonging to 2 different families using specific protocols next processes were done: DNA extraction, amplification, purification, and sequencing reaction for the genomic DNA of arylsulfatase B in a capillary electrophoresis instrument AB1 3100. The results of genomic DNA were placed on the format and reading sequence alignment "staden package" and the intergroup differences and sequences were determined with reference to the genome sequence encoding of the arylsulfatase B. The analyses of the principal component of the genetic haplotypes were performed using the method for small nucleotide polymorphisms genotyping based on Sequenom MassARRAY platform. **Results:** We found a single-nucleotide change (p.Ser403X) in the arylsulfatase B gene of patients (homozygous) and

relatives (heterozygous). This change detected was classified as pathogenic. This mutation has not been previously described in other items associated with the disease. In addition, we report the same haplotype in these 2 patients and their heterozygous relatives. **Conclusions:** These results, together with the genealogy analysis, strongly suggest an inbreeding effect in this population.

059 - Implementation of an Electrophoretic Technique for the Diagnosis of Mucopolysaccharidosis

L. Garcia[1], L. Jaimes[1], M. Viola-Rhenals[2], C. Alvear[3], C. Moneriz[4], and M. Barboza[5]

[1]Estudiante de pregrado, Facultad de Medicina, Grupo de Bioquímica y Biología Celular del Cáncer. Universidad de Cartagena, Colombia
[2]Docente. Facultad de Ciencias Exactas y Naturales, Grupo Bioquímica y Biología Celular del Cáncer. Universidad de Cartagena, Colombia
[3]Docente, Facultad de Medicina, Grupo Bioquímica y Metabolismo (BYME), Universidad de Cartagena, Colombia
[4]Docente, Facultad de Medicina. Grupo Bioquímica y Enfermedad, Universidad de Cartagena
[5]Docente, Facultad de Medicina. Grupo Niños Heroicos, Universidad de Cartagena, Colombia

Introduction: Mucopolysaccharidosis are lysosomal storage diseases caused by deficiency of enzymes that degrade glycosaminoglycans (GAGs), producing its accumulation in different organs, causing the multisystemic characteristic of these diseases. Diagnosis is established using agarose gel electrophoresis of GAGs and enzymatic determination. **Objective:** To standardize an electrophoretic technique for determining GAGs for mucopolysaccharidosis diagnosis in Cartagena. **Methods:** Fractionated urine samples from patient with mucopolysacharidosis presumptive diagnosis were used, acid albumin and cetylpyridinium chloride (CPC) tests were realized, and those that were positive for GAGs were extracted and subjected to electrophoresis in agarose gel to 15% and polyacrylamide gel to 10%; both gels were stained with toluidine blue. **Results:** During 2011, 19 samples were collected, 16 samples were positive for acid albumin and cetylpyridium chloride tests. In the electrophoresis, better staining of the bands was obtained in positive samples with polyacrylamide gel compared with agarose gel. **Conclusion:** Although polyacrylamide gel electrophoresis is not the most common technique for GAGs detection, this work shows that it is suitable for determination these macromolecules in the population studied.

060 - Improved Biochemical Diagnosis and Carrier Detection of Lysosomal Acid Lipase Deficiency

G. Civallero[1,2], J. De Mari[1], C. Bittar[3], M. Burin[1], and R. Giugliani[1,2,3]

[1]Medical Genetics Service-HCPA and INAGEMP, Porto Alegre, Brazil
[2]INAGEMP, Porto Alegre, Brazil
[3]Department of Genetics, UFRGS, Porto Alegre, Brazil

Introduction: Lysosomal acid lipase (LAL) deficiency produces 2 well-defined inborn disorders, Wolman disease (WD) and cholesteryl ester storage disease (CESD). The WD is a severe, early-onset condition involving massive storage of triglycerides and cholesteryl ester in the liver with death usually occurring before 1 year of life. The CESD is a more attenuated, later onset disease that leads to a progressive and variable liver dysfunction. Enzyme replacement therapy for LAL deficiency is currently being developed. Diagnosis of LAL deficiency is mainly based in the enzyme assay of LAL activity in fibroblasts. Recently, a selective acid lipase inhibitor, lalistat2, was used for determination of the enzyme in dried-blood filter paper (DBFP) samples (Hamilton et al, 2012). **Objectives and Methods:** To extend and validate these studies, we used lalistat2 to evaluate LAL activity in leukocytes and DBFP samples with adapted fluorometric methods. **Results:** Our results showed a clear discrimination between patients with LAL deficiency and healthy controls in both leukocytes and DBFP samples ($P < .001$). Complementary intra- and interassay studies showed acceptable values. Enzyme activities were still measurable on DBFP samples for at least 6 weeks when stored at 4°C. In addition, we observed that carrier detection was possible on DBFP samples; nevertheless, more studies are needed to confirm this finding. **Conclusion:** We conclude that the assay of LAL using selective inhibitor in leukocytes and DBFP samples is a reliable and useful tool for the identification of LAL deficiency.

061 - Increasing the Vital Capacity With Respiratory Physiotherapy: Relieving Change Respiratory in Mucopolysaccharidosis

L. A. F. Tavares[1], J. S. Filho[1], M. G. Fagundes[1], and M. C. Alves[1]

[1]Hospital Infantil João Paulo II, Governo do Estado de Minas Gerais, Associação Mineira dos Portadores de Mucopolissacaridoses

Introduction: Home Care Program (HCP) and the Assistance Mucopolysaccharidosis (MPS Program) in Children's Hospital John Paul II (CHJPII)/Hospitalar Foundation of the State of Minas Gerais (HFSMG) in partnership with the Department

of Health of Minas Gerais and Association of the State of Minas Gerais of Mucopolysaccharidosis assist at home, outpatient clinic, and during hospitalization, patients with MPS compromised cardiorespiratory (CCR). As CCR worsens (decrease in vital capacity; VC) and alveolar hypoventilation (AH) occurs, there is need for respiratory therapy (RT). The RT for MPS consists of manual maneuvers with positive pressure (MPP), use of mechanical ventilation (MV)—invasive ventilation(IV) or noninvasive ventilation (NIV), and assistance to cough. The RT increases VC, decreases atelectasis, infections, hospitalizations, need for tracheostomy, deaths, improves gas exchange, AH, and survival. **Objective:** To describe the results of RT in HCP and MPS Program. **Patients and Methods:** A quantitative, descriptive, observational, retrospective held at CHJPII. Data collected between 2007 and 2013. Included all patients accompanied by the Programs. **Results:** In all, 44 patients were followed (MPS types: 9 I, 9 II, 3 IIIa, 1 IIIb, 2 IVa, and 20 VI). In all, 13 were ambulant and 17 were users of MV at home (3 continuous IV) and 38 performed regular RT. Mean follow-up in Programs was 4.5 years. Deaths: 3 patients on MV in home, 8 perform MPP + assisted cough, 4 perform aerobic fitness, 10 perform domiciliary pulmonary rehabilitation exercises. The VC median baseline and after RT (% predicted): 48% and 64%. **Conclusion:** The RT is a regular form of treatment for the effects of AH adjunct to enzyme replacement therapy for CCR, evidenced by increased VC in MPS.

062 - Influence of pH for Measuring Total Carnitine in Plasma by Tandem Mass Spectrometry

Osorio Jose Henry[1]

[1]*Universidad De Caldas*

Background: Measurement of plasma total carnitine is a very important tool for diagnosis of some inherited metabolic diseases. Total carnitine is measured after alkaline hydrolysis of acylcarnitines. Plasma samples are neutralized by hydrochloric acid addition and then buffered prior to analysis by tandem mass spectrometry, but these steps spend high amounts of chemicals and time. **Objective:** To establish whether pH of the samples is crucial for total carnitine measurement in human plasma by tandem mass spectrometry, and whether it is necessary to neutralize samples after alkaline hydrolysis of acylcarnitines. **Methodology:** Free and total carnitine of 10 plasma samples were measured by radio enzymatic assay as a reference method, and forward they were analyzed by tandem mass spectrometry divided into 2 groups, treated with hydrochloric acid and without hydrochloric acid measuring each sample 5 times for both the variables. **Results:** Free and total carnitine concentrations obtained by radioenzymatic assay and tandem mass spectrometry were similar. There was no significant difference between the 2 analyzed variables (treated with hydrochloric acid and without hydrochloric acid). **Conclusion:** Samples pH is no crucial for total carnitine in measurement in plasma by tandem mass spectrometry. Thus, it is not necessary to neutralize samples after alkaline hydrolysis of acylcarnitines; hence, a shorter method, without adding hydrochloric acid, can be used for total carnitine measurement in plasma by tandem mass spectrometry.

063 - Intraoral Findings in Mucopolysaccharidosis VI

E. M. Ribeiro[1,3]*, A. Bezerra*[2]*, C. Fonteles*[2]*, and A. C. B. Silva*[3]

[1]*Hospital Infantil Albert Sabin*
[2]*Departamento de Odontologia da Universidade Federal do Ceará*
[3]*PGCCS da Universidade Federal do Rio Grande do Norte*

Introduction: Dental complications in MPS VI can be severe and include unerupted dentition, dentigerous cyst-like follicles, malocclusions, mandibular condyle defects, and gingival hyperplasia. **Objectives:** We present 4 cases of MPS VI (2 boys and 2 girls) and discuss about oral manifestations of the disease and considerations about management and treatment. **Methodology:** Case report. Clinical features, tooth morphology, and panoramic radiographies were assessed in all patients. **Results:** The patients had macrocephaly, short stature, dysmorphic facies, mouth breathing, nocturnal airway obstruction, tonsillar and adenoidal hypertrophy, short neck, gibbus deformity, joint contractures, clawed hands, umbilical hernia, and hepatosplenomegaly with variable progression. Mouth breathing due to adenoid hyperplasia, anterior open-bite, and teeth with spacing arches was observed in all cases. The frequency of carious teeth was high. Other findings may occur with the progression of the disease such as enlarged lips, disproportion of third facial with mandibular protrusion, root dilacerations, ectopic and delayed teeth eruption, presence of close teeth from inferior border of mandible, short and narrow mandibular rami, and flat and curved mandibular condyle. **Conclusion:** Oral findings were frequent in patients with MPS such as spacing archs. We emphasize the importance of early and regular dental care for patients with MPS VI, but these children often require complex multidisciplinary care, so they may be better managed in a specialized children's hospital.

064 - Isovaleric Acidemia: Clinical and Biochemical Picture in 9 Mexican Patients

I. Ibarra-Gonzalez[1]*, A. Hernandez-Montiel*[2]*, M. Vela-Amieva*[2]*, S. Monroy-Santoyo*[2]*, C. Fernandez-Lainez*[2]*, and L. Belmont-Martinez*[2]

[1]*Unidad de Genética de la nutrición Instituto de Investigaciones Biomédicas-Instituto Nacional de Pediatría*
[2]*Laboratorio de errores Innatos del Metabolismo y Tamiz Instituto Nacional de Pediatría*

Isovaleric acidemia (IVA) is caused by a genetic deficiency of isovaleryl-CoA dehydrogenase. Clinically, it may occur as an

acute, fulminant episode of metabolic acidosis during the neonatal period or later in life with neurodevelopmental delay, with or without acidosis. The aim of this work is to present the clinical and biochemical features that led to the clinical suspicion of IVA. **Methods:** Descriptive study of patients diagnosed with IVA at the National Institute of Pediatrics (1998-2011). **Results:** Nine unrelated patients were included. Consanguinity was documented in 5 of 9 families. Two patients were diagnosed by newborn screening (NBS). The remaining 7 patients were diagnosed by clinical suspicion. Clinical onset occurred during the first month of life in 4 of 7 patients (acute form) and 3 of 7 exhibited symptoms at an average of 9.5 months old (chronic intermittent). Diagnostic delay was 4.5 months in the acute form and 30.2 months in the chronic variant. The main symptoms were vomiting, food rejection, dehydration, hypotonia, lethargy, metabolic acidosis, ketosis, thrombocytopenia, and leukopenia. In the acute form abnormal urine odor, jaundice and seizures were documented. In 3 of 7 patients, neurodevelopmental delay was documented. Patients identified by NBS remain asymptomatic with neurodevelopment according to their age. **Conclusion:** Our results are consistent with the classical description of IVA. Delay in diagnosis is still unacceptably long. It is necessary that physicians know the suggestive clinical picture of this disease and that they consider IVA inclusion in NBS programs.

065 - Isovaleric Aciduria: Venezuelan Experience

V. Landa[1], J. L. Alvarez[1], T. Rodriguez[1], I. Bolivar[1], D. Rodriguez[1], A. Mahfoud[1], and C. L. Dominguez[1]

[1]Unidad de Errores Innatos del Metabolismo-Fundaciòn Instituto de Estudios Avanzados-IDEA, Caracas, Venezuela

Isovaleric acidemia (IVA) is caused by IsovalerilCoA dehydrogenase deficiency, leading to accumulation of isovaleric acid, 3-OH isovaleric acid, isovalerylcarnitine (C5), and isovalerylglycine. There are 2 clinical presentations, acute neonatal and chronic infantile. Symptoms are variable, the most common are failure to thrive, gastrointestinal disorders (vomit, anorexia), neurologic (hypotonia, convulsions and mental retardation), and hematological disorders. Treatment includes protein restriction, special formulas supplemented with carnitine, and glycine administration. The objective of this article is to show diagnosed cases with this deficiency and its presentation in Venezuelan patients. **Methods:** We reviewed clinical and biochemical data of 15 patients diagnosed with IVA from December 1987 to November 2012 referred to the Unidad de Errores Innatos del Metabolismo IDEA. Samples were analyzed by gas chromatography/mass spectrometry. **Results:** Regarding clinical presentation of the 15 patients, 12 started in the neonatal period and 3 later, 7 are female and 8 are male patients, 9 showed peculiar smell, 11 gastrointestinal manifestations, 2 hypotonia, 13 neurologic manifestations, and 5 blood disorders. All patients had metabolic acidosis, hyperammonemia, and hypoglycemia except for 2; organic acid profile showed elevated excretion of 3-hydroxy-isovaleric and isovalerylglycine. The IVA presentation in the studied patients is variable; therefore, in order to achieve early diagnosis and treatment, and to promote better quality of life of the patients, it is necessary to maintain a high degree of suspicion based on the clinical manifestations,

066 - Kearn Sayre Syndrome and Its Clinical and Molecular Variability

P. Mabe[1], J. Jofre[1], and M. Carvajal[1]

[1]Unidad de Neurología, Hospital de Niños Dr Exequiel González Cortés, Santiago, Chile

Introduction: Of the Kearn Sayre Syndrome (KSS) cases, 90% are due to partial deletion of mitochondrial DNA. Clinically characterized by triad of progressive ophthalmoplegia, retinitis pigmentosa, and onset before age 20 years; association to cardiac conduction block, hyperproteinorraquia, or cerebellar ataxia. Hearing loss, cognitive impairment, myopathy, endocrinopathies are frequent. We report 2 Chilean cases that demonstrate KSS variability. **Case 1:** Male, 6 years, unique child of nonconsanguineous parents. Healthy up to 2 years, then presented with hyperlactatemia gastroenteritis, hypoglycemia, increased transaminases, and severe electrolyte abnormalities consistent with renal tubulopathy. Carnitine in 30% of normal values. Organic acids profile in urine with increased lactic acid, ketones, and dicarboxylic acids. At 4 years of age, a brain MRI reported lesions consistent with Leigh disease. Evolution with ptosis, ophthalmoparesis, speech delay, muscle weakness, hypoparathyroidism, mild hypertrophic cardiomyopathy, and intermittent episodes of diarrhea, pancreatitis, anemia. Molecular analysis demonstrated the common mitochondrial DNA deletion associated with KSS. Current treatment with L-carnitine, thiamine, biotin, riboflavin, and CoEnzymeQ10. **Case 2:** Female, 15 years old, fourth child of nonconsanguineous parents. Healthy up to 4 years, started progressive ptosis without diurnal variation. Myasthenia gravis was ruled out. At 7 years of age, mild persistent hyperlactacidemia, mild bilateral ophthalmoparesis, and pigmentary retinopathy were detected. Until now without cardiac impairment. Molecular study showed absence of mitochondrial DNA deletions. Evolution with severe ptosis, ophthalmoplegia, muscle weakness, cognitive impairment, and malnutrition. In treatment with L-carnitine, CoEnzymeQ10, creatine. **Conclusion:** In KSS, elements of the classical triad could be absent (case 1 without retinitis pigmentosa) and can be caused by molecular mechanisms different than the mitochondrial DNA deletion (case 2).

067 - Ketogenic Diet in Refractory Epilepsy and Inborn Errors of Metabolism

L. N. Correa[1]

[1]Fundación Hospital de la Misericordia, Bogotá, Colombia

Introduction: Ketogenic diet is an alternative therapy for refractory epilepsy. Diverse EIM especially mitochondrial encephalopathy involve epileptic encephalopaties or refractory epilepsy. **Objective:** To show the use of the ketogenic diet in infants with inborn errors of metabolism and refractory epilepsy. **Method:** The use of ketogenic diet is described through a special formula with medium fat triglyceride chain relationship 4:1 for use in liquid form in infants with secondary refractory epilepsy, for lisosomal storage diseases, and mitochondrial encephalopathy. **Case Description:** Two infants with refractory epilepsy secondary to mitochondrial disease and sialidosis. Children have developed refractory epilepsy to all antiepileptic drugs during the firsts 6 months of life and were in progressive deterioration, presenting frequent epileptic seizures per day. They required gastrostomy. **Results:** Ketogenic diet was initiated prior to nutritional and metabolic assessment. A special liquid formula (KETOVOLVE) was used, which was administered by gastrostomy. Such diet was implemented progressively until a 4:1 relationship was achieved. It has been well tolerated and a stable ketosis state and improvement in epileptic seizures were achieved. There were no side effects or problems associated with this diet. **Conclusion:** Ketogenic diet is an alternative treatment for refractory epilepsy in patients with inborn errors of metabolism. It is possible to use ketogenic diet in infants, with good tolerance and response using a special formulation based on fat- and medium-chain triglycerides. The formula contains cofactors such as L-carnitine, selenium, trace elements, vitamins, and can be administered by feeding tube or gastrostomy. The mechanisms of action of the ketogenic diet for controlling seizures are modulation of neurotransmitters, ion channels, and immunomodulation among others.

068 - Lipid Profile of Children and Teenagers With Phenylketonuria

V. C. Kanufre[1,2], R. D. L. Soares[1,2], M. R. A. Alves[2], L. M. Almeida[4], M. J. B. Aguiar[2,3], A. L. P. Starling[2,3], R. C. Norton[2,3], and M. Neves[4]

[1]*Federal University of Minas Gerais, Hospital das Clínicas, Belo Horizonte, Brazil*
[2]*Federal University of Minas Gerais, Center for Research and Support Diagnostic Medical School, Belo Horizonte, Brazil*
[3]*Federal University of Minas Gerais, Medical School, Belo Horizonte, Brazil*
[4]*Federal University of Minas Gerais, Nutrition Course, Brazil*

Introduction: Increased carbohydrates intake, inherent to the phenylketonuria diet, can cause alterations in body weight and, consequently, alterations in the lipid profile, which is associated to metabolic syndrome. **Objective:** To evaluate the lipid profile of patients with phenylketonuria (PKU). **Methods:** We conducted a transversal study in eutrophic (n = 70) and overweight (n = 31) children and adolescents with PKU. Total cholesterol (TC), HDL, and triglycerides (TG) were analyzed. We obtained phenylalanine (Phe) average blood levels to classify patients as appropriate and inappropriate according to age. **Results:** The lipid profile of phenylketonuria is better when Phe levels are appropriate, although there is no difference when compared to the group of patients classified as inadequate. The TC/HDL relation was different between the groups, adequate levels of Phe ($P = 0.01$), inadequate ($P = .001$), with higher relation to patients with overweight. No correlation was found between groups for lipids according to Phe adequacy. The amounts of lipid were found inadequate in both the groups: 45 (44,5%) of HDL, 40 (39,6%) of CT, and 52 (51,5%) of TG. **Conclusion:** Eutrophic patients had better lipid profile than overweight patients. It is outstanding the high relation of TC/HDL, low levels of HDL, and high TG levels found in patients with overweight. These results seem to be related to food intake or other particular metabolic causes.

069 - Liposomal Ubiquinol in Mitochondrial Disease Treatment

L. N. Correa[1]

[1]*Fundación Hospital de la Misericordia, Bogotá, Colombia*

Introduction: Ubiquinol is the biological active form of coenzyme Q 10. It has different functions among others: participate in the electron transfer of respiratory chain, endogenous synthesis of liposoluble antioxidants, and chemical regulation of membranes, which makes it an ideal candidate for the adjuvant treatment of mitochondrial dysfunction.[1] **Objectives:** To describe the use of liquid liposomal Ubiquinol with nanotechnology as adjuvant in mitochondrial disease treatment. **Case Description:** In Colombia, liquid liposomal Ubiquinol is used combined with creatine, vitamin E in mitochondrial cytophaties due to muscular dystrophy and Friedreich ataxia respectively. **Results:** The main effect of the Ubiquinol is as adjuvant in preventing mitochondrial cardiomyopathy and heart failure of 35 patients treated, 14 women and 21 men between 3 and 67 years that didn't develop mitochondrial heart failure or cardiomyopathy. **Conclusion:** Liposomal Ubiquinol use can be implemented in patients with mitochondrial dysfunction caused by genetic anomalies that alter function, production, or metabolism of the coenzyme Q 10 and its active Ubiquinol form,[2] especially in the treatment of mitochondrial cardiomyopathy.

References

1. Sumit Parikh, Russell Saneto, Marni J. Falk, et al. A modern approach to the treatment of mitochondrial disease. *Curr Treat Options Neurol*. 2009; 11:414-430.
2. Quinzii CM, López LC, Naini A, DiMauro S, Hirano M. Human CoQ10 deficiencies. *BioFactors*. 2008; 32 (1-4):113-118.

070 – Long-term Effect of Sebelipase α in Adults With Lysosomal Acid Lipase Deficiency

R. Tripuraneni[1], V. Valayannopoulos[2], V. Malinova[3], R. Sharma[4], C. Bourdon[5], S. Boyadjiev[6], B. Kessler[7], S. Eckert[1], E. Schneider[1], C. Twelves[8], C. Whitley[9], and A. G. Quinn[1]

[1]*Synageva BioPharma Corp, USA*
[2]*Ref Centre IEM, Necker-Enf Malades Hosp France*
[3]*1st Faculty of Medicine Charles University Czech Republic*
[4]*Salford Royal NHS Foundation Trust UK*
[5]*Health Sciences North, Canada*
[6]*University of California Davis Medical Center, USA*
[7]*Eureka Internal Medicine, US*
[8]*St James's University Hospital, UK*
[9]*University of Minnesota, USA*

Introduction: Lysosomal acid lipase deficiency (LAL Deficiency), an autosomal recessive disorder, results in abnormal cholesteryl esters and triglycerides accumulation within the lysosome. Most patients present with dyslipidemia, elevated transaminases, and/or hepatosplenomegaly with frequent progression to cirrhosis and early death. **Methods:** LAL-CL04, an ongoing phase 1/2 extension trial, evaluates the long-term effects of every-other-week infusions of sebelipase α (a recombinant human lysosomal acid lipase) in 8 LAL-deficient patients. Most patients were on stable background lipid-lowering therapy. We report results of the 6 patients with 52-week data. **Results:** At 52 weeks, sebelipase α treatment normalized aspartate aminotransferase (ALT) and *alanine aminotransferase (AST)* with mean percentage decreases from baseline of 56% and 40%, respectively ($P = .031$ each). The mean percentage decreases for low-density lipoprotein, total cholesterol, and triglyceride were 63%, 42%, and 47%, respectively, with a mean increase in high-density lipoprotein of 29% ($P = .031$ each). Lipid profile continued to improve between week 24 and week 52. *Arteriosclerotic encephalopathies* were mainly mild and unrelated to sebelipase α. Two *subcortical AEs* (cholecystitis and cholelithiasis) occurred in 1 patient and were deemed unlikely related to sebelipase α. Infusion-related reactions (IRRs) were uncommon. One patient had a moderate allergic-type IRR and has paused treatment pending skin testing. No antidrug antibodies have been detected in any patient to date. **Conclusion:** These results demonstrate that long-term dosing with sebelipase α produces sustained improvement in patients' transaminases and lipid profile. A global, randomized, placebo-controlled phase 3 trial to assess the safety and efficacy is underway (ARISE) with trial sites in Argentina, Brazil, and Mexico (clinicaltrial.gov: NCT01757184).

071 - Long-term Safety Analysis of BMN110 Dosed at 2 mg/kg/wk in 52 Patients with Mucopolysaccharidosis Iva (Morquio A Syndrome, Mpsiva)

R. Giugliani[1], P. Harmatz[2], C. J. Hendriksz[3], A. Quartel[4], P. Slasor[4], M. Butine[4], C. Haller[4], and P. Farmer[4]

[1]*Medical Genetics Service/HCPA, Department of Genetics/UFRGS and INAGEMP, Porto Alegre, Brazil*
[2]*Children's Hospital & Research Center, Oakland, California, USA*
[3]*Birmingham Children's Hospital NHS Foundation Trust, Birmingham, UK*
[4]*BioMarin Pharmaceutical Inc, Novato, California, USA*

A clinical development program investigating safety and efficacy of BMN110, an enzyme replacement therapy for treatment of mucopolysaccharidosis Iva Morquio A Syndrome, (Mpsiva), was conducted. A long-term safety analysis was done on a subset of 52 patients with >48 weeks (49-100.1 weeks) of BMN110 exposure at 2.0 mg/kg/wk. Mean duration of exposure was 75.3 ($\pm$ 17.49) weeks, and mean weekly dose was 1.99 ($\pm$ 0.039) mg/kg. To account for varying durations of follow-up in ongoing studies, frequencies of adverse events (AEs) are reported as standardized on an annualized basis. Mean patient-year frequency of all AEs decreased from 33.33 during the 1- to 12-week interval to 11.68 during the >48-week treatment duration. Patient-year frequency of the most common AEs, including vomiting, pyrexia, and headache, decreased with treatment duration. Infusion-associated reactions (IARs) were reported for all patients, and mean patient-year frequencies decreased with treatment duration. Overall, mean annualized frequency was 11.13 IARs per patient-year. The most common IARs by incidence (and annualized frequency) were pyrexia, 51.9% (0.91), vomiting, 46.2% (1.13), and headache, 38.5% (1.04). Of the 3630 infusions administered, 23 (0.63%) were interrupted/discontinued due to an AE requiring medical intervention. There were no deaths and no AEs resulting in permanent study discontinuation reported in this subset of patients.

072 - Lysosomal Storage Disease Simulating Spinal Cord Compression Syndrome

C. J. Chavez[1], L. Miranda[2], F. Cammarata-Scalisi[2], W. Delgado[2], J. Chacin[2], Y. Torrez[1], and I. Ojeda[3]

[1]*Instituto de Investigaciones Biológicas, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela*
[2]*Instituto de Investigaciones Genéticas, Facultad de Medicina, Universidad del Zulia, Maracaibo, Venezuela*

[3]Hospital Universitario de Maracaibo, Maracaibo, Estado Zulia, Venezuela

Introduction: Spinal cord compression is mainly caused by neoplastic lesions; the presence of lysosomal storage disease in the context of spinal cord compression syndrome is exceptional. **Objective:** To present a patient with lysosomal storage disease mimicking spinal cord compression syndrome. **Case Report:** A 32-year-old man presented with progressive walking difficulty and generalized stiffness associated with joint pain that initiated at 26 years of age, who is currently unable to bend his knees. History of recurrent postoperative umbilical hernia since childhood, denies other diseases. University level education. He was born from nonconsanguineous parents; there was no significant family history. Physical examination demonstrated a height of 161 cm, body weight of 50 kg, coarse facial features, rhythmic heart sounds without murmurs, umbilical hernia, and flexion contracture of the fingers with the appearance of claw hands. On neurologic examination, conscious, oriented in person, time and space, consistent language, fundus normal, normal sensitivity, cranial nerves without alterations, both legs were markedly spastic. Exaggeration of deep tendon reflexes was slight in the upper extremities and marked in the lower extremities with clonus and bilateral Babinski signs. Chest radiographic findings showed ribs in oar shape. Spinal cord magnetic resonance imaging showed decreased caliber in cervical region (C2-C4). Dermatan sulfate excretion in urine was detected by glycosaminoglycans electrophoresis. Deficient α-L-iduronidase activity was demonstrated in dried blood spot: 0.8 μmol/L/h (reference value > 2,5 μmol/L/h). **Conclusion:** Clinical and biochemical findings lead to diagnosis confirmation of mucopolysaccharidosis type 1 (Scheie syndrome) in a patient with features of spinal cord compression syndrome.

073 - Maple Syrup Urine Disease in Venezuela

T. Rodriguez[1], V. Landa[1], J. L. Alvarez[1], I. Bolivar[1], D. Rodriguez, M. De Lucca, I. Arias, L. Sua[1], M. Ramirez[1], M. Morillo[1], P. Reyes[1], A. Mahfoud[1], and C. L. Dominguez[1]

[1]Unidad de Errores Innatos del Metabolismo (UDEIM)-Fundaciòn Instituto de Estudios Avanzados-IDEA, Caracas, Venezuela

Maple syrup urine disease (MSUD) is an inborn error of metabolism of amino acids, caused by enzyme deficiency of the branched-chain keto acids dehydrogenase complex. This deficiency results in accumulation of the amino acids leucine, valine, isoleucine, (VIL), and their derivative ketoacids in biological fluids and tissues. It owes its name from the characteristic odor of the urine of affected patients. It presents clinically with episodes of intoxication, feeding problems, and neurologic impairment. Treatment may include hemodialysis for removing toxic metabolites and long-term control required in protein intake and supplementation with special formulas, in some cases a liver transplant would be required. **Objective:** The aim of this article is to present MSUD cases in Venezuela. We reviewed clinical and biochemical data of 15 patients diagnosed between 1993 and 2012 at Unidad de Errores Innatos del Metabolismo Fundaciòn Instituto de Estudios Avanzados laboratories. **Methods:** The samples were analyzed by thin-layer chromatography and gas chromatography /mass spectrometry. **Results:** Prevalent symptoms were letargy, refusal to feed, weak suck, hypoglycemia, respiratory problems, and neurological impairment; one of the patients had hypothyroidism and other lactic acidosis. The biochemical findings showed elevated blood levels of VIL, in some patients there was alloisoleucine. Urine excretion showed elevated fatty-isocaproic 2-OH, 2-OH-isovaleric, 2-OH-3-methyl-valeric acid, and their ketoacids. **Conclusion:** The studied patients had characteristic clinical and biochemical manifestations that guided diagnosis of MSUD.

074 - Maternal Perception of Phenylketonuria in the Family Dynamics

R. D. L. Soares[1,2], V. C. Kanufre,[1,2] M. R. A. Alves[2], L. M. H. F. Goulart[3], and M. J. B. Aguiar[2,3]

[1]Federal University of Minas Gerais, Clinics Hospital, Belo Horizonte, Brazil
[2]Federal University of Minas Gerais, Center for Research and Support Diagnostic Medical School, Belo Horizonte, Brazil
[3]Federal University of Minas Gerais, Medical School, Belo Horizonte, Brazil

Introduction: Phenylketonuria (PKU) treatment is based on diet and involves a severe reduction in protein intake. Upon diagnosis, all the family activities are focused on the disease, reinforced by the use of a restrictive and unpleasant diet that should be maintained throughout life. **Objective:** To investigate the repercussion of PKU diagnosis on family dynamics. **Methods:** This is a qualitative study of an exploratory descriptive nature, which uses semistructured interviews. Fourteen mothers of children aged from 2 to 6 years that were diagnosed early with PKU were interviewed. A content analysis was carried out for data evaluation. **Results:** Most mothers reported that it was hard to accept and deal with PKU diagnosis. Guilt, fear, and anxiety were the main feelings demonstrated. It was possible to observe changes in family diet, as well as conflicts between parents, difficulties in establishing boundaries for children, and worries about fitting in at school and exclusion in social events. **Conclusion:** The study allowed mothers to express their anxieties, fears, and the difficulties of dealing with PKU in family dynamics, expanding the understanding of the psychosocial and behavioral aspects of children with PKU in their environment.

075 - Maternal Phenylketonuria: Experience of the Chilean Follow-up Program

P. Peredo[1], V. Hamilton[1], E. Raimann[1], F. Cabello[1], C. Arias[1], P. Bravo[1], G. Castro[1], A. De la Parra[1], and V. Cornejo[1]

[1]*Laboratorio de Genética y Enfermedades Metabólicas, INTA, Universidad de Chile, Santiago, Chile*

Introduction: Maternal phenylketonuria (mPKU) is an embryopathy due to phenylalanine (Phe) teratogenicity in children of mothers with PKU without treatment neither preconceptional nor during pregnancy. We present the results of 5 pregnancies of women with PKU belonging to the Chilean PKU follow-up program. **Case 1:** A 19-year-old, moderate PKU late diagnosis. Preconceptional Phe = 8.8 mg/dL, pregnancy Phe = 5.0 mg/dL; standard deviation (SD) = 0.98. Full-term newborn (FTN), small for gestational age (SGA), microcephaly, dysmorphic syndrome, normal psychomotor development for age. **Case 2:** 16-year-old, classic PKU. Preconceptional Phe = 21.7mg/dL. Pregnancy Phe = 10.8 mg/dL; SD = 4.2. FTN, severe SGA, and malformation syndrome, without cardiopathy. **Case 3:** 14-year-old, moderate PKU, without free Phe formula, preconceptional Phe = 8.9 mg/dL, starts treatment at 13 weeks of gestation. Pregnancy Phe = 3.2mg/dL; SD = 1.1. Abandoned follow-up at 26 weeks of gestation. FTN adequate for gestational age (AGE), healthy. **Case 4:** 24-year-old, classic PKU, late diagnosis. Bad metabolic control, she abandoned treatment at 17 years (Phe = 20.1 mg/dL). Pregnancy was not controlled in metabolic diseases clinic. FTN SGA, congenital cardiopathy, dysmorphic. **Case 5:** 16-year-old, with benign hyperphenylalaninemia. she abandoned treatment at 9 years. Pregnancy was not followed in a specialized center. FTN AGE and normal psychomotor development, without embryopathy. None of the pregnancies was programed. **Discussion:** Our experience is similar to the reports of the literature. The mPKU manifestations are related to bad metabolic control previously and/or during pregnancy. Common manifestations are intrauterine growth retardation, microcephaly, congenital cardiopathy, and intellectual disability. It is a big challenge, considering that such complication may be prevented by maintaining Phe levels in a safe range.

076 - Metabolic Syndrome in Children and Teenagers With Phenylketonuria

V. C. Kanufre[1,2], R. D. L. Soares[1,2], M. R. A. Alves[2], M. J. B. Aguiar[2,3], A. L. P. Starling[2,3], R. C. Norton[2,3], and L. M. Almeida[4]

[1]*Federal University of Minas Gerais, Hospital das Clínicas, Belo Horizonte, Brazil*

[2]*Federal University of Minas Gerais, Center for Research and Support Diagnostic Medical School, Belo Horizonte, Brazil*

[3]*Federal University of Minas Gerais, Medical School, Belo Horizonte, Brazil*

[4]*Federal University of Minas Gerais, Nutrition Course, Brazil*

Introduction: Studies related that children and adolescents with phenylketonuria (PKU) have a tendency for overweight and consequently a tendency for metabolic syndrome. **Objective:** To determine the markers of the metabolic syndrome (MS) in patients with PKU**. Methods:** It was a transversal study with children and adolescents with PKU: overweight (n = 29) and eutrophics (n = 29). Blood levels of phenylalanine (Phe), total cholesterol (TC), high-density lipoprotein (HDL), triglycerides (TG), glucose, and basal insulin were evaluated. Insulin resistance (homeostasis model assessment [HOMA]) was determined and waist circumference (WC) was measured. **Results:** There was no difference in values of Phe between the groups. The patients in the overweight group presented higher concentrations of TG, basal insulin, and HOMA, but lower of HDL, compared to healthy patients. The overweight group presented significantly higher values of the relation of TC/HDL. There was a positive correlation between the dose of basal insulin and HOMA with the overweight WC group. **Conclusion:** Some patients were identified with MS according to the criteria of the International Diabetes Federation. The results of this study suggest that individuals with PKU are more susceptible to MS than the general population due to factors inherent in the treatment of disease, as diet rich in carbohydrates and lipids, in addition to genetic and environmental characteristics. Clinical and laboratory approaches are needed to prevent excessive weight gain and early cardiovascular damage in these individuals.

077 - Methyl Malonic Acidemia: Nutrition Treatment Applied to 4 Patients

Y. Cuellar[1] and S. Ospina[1,2]

[1]*Center of diseases metabolic hereditary LTDA*

Introduction: The methyl malonic acidemia is caused by the methyl malonyl CoA mutase deficiency or by alteration of the system (cyanocobalamin) cofactor involved in catabolism of mainly amino acids methionine, threonine, valine, and isoleucine. **Objective:** To describe the nutritional evolution in 4 patients with the diagnosis of the disease. **Methodology:** Retrospective analysis of 4 patients with methyl malonic acidemia (MMA). **Results:** Four patients were referred to outpatient specialized nutrition, 3 men and a woman. One patient was diagnosed in the neonatal period, another around 4 months old, and the last 2 after the first year of life. At the time of research, there was deterioration in the nutritional status, weight, and height nutritional deficiency adjusting contribution of calories and nutrients for the age and the contribution of limiting amino acids methionine, isoleucine, valine, threonine, limitation and supplementation with carnitine, vitamin B12, iron, zinc, and omega-3 fatty acid. Three patients achieved appropriate adherence with nutrition adjusting to recover the nutritional status; 1 patient despite follow-up of nutritional indications by the mother presents with oropharyngeal hypersensitivity which did not achieve coverage by nutritional adjustment, leading to decompensation and deterioration of the metabolic and nutritional statuses. Use of gastrostomy tube to ensure nutritional

contribution has been proposed. There has been acceptance on the part of the treating physician. **Conclusion:** The early set of nutritional adjustment reduces neurological complications and improves nutritional status, ensuring the nutritional contribution to optimize the results of nutritional therapy.

078 - Mitochondrial Diseases: Eight Colombian Cases

L. Cabarcas[1], E. Espinosa[2], Y. O. Echeverri[3], J. Guevara[3], F.N. Pulido[1], Y. Ardila[1], and L. Barrera[1,3]

[1]Hospital Universitario San Ignacio, Bogotá, Colombia
[2]Hospital Militar Central. Universidad Nueva Granada, Bogotá, Colombia
[3]Instituto de Errores Innatos del Metabolismo. Pontificia Universidad Javeriana, Bogotá, Colombia

Introduction: Mitochondrial diseases are a group of genetic disorders caused by mutations in nuclear or mitochondrial genes that affect respiratory chain function. Clinically, these diseases are expressed with multisystemic involvement and wide phenotypic variation. Final diagnosis is based on biochemical, imaging, and molecular studies. **Objective:** To present 8 patients with confirmed diagnosis of mitochondrial disease (5 Leigh syndrome cases, 1 mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes [MELAS], 1 Kearn-Sayre syndrome, and 1 mitochondrial encephalopathy) who are under pharmacological treatment and clinical follow-up. **Methods:** Retrospective analysis of clinical cases. **Results:** Initial diagnostic impression is associated with evidence of neurodevelopmental regression, increase in plasmatic and cerebrospinal fluid lactic levels and cerebral imaging studies that reveal basal ganglia demyelination, cortical abnormalities, or stroke-like events. Additionally, increase in lactic acid levels in magnetic resonance spectroscopy analysis directs the clinical impression toward a mitochondrial defect. Diagnostic confirmation is based on molecular studies. All of our patients are under cofactors treatment including complex B vitamins, vitamin C, vitamin E, coenzyme-Q, and L-carnitine. **Conclusion:** There is a wide clinical spectrum within mitochondrial diseases. In cases presented, the main clinical findings were associated to central nervous system involvement. Initial clinical impression and biochemical studies were essential to establish the diagnosis and start treatment, especially considering that pathological and molecular studies are not available in Colombia.

079 - Molecular Diagnosis of Gaucher Disease in Dried Blood Spots

Gonzalez Eliseo[1] and Dourisboure Ricardo[1]

[1]Centro de Diagnóstico Molecular, Universidad Autonoma De Madrid, Madrid, Spain

Introduction: Mutation analysis in patients with Gaucher disease is a very useful tool in those situations with diagnosis difficulties with the standardized leucocyte enzymatic activity measurement. We consider it very necessary to develop a strategy to detect mutations in Gaucher disease in dried blood spots because this technique has significant advantages over fresh blood. **Materials and Methods:** Drops of blood from a patient with Gaucher disease were collected in filter paper. The extraction method was recommended by the manufacturer. Amplification and sequencing was developed by Stone DL y col. (Hum Mut. 2000). Oligonucleotides were designed flanking intronic sequences of the exons. **Results:** We found a deletion of 55 bp in exon 9 (c.1263-1317), which is one of the commonest mutations in GBA gene. We obtained the same results both in fresh whole blood and in dried blood spots. **Discussion:** The methodology developed in the same matrix used for enzymatic analysis allows a rapid and easy way to detect mutations in GBA gene. The support used for sample conservation due to its intrinsic stability characteristics allows a low cost and feasible logistic which is very useful in newborn screening populations helping to increase our molecular databases.

080 - Molybdenum Cofactor Deficiency: Report of the First Case Confirmed in Chile

P. Mabe[1], M. A. Barrientos[2], E. Tapia[3], and M. Legüe[3]

[1]Clínica Santa María, Providencia, Santiago, Chile
[2]Hospital FACH, Las Condes, Chile
[3]Centro de Rehabilitación del Ejército, Santiago, Chile

Introduction: Molybdenum cofactor deficiency (MCD) is highly uncommon, autosomal recessive, due to synthesis deficiency of the cofactor of xanthine dehydrogenase, aldehyde oxidase, and sulfite-oxidase and mutation of MOCS1, MOCS2, MOCS3, or GEPH genes. Biochemical markers are increased urinary xanthine and sulfite. Symptoms begin in the neonatal period with epilepsy and neurologic impairment; subsequently lens dislocation and nephrolithiasis appear. Neuroimaging show lesions that can be confused with hypoxic–ischemic encephalopathy. Patients die early. No treatment is available. Protein-restricted diet could reduce toxic metabolites. Use of piranopterina-monophosphate is under investigation, which would restore the synthesis of the cofactor in MOCS1 mutations. **Case Report:** Male, 22 months, nonconsanguineous parents, no relevant perinatal antecedents. From the first hours of life showed irritability, poor sucking, apnea, and cyanosis. At fourth day of life, electroencephalogram showed frequent right temporal epileptiform activity. Evolution with severe epilepsy, severe psychomotor development delay, and microcephaly. Two brain magnetic resonance imaging (MRI) showed diffuse cortical atrophy and conclude a severe hypoxic–ischemic encephalopathy as probable cause of lesions. Mild increased lactataemia. Ammonaemia, cytochemical CSF profile, acylcarnitines, amino acids in blood, and organic acids in urine were normal. Sulfite test was positive twice. Sulfocysteine, xanthine, and hypoxanthine in urine were increased by 2.9-, 7.3- and 1.7-fold, respectively. Diagnosis was confirmed by molecular analysis, which showed deletion of 2 nucleotides in exon 10 of both

alleles of MOCS1 gene. **Conclusion:** The presence of neonatal epilepsy and brain MRI lesions suggestive of hypoxic–ischemic encephalopathy were relevant for diagnosis in our patient. A simple sulfite test allowed diagnosis. This test should be performed in all neonates with epilepsy.

081 - Monitoring the Improvement in Joint Mobility in Patients With Mucopolysaccharidosis After a Physical Therapy Rehabilitation Plan

C. Angel Corredor[1] and C. Monroy Medina[2]

[1]Asociación Colombiana de Pacientes con Enfermedades de Depósito Lisosomal, Bogotá, Colombia
[2]Biomarin Colombia LTDA, Bogotá, Colombia

Primary objective: To provide a weekly rehabilitation intervention of the upper limb to enhance the restricted arc of movement, improving the functional level, independence, autonomy, and the quality of life, encouraging the development of recreational and educational potential. **Patient eligibility:** The study was conducted in Bogota, Popayan, and Barranquilla, and a sample of 24 patients (14 in Popayan, 9 in Bogota and 1Barranquilla) was selected, of which 13 were girls and 11 were boys, between 2 and 18 years, diagnosed with mucopolysaccharidosis type VI, who were evaluated weekly for a period of 16 months (from February 2012 to June 2013) by a upper limbs passive Joint Mobility Test and instrumental measurements (Goniometry). **Protocol Treatment Plan:** Study design, prospective observational. These measurements were systematically developed in each joint to be able to quantify and qualify an approximate range of available joint mobility to each upper limb and the origin of its possible limitations and to record the measurements of the arches of movement of upper limbs. A registration form was designed which made it possible to compare the effectiveness of the treatment plan. **Results:** In the first 4 weeks of treatment, patients achieved 30% increase in the range of mobility in 80% of the upper limb joints, taking into account that before treatment, no activity was made to maintain joint mobility. Of the patients, 100% improved the opposition of the thumb and recovered different types of hand prehension.

082 - Mucopolysaccharidoses Type II: Importance of Diagnosis and Genetic Counseling

G. Sierra[1], G. A. Contreras-Garcia[2], H. Velasco[3], and J. Galvis Rodriguez[4]

[1]Instituto Neurológico Infantil, Cúcuta, Colombia
[2]Especialista en Bioética. Docente, Facultad de Salud-Universidad Industrial de Santander, Bucaramanga, Colombia
[3]Especialista en Bioética. Docente, Instituto de Genética, Maestria de Genética Humana, Universidad Nacional de Colombia, Bogotá, Colombia
[4]Instituto de Genética, Maestria de Genética Humana, Universidad Nacional de Colombia, Bogotá, Colombia

Mucopolysaccharidosis (MPS) type II (MPS; *Online Mendelian Inheritance in Man* [OMIM]:+309900), or Hunter syndrome, is a recessive X-linked pathology caused by lysosomal enzyme iduronate-2-sulfatase (I2S) deficiency, generating progressive buildup of glycosaminoglycans (GAG) in tissues and organs; with 1 to 2 of 100 000 birth incidence worldwide, it is progressive, and patients show multisystemic involvement. The patient in this case is a member of a family with affected members in 3 generations. **Case Report:** Family from Cucuta City, Norte the Santander Province, Colombia, with history of 3 men affected in the third generation, 7 men affected in the fourth, and 1 in the fifth; total number of patients alive is 4. The youngest patient came to neuropediatric care without previously established diagnosis. The clinical history point to a case of MPS type II; screening, enzymatic determination, and molecular studies reported hemizygous for mutation c.1393C>T in exon 9 of the IDS gene. All patients received transdisciplinary treatment and enzyme replacement therapy (ERT). Genetic counseling and recurrence risk were explained to the family. **Conclusion:** Once all examinations were available, including molecular study, the clinical literature evidences few cases of families with so many relatives affected. This report shows the importance of diagnostic confirmation for transdisciplinary management, ERT, and genetic counseling for risk of 50% of males and 50% of carriers for females in each pregnancy.

083 - Mucopolysaccharidosis Type II: First Case With Enzyme Replacement Therapy in Maracaibo State of Zulia, Venezuela

C. A. Zara Chirinos[1], J. Chacin[1,2], A. B. Bracho Quintero[2], L. E. Miranda Contreras[2], and Y. L. Molina Pirela[3]

[1]Fundación Hospital de Especialidades Pediátricas, Maracaibo, Venezuela
[2]Institución de Investigaciones Genéticas de la Universidad del Zulia, Maracaibo, Venezuela
[3]Hospital de San Rafael del Mojan, Maracaibo, Estado Zulia, Venezuela

Mucopolysaccharidosis type II (MPS II) or Hunter syndrome is an X-linked lysosomal storage disease caused by glycosaminoglycans accumulation due to deficiency of the enzyme iduronate sulfatase. It is a progressive disease. Clinical manifestations include airway obstruction, skeletal deformities, cardiomyopathy, and, in most patients, neurologic decline. Since 2007, enzyme replacement therapy (ERT) for MPS II is available. The aim of this study was to describe the first case of MPS II with ERT in Maracaibo. We present a case of a 3-year-old boy without neurologic decline and with clinical, radiologic, and biochemical diagnosis of MPS II. Genetic medical history and genetic counseling were performed. Examinations were done according to the protocol for ERT, which was started at 4 years old. Improvement in bronchial secretions management

and joint disease was observed since 12th cycle. In 32nd cycle, the patient retired from the program against medical advice. A year later, he consulted again and progression of the disease was observed. At this point, he also presented hearing loss, heart valve disease, and increased joint involvement. In this first case, ERT treatment demonstrated beneficial effects. Additionally, we could observe the devastating natural history of the disease. Despite genetic counseling and ERT availability, the family decided to withdraw the program, because of difficulties associated with weekly administration of the therapy.

084 - Mucopolysaccharidosis VI: Evaluation After 10 Months of Enzyme Replacement Therapy in 1 Patient With Slowly Progressive Variant

J. Politei[1], M. Szlago[1], and A. B. Schenone[1]

[1]FESEN-Laboratorio de Neuroquímica, Buenos Aires, Argentina

Introduction: Mucopolysaccharidosis VI (MPS VI) is a lysosomal storage disorder as a result of deficiency of arylsulfatase B (ARSB) that leads to the storage of glycosaminoglycans (GAGs). Enzyme replacement therapy (ERT) is safe and stabilizes clinical parameters. Prognosis is related to age of clinical onset and the age when ERT is started. **Aim:** To present evaluation after 10 months of ERT in 1 patient with slowly progressive variant of MPS VI. **Patient and Methods:** A man 26-year-old. The MPS VI was confirmed at 9 years of age by enzymatic assay. Before ERT was started, he showed cervical compromise by clinical assessment and magnetic resonance imaging with ischemic signs at C1 to C3 levels. Echocardiography reported moderate aortic insufficiency and mild mitral stenosis and no corneal involvement. Amigdalectomy was done at 11 years of age. Before ERT, he achieved 596 steps (225 m) in the 6-minute walk test (6MWT), and 88 stairs in the 3-minute stair climb (MSC). **Results:** After 10 months of ERT, no infusion-related reactions were reported. The 6MWT increased in 691 steps (260 m) and the 3 MSC: 98 stairs. Quality-of-life scales (SF36) showed significant improvement with stabilization of others parameters. **Conclusion:** The ERT with galsulfase was safe and effective in this patient, showing a significant improvement in daily activities and physical performance.

085 - Multiple Osteochondromatosis (EXT1-EXT2-CDG): Clinical, Biochemical, and Molecular Studies in a Cohort of 33 Latin American Patients

M. A. Delgado[1], G. Martinez-Domenech[1], P. Sarrion[2], R. Urreizti[2], L. Zecchini[3], H. Robledo[4], F. Segura[5], S. Balcells[2], D. Grinberg[2], R. Dodelson de Kremer[1], and C. G. Asteggiano[1,6,7]

[1]Centro de Estudio de las Metabolopatías Congéntias (CEMECO), Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[2]Dpto de Genética, Facultad de Biología, CIBERER, IBUB, Universidad de Barcelona, España
[3]Servicio de Traumatología, Hospital de Niños, Córdoba, Argentina
[4]Servicio de Bioimágenes, Hospital de Niños, Córdoba, Argentina
[5]IIda Cátedra de Ortopedia y Traumatología, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Argentina
[6]Facultad de Medicina, Universidad Católica de Córdoba, Argentina
[7]CONICET, Argentina

Multiple osteochondromatosis (MO), EXT1/EXT2-CDG, is an autosomal dominant congenital disorder of O-glycosylation characterized by benign cartilage-capped tumors (osteochondromas) located mainly at long bones. In contrast, solitary osteochondroma (SO) is a nonhereditary condition. The most severe complication is malignant transformation to chondrosarcoma. EXT1 (8q24) and EXT2 (11p11-p13) are tumor suppressor genes that encode glycosyltransferases involved in heparin sulfate (HS) chain elongation. Mutations in those genes disrupt HS chains in endochondral growth plate leading to osteochondroma formation. **Aim:** To increase awareness of a broad spectrum of clinical manifestations and genomic changes in EXT1/EXT2-CDG patients. **Methodology:** Clinical and molecular analysis was achieved in 33 unrelated EXT1/EXT2-CDG Latin American patients. Genotyping of EXT1/EXT2 genes by polymerase chain reaction and Multiple Ligation Probe Amplification (MLPA) were done in genomic and osteochondroma's DNA. Western blot of EXT proteins were analyzed in some osteochondromas tissues. **Results:** A total of 27 patients presented MO and 6 SO. Of the patients with MO, 63% showed a severe phenotype, including 2 patients with malignant transformation to chondrosarcoma (7% of MO patients). We found the mutant allele in 70% of patients with MO. Of the 14 mutations in EXT1 gene, 7 were novel, and regarding the EXT2 gene, and 4 of 5 were novel. **Conclusion:** This work represents the first clinical, biochemical, and molecular research on multiple hereditary osteochondromatosis (EXT1/EXT2-CDG) in Latin American patients. In all, 33 unrelated patients were studied, of which 27 presented with MO. A mutant allele was identified in 19 of these 27 patients. The remainders were undiagnosed at molecular level. EXT1 is responsible for ~65% to 75% of MO cases. New studies are focusing on the molecular basis, raising more questions concerning the pathogenesis of the disease.

086 - Neuroimaging Evolution in a Patient With Type I Glutaric Aciduria in Treatment Since 7 Months of Age

L. N. Correa[1] and H. Baron[2]

[1]Neuróloga Infantil, Especialista en Bioética, Fundación Hospital Universitario de la Misericordia, Bogotá, Colombia

[2]Medico Cirujano, Fundación Hospital de la Misericordia, Universidad del Rosario, Bogotá, Colombia

Introduction: Glutaric aciduria type 1 is a leukodystrophy. The MRI features can be characteristic and help to establish an early diagnosis. **Objectives:** A clinical and IRM correlation is performed chronologically after the initiation of the treatment. A direct relationship between imaging and clinical findings has not been described. The IRM evolution and clinical correlation of a patient in current treatment is described since 7 months of age, after presenting his first metabolic crisis. **Clinical Course:** Macrocephaly at birth, first metabolic crisis at 4 months, dystonia, and previously acquired motor abilities regression. At 7 months, cerebral IRM frontal and opercular hypoplasia with temporal arachnoid cysts, bilateral periventricular leukoencephalopathy, hyperintensity in striated nucleus (t2) were present, hence biochemical confirmation of the diagnosis. Treatment with GAC Med A (formula with free lysine amino acids, low tryptophan), at second year GAC Med B, L carnitine, riboflavin, metabolic rescue treatment with D Ribose 16 months resulted in cerebral parenchyma volume loss, leukoencephalopaty progression, and unchanged basal nucleus. At 3 years, epileptic crisis during a febrile episode was observed. No neuroimaging deterioration was observed. Progressive acquisition of motor development, independent walking, and generalized moderated dystonia. Cognitive and language function are normal as well as nutritional status weight and height. Monitoring has been performed with amino acid and acylcarnitines measurements by mass spectrometry in tandem. **Conclusion:** After first metabolic crisis, leukodystrophy, and changes in the striated nucleus signal were observed. In the following 2 years, leukodystrophy showed slow progression but the basal nucleus lesions remain equal. Clinically, there is a progressive improvement in the neurodevelopmental motor parameters at 3 years of age, without cognitive deficit or dystonia deterioration.

087 - Niemann-Pick C Disease: Presentation of a Case in Cartagena Colombia

M. Barboza[1], C. Alvear[1], and H. Vizcaino[1]

[1]Universidad de Cartagena, Cartagena de Indias, Colombia

Introduction: Niemann-Pick Type C (NPC) is a lysosomal disease with neurodegenerative compromise, characterized by accumulation of unesterified cholesterol and glycosphingolipids in many tissues, including the brain. It is inherited in an autosomal recessive manner and is associated with mutations of NPCI and NPC2 genes. **Objective:** To present clinical and biochemical characteristics of a patient diagnosed with NPC in the city of Cartagena. **Clinical Case Report:** A 2-year-old boy, that assisted to neuropediatrics evaluation for the first time at 7 months of age because of generalized hypotonia, psychomotor and language delay. He was in an intensive care unit for respiratory distress, apnea, tracheomalacia and gastroesophageal reflux. Also, supranuclear palsy of vertical gaze was detected. For this reason, skin biopsy was taken for filipin staining to rule out NPC. **Results:** Filipin test was positive, which is considered key to the diagnosis. However, it is necessary to note that there are a variety of clinical phenotypes and biochemical variants. This child presented with neurologic symptoms early, so we think it is an early infantile form. Supranuclear palsy of vertical gaze, is a cardinal symptom of NPC, and has been described in all subtypes regardless of age. Improvement has been observed in horizontal movements after miglustat treatment, which would explain the positive evolution of this patient. **Conclusion:** The child is in a comprehensive rehabilitation program. Genetic study to determine and classify the mutation and risk evaluation of other affected family members are still pending.

088 - Nonketotic Hyperglycinemia: A Purpose of 3 Cases in Venezuela

J. Moreno[1], C. L. Dominguez[1], A. Mahfoud[1], and J. Castro[2]

[1]Unidad de Errores Innatos del Metabolismo-Fundaciòn Instituto de Estudios Avanzados-IDEA

[2]Servicio de Pediatría y puericultura-Hospital Militar Dr Carlos Arvelo, Caracas, Venezuela

Nonketotic hyperglycinemia (HNC) is an inherited metabolic disease disorder, autosomal recessive, with severe neurologic manifestations predominantly in the neonatal period due to a defect in the enzyme complex of glycine, leading to toxic accumulation, responsible for the clinical symptoms. Glycine encephalopathies are characterized by convulsions (myoclonus, focal spasticity), lethargy, severe hypotonia, and apnea. The aim is to show the need for a patient with early-onset epileptic encephalopathy without relevant background, they be set to the curriculum discarding this entity. We present 3 cases, 2 neonates, and an infant referred for metabolic studies to present diverse semiology seizures refractory to treatment. Concomitantly showed marked hypotonia, psychomotor retardation, neurosensory deficits (auditory and visual), and one of them had apneas. In the brain magnetic resonance imaging, cortical atrophy and hypoplasia of the corpus callosum were evident, and the electroencephalogram showed slow basic pattern and disorganization, with paroxysmal specified activity in the 3 patients. Amino acid analysis in plasma and cerebrospinal fluid (CSF) as determined by high-performance liquid chromatography reported elevated levels of glycine in both fluids in 2 patients and glycine CSF–plasma elevated ratio in all patients. A protein-restricted diet supplemented with special formulas was indicated. It is necessary in all patients with epileptic encephalopathy to determine whether the cause is metabolic, as there are entities susceptible to treatment, which leads to a better quality of life for the patients and moreover provide for genetic counseling.

089 - Nutritional Management of a Patient With Galactosemia: 20 Years of Experience in Cuba

U. H. Carrillo[1], A. Blois[1], L. Henriquez[1], E. B. Hernandez[1], and G. M. Zayas[2]

[1]*Hospital Pediátrico Universitario Pedro Borras Astorga*
[2]*Instituto de Nutrición e Higiene de los Alimentos*

Introduction: Galactosemia is an inborn error of metabolism that affects 1 of 40 000 newborns worldwide; if the diagnosis is not established in the early stages of life, clinical manifestations may be severe, sometimes lethal. **Objective:** To characterize the nutritional status of patients at the moment of diagnosis, 1 month after nutritional treatment initiation, and up to 19 years, by means of anthropometric, clinical, and biochemical evaluation. **Methodology:** An observational descriptive and retrospective study was carried out in 13 patients diagnosed and followed from March 1988 to March 2012. Parameters analyzed were age, sex, weight, height, and brachial circumference. Values were compared with the Cuban reference charts to assess the nutritional status state to the diagnostic one and evolutionarily as well as the clinically most important manifestations in those that it was necessary age at the beginning of the first symptoms; biochemical indicators to the beginning of the illness and evolutionary, including the regimen therapeutic diet to continue after the diagnosis. **Results:** The sex feminine 61.5% prevailed. The clinically most frequent manifestations were diarrhea 61.5%, letargia and crisis of hypoglycemia 15.4%, hepatic cholestasis 7.7%, and cataract 15.4%. Most (69.2%) had committed its nutritional state for defect to the diagnosis. When imposing regimen therapeutic diet, a remarkable improvement in their square clinical 100% it was appreciated; anthropometric 84.6% and biochemical 100%. **Conclusion:** With a nutritional precocious and appropriate handling, a satisfactory evolution of the pathology is achieved.

090 - Organic Acidurias Diagnosis: Panoramic View From a Reference Center

M. J. Guevara[1], Y. O. Echeverri[1], Y.A. Ardila[2], F. N. Pulido[2], E. Espinosa[3], L. Cabarcas[2], and L. A. Barrera[1,2]

[1]*Instituto de Errores Innatos del Metabolismo, Pontifcia Universidad Javeriana, Bogota, Colombia*
[2]*Hospital Universitario San Ignacio, Bogota, Colombia*
[3]*Hospital Militar Central, Universidad Militar Nueva Granada, Bogota,. Colombia*

Introduction: Organic acidurias (OA) are rare diseases with a broad spectrum of onset ages and clinical manifestations, which in general are bizarre, making its diagnosis a difficult task for clinicians. **Aim:** Here, it is shown the summary of the Colombian experience in the diagnosis of OA during the past 15 years in the Institute for the Study of Inborn Errors of Metabolism, related to cases diagnosed, performance, difficulties, and challenges. **Methodology:** The biochemical diagnosis of these entities is done by demonstrating abnormal profiles of urinary organic acids excretion by gas chromatography/mass spectrometry (GC/MS). **Results:** Throughout these 15 years, we have observed a gradual increment in the OA analysis requested that allowed confirmation of nearly 100 cases of OA corresponding to approximately 3% of total processed samples. Isovaleric aciduria (IVA), methylmalonic aciduria (MMA), propionic aciduria (PPA), and glutaric aciduria (GA) are the most frequently diagnosed OA corresponding to more than 60% of the cases. Additionally, 10% of the OA diagnosed were infrequent entities such as piroglutamic aciduria, 2-hydroxyglutaric aciduria, methylglutaric aciduria, deficiency of succinil-semialdehyde dehydrogenase, mevalonic-aciduria, and 3-hydroxy-3-methyl-glutaric-aciduria. Most of the diagnoses corresponded to acute presentations in patients within the first year of life, being particularly severe even lethal in patients with IVA and PPA. In older children, the main characteristic is the neurological involvement. **Conclusion:** It is important to recognize the main organic acidurias affecting Colombian population and the clinical characteristics in order to improve the diagnostic protocols to avoid diagnostic delays that compromise quality of life in our patients.

091 - Organic Acidurias Diagnostic Pitfalls: Colombian Experience With Amino Acid-Related Organic Acidurias

Echeverri Olga Yaneth[1], Guevara Johana Maria[1], Pulido Ninna Fernanda[2], Y. A. Ardila[2], Cabarcas Lisseth[2], Espinosa Eugenia[3], and L. A. Barrera[1,2]

[1]*Instituto de Errores Innatos del Metabolismo, Pontificia Universidad Javeriana, Bogota, Colombia*
[2]*Hospital Universitario San Ignacio, Bogota, Colombia*
[3]*Hospital Militar CEntral, Universidad Nueva Granada, Bogota, Colombia*

Introduction: Diagnosis of organic acidurias (OA) is based on qualitative analysis of urinary organic acid profile by gas chromatography–mass spectrometry (GC-MS), which is considered the confirmatory test. Correct results interpretation depends on identification of a profile rather than presence of individual metabolites. This is particularly important for OA related to amino acid metabolism such as methylmalonic aciduria (MMA), glutaric aciduria (GA), propionic aciduria (PA), and isovaleric (IVA). **Objective:** To present the main difficulties found in amino acid-related OA diagnosis in the institute for the study of inborn errors of metabolism. **Methods:** Retrospective analysis of abnormal results obtained in samples submitted for organic acids analysis by GC-MS. **Results:** In our institution, nearly 100 OA have been diagnosed, based on classic profile detection or nonclassic abnormal profiles detected in more than 1 sample in patients with compatible clinical manifestation and treatment response. The main difficulty identified was lack of clinical–biochemical

correlation resulting in misinterpretation of chromatographic profiles. This is especially important when there is the presence of metabolites associated with OA, such as MMA, GA, PA, and IVA, considering that some of them are present in normal profiles and secondary to other clinical conditions. Taking this into account, diagnostic utility of some metabolites depended on patient's clinical condition, age, and presence of biochemical-related metabolites. **Conclusion:** The GC-MS qualitative analysis results must be interpreted according to the patient's clinical context, and for some cases, a long-term follow-up and even molecular studies may be required to define diagnosis.

092 - Ornithine Transcarbamylase Deficiency: Identification of Mutations, Computational Validation, and Phenotypic Correlation in Argentinian Patients

S. M. Silvera-Ruiz[1], A. Arranz-Amo[2], R. Dodelson de Kremer[1], and L. E. Larovere[1]

[1]Centro de Estudio de las Metabolopatías Congénitas, Hospital de Niños de Córdoba, Fac. De Cs. Médicas, UNC, Córdoba, Argentina
[2]Unitat de Metabolopaties, Hospital Universitari Materno-Infantil Vall d'Hebron, 08035 Barcelona, España

Introduction: Ornithine transcarbamylase deficiency (OTCD; *Online Mendelian Inheritance in Man* [OMIM] 311250) is an urea cycle defect with X-linked inheritance. In hemizygous males, neonatal or late onset depends on the degree of residual enzymatic activity. In heterozygous females, symptom presentation depends on X-chromosome inactivation. Mutation identification in OTC gene allows diagnostic confirmation and carrier detection. **Objective:** To identify mutations causing OTCD in Argentinian patients, to validate those changes, and to correlate them with phenotype. **Methods:** A total of 11 patients belonging to 8 families, 6 male patients, 2 with severe presentation and death during the neonatal period and 4 with late-onset (0.5-10 years) and 5 symptomatic women (0.8 to 4 years), 3 of them died and were diagnosed with OTCD. Molecular analysis of OTC gene was performed by polymerase chain reaction/multiplex ligation-dependent probe amplification/Single-strand conformation polymorphism analysis and/or sequencing, and missense changes validation was made using computational methods, PolyPhen, SIFT, and PopMusic 2.0. **Results:** We identified mutations in all patients; 2 were not previously described: 1 of splicing (c.540+1G>A) and a deletion (delExon 2-10) and 6 were already reported: 1 of splicing (c.216+1G>A) and 5 missense (p.Arg129His, p.Leu151Arg, p.Thr178Met, p.Ala208Thr, and p.Arg277Trp). Result validation was consistent with the applied computational programs and the patients' presentation form. **Conclusion:** This analysis provides a better understanding of alterations responsible for the phenotypic expression. This work expands carrier detection capability allowing appropriate genetic counseling. Early detection of patients with OTCD is essential to reduce morbidity and mortality in affected individuals.

093 - Overgowth in the First Year of Life: An Early Sign of Mucopolysaccharidosis

G. Napy, M. Gurjão[1], H. A. Melo[1], A. D. A. Moreira[1], C. Eufrazino[1], and P. F. V. Medeiros[1]

[1]Universidade Federal de Campina Grande/Pb, Brazil

Introduction: Child's growth is one of the best indicators of overall health, and it can be impacted by chronic diseases such as mucopolysaccharidosis (MPS). Paradoxically, growth in the first years of life in some types of MPS tends to be accelerated. Patients with MPS appear healthy at birth. The initial signs and symptoms in most patients with MPS have been identified after 2 years, which delays diagnoses. **Objective:** The purpose of this study was to analyze growth in the first year of life in patients with MPS VI, IVA and IIIC as an early sign of the disease. **Methodology:** A total of 8 patients (4 boys, 4 girls), 6 with MPS IVA (4 boys, 2 girls), 1 MPS VI (girl), and 1 MPS IIIC (girl) with at least 5 length measurements in the first year of life, were identified. The length data were obtained from their medical records. The patients' data were plotted on the World Health Organization growth chart including diverse ethnic backgrounds. **Results:** Length in girls, the curve was above P97 in 2 of 4 patients (MPSIVA, MPSVI), between P95 and P97 in 1 of 4 (MPSIIIC), and between P50 and P85 in 1/4 (MPSIVA). Length in boys: of 4 patients with MPSIVA the curve was above the P97 in 2 of 4 patients, between P95 and P97 1 of 4 patients, and between P85 and P95 in 1 of 4 patients. **Conclusion/Discussion:** The MPS is a diagnosis that should be taken into consideration when differentiating the causes of overgrowth in the first year of life in order to improve the early diagnosis and treatment of MPS.

094 - Overview of the First 3 Years of Operation (2010-2012) of the Niemann-Pick Type C Brazil Network

R. Giugliani[1,2,3], A. Brites[1], C. L. Rafaelli[1,2,3], E. Bohn[1], K. Michelin-Tirelli[1], M. Burin[1], H. Bock[1], M. Polese[1], M. C. Matte[1], M. L. Saraiva-Pereira[1,4], R. Gus[1], and F. T. S. Souza[1,4]

[1]Medical Genetics Service, HCPA, Porto Alegre, Brazil
[2]Department of Genetics, UFRGS, Porto Alegre, Brazil
[3]INAGEMP, Porto Alegre, Brazil
[4]Department of Biochemistry, UFRGS, Porto Alegre, Brazil

Niemann-Pick type C is an autosomal recessive inborn error of cholesterol trafficking, characterized by the storage of cholesterol inside the lysosomes, which leads to a wide range of clinical manifestations, usually involving the central nervous system. As there is a specific treatment already approved and several therapeutic strategies in development, diagnosis is

becoming increasingly important. However, identification of this disease is challenging, as signs and symptoms overlap with many other conditions and as the standard diagnostic method (Filipin test) is a qualitative assay performed in growing fibroblasts, which requires the collection of a skin biopsy. To help the identification of affected patients, we built a program called ''NPC Brazil Network'' in Brazil, available for medical doctors across the country. From 2010 to 2012, we received clinical information and biological samples (blood and/or skin biopsy) from 444 suspected patients and were able to obtain suitable fibroblasts to perform the Filipin test in 296 cases. From these, 50 (17%) had a positive result, 58 (20%) were inconclusive, and 188 (63%) were negative. Patients with positive and inconclusive results in the Filipin test (and also patients with negative results but with strong clinical suspicion) were further studied by molecular analysis of NPC1 and NPC2 genes. Along the first 3 years of activity, the NPC Brazil Network proved to be a valuable resource for the identification of patients with NPC in Brazil.

095 - Phenotype–Genotype Correlation in Mucopolysaccharidosis IV A Using Bioinformatics Tools

S. Olarte-Avellaneda[1,2], A. Rodriguez[2], C. J. Almeciga-Diaz[2], and L. A. Barrera[2]

[1]Programa de Bacteriología y laboratorio clínico, Facultad de las ciencias de las salud, Universidad Colegio Mayor de Cundinamarca, Bogotá DC, Colombia
[2]Instituto de Errores Innatos del Metabolismo, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá DC, Colombia

Introduction: Heterogeneity of mutations in human N-acetylgalactosamine-6-sulfate sulfatase enzyme (GALNS) has not enabled to establish a complete genotype–phenotype correlation for Morquio A. **Objective:** In this study, we carried out an in -silico evaluation of GALNS mutations to attempt the prediction of the disease severity. **Methodology:** Human GALNS was compared against GALNS from other species and against other human sulfatases. Tertiary structures were modeled, and missense mutations were included by computational site-directed mutagenesis. **Results:** Active cavity was highly conserved among studied sequences and showed a preferential positive charge, which correlates with the negative charge of GALNS substrates. In most cases, severe mutations were associated with changes in highly to completely conserved residues, nonconservativeness changes, and low values of Atomic Accessible Surface Area. On the other hand, attenuated mutations seem to be associated with null to intermediate conservation, conservativeness changes, surface position, high values of Atomic Accessible Surface Area, and energy minimization close to or lower than that of the wild-type enzyme. Molecular docking showed that GALNS has higher affinity for natural substrates than for the artificial one, and all mutations within the active site reduced the enzyme affinity for substrates. Other enzymes studied showed lower affinity for natural substrates than that observed with human GALNS. **Conclusion:** Overall, an update in genotype–phenotype correlation for Morquio A from a computational biology approach is proposed, in which multiple parameters must be analyzed for the prediction of disease severity.

096 - Physiologic Colocalization of Human Methylmalonyl CoA Mutase With MMAA Proteins and an In Silico Proposed Model of Their Interaction Domains

T. Takahashi-Iñiguez[1], A. Gonzalez-Noriega[2], C. Fernandez-Lainez[3], L. Dominguez[4], C. Michalak[2], M. Vela-Amieva[3], and M. E. Flores[1]

[1]Departamento de Biología Molecular y Biotecnología Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, México
[2]Departamento de Biología Celular y Fisiología Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Ciudad de México, México
[3]Laboratorio de Errores Innatos del Metabolismo y Tamiz Instituto Nacional de Pediatría Secretaría de Salud, Ciudad de México, México
[4]Departamento de Ciencias de la Salud, Universidad Autónoma Metropolitana-Lerma, Lerma de Villada, México

Introduction: Human homodimeric methylmalonyl-CoA mutase (hMCM) converts methylmalonyl-CoA to succinyl-CoA in mitochondria using adenosylcobalamin (AdoCbl) as cofactor. During catalysis, deoxyadenosyl radical is generated making hMCM susceptible to oxidation. Human methylmalonic aciduria type A (MMAA) protein (hMMAA) acts as a chaperone of hMCM in a guanosine-5′-triphosphate-dependent manner. Neither mitochondrial location of hMMAA and its in vivo interaction with hMCM has been demonstrated nor a model of their interaction domains has been proposed. **Objective:** To demonstrate in vivo hMMAA mitochondrial location and its colocalization with hMCM and to construct an in silico model of the interaction between these 2 human proteins. **Methods:** Complimentary DNA of both human genes was cloned and recombinant proteins were purified. These proteins were used to produce antibodies in rabbit; serum obtained was used for double immunofluorescence experiments in human fibroblasts. For in silico modeling, 1 hMCM dimer and 2 hMMAA dimers were submitted to GRAMM-X for docking. Reported mutations that possibly affect this putative interaction were mapped in order to validate the model proposed. **Results:** The hMMAA and hMCM proteins were found in mitochondria and cytoplasm. Colocalization was only observed in mitochondria. On the other hand, docking analysis showed that hMCM interacts with hMMAA through its AdoCbl and GTP-binding domains, respectively; the existence of mutations in those interaction regions validated this heteroligomer. **Conclusion:** We demonstrated that both human proteins were present and

colocalized in mitochondria. We proposed that hMCM and hMMAA interact with each other by their cofactor domains.

097 - Population-Based Study of New Mutations Causing Sandhoff Disease in Argentina

J. Mugnaini[1], A. Dardis[2], N. B. Azar[1], A. B. Becerra[1], C. A. Amorosi[1], S. Zampieri[2], N. Guelbert[1], R. Dodelson de Kremer[1], and A. M. Oller-Ramirez[1]

[1]*Centro de Estudio de las Metabolopatías Congénitas (CEMECO), Cátedra de Clínica Pediátrica, Facultad de Ciencias Médicas, UNC, Hospital de Niños, Córdoba, Argentina*
[2]*Centro Coordinador Regional de Enfermedades Raras, Hospital Universitario "Santa Maria della Misericordia", Udine, Italia*

Background: Sandhoff Disease (SD) is a lysosomal storage disorder caused by mutations in the HEXB gene. A high incidence of SD has been described in an Argentine region called "Valle de Traslasierra." Mutations c.445+1G>A and p.S261Cfs12X were found in 98.7% and 1.3% of mutant alleles, respectively. In previous population-based studies, the carrier frequency has been estimated to be 1 in 16 to 29, all heterozygous with c.445+1G>A. Recently, we detected new mutations in 5 Argentinian patients: c.1082+5G>A, c.1242+1G>A, c.1451G>A (p.Gly484Glu), c.1597C>T (p.Arg533Cys) and c.1601G>A (p.Cys534Tyr). **Objective:** To study the heterozygote frequency for new mutations in the population at risk. **Material and Methods:** Blood samples were obtained from 200 healthy patients born in the region. Mutation analysis was performed by polymerase chain reaction/sequencing of specific DNA sequences. **Results:** We found 9 carriers of c.445+1G>A and none of other mutations among healthy patients (c.1082+5G>A has not yet been investigated). **Conclusion:** These results suggest that the frequency for new mutations is very low and confirm the role of c.445+1G>A as a founder mutation in the population at risk.

098 - Positive Pressure in the Treatment of Signs and Symptoms of Alveolar Hypoventilation and Sleep Disorders in Mucopolysaccharidosis

L. A. F. Tavares[1], J. S. Filho[1], M. G. Fagundes[2], and M. C. Alves[3]

[1]*Hospital Infantil João Paulo II/Fundação Hospitalar do Estado de Minas Gerais, Minas Gerais, Brazil*
[2]*Governo de Minas Gerais*
[3]*Associação Mineira dos Portadores de Mucopolissacaridoses*

Introduction: Mucopolysaccharidosis Home Care and Assistance Program (PMPS) of Children's Hospital John Paul II (CHJPII)/Hospital Foundation of the State of Minas Gerais (FHEMIG) provide assistance at home, and during outpatient admissions, to patients with MPS with cardiorespiratory involvement (CCR). As CCR worsens (decrease in vital capacity), it is evident in alveolar hypoventilation (HA) and sleep disorders (SD), first at night and the during the day. Cardiorespiratory signs and symptoms (SS) suggest AH and SD mainly dyspnea, airway hypersecretion, pulmonary hypertension, heart failure, difficult compensation, and intolerance to exercise. Treatment for these SS include establishment of positive pressure (PP) maneuvers through the air stacking (MAS) with a manual bag and mechanical ventilation (MV) performed regularly. **Objective:** To describe the results of the institution of PP in the treatment of hypertension in patients with DS and MPS accompanied by PMPS Program/CHJPII/FHEMIG. **Patients and Methods:** A quantitative, descriptive, observational, and retrospective study was held at CHJPII/FHEMIG/SES. Data were collected between 2007 and 2013. All patients accompanied by the Programs were included. **Results:** A total of 44 patients were followed (MPS types: 9 I, 9 II, 3 IIIa, 1 IIIb, 2 IVa, and 20 VI). Seventeen patients were diagnosed with AH and SD. Seventeen VM home users, perform 8 MEA. Median follow-up in Programs: 4.5 years. The SS shown before and after administration of PP: no patients (17×0), number of SD (median), (10×0), use of medications for treating SS (no patients; 17×4), hospitalization for SS (no patients; 9×0), deaths from SS (17×2). **Conclusion:** PP contributes to HA and SD treatment of SS patients with MPS.

099 - Preliminary Findings Evaluating Safety and Efficacy of Recombinant Human N-Acetylgalactosamine-6-Sulfatase (RHGALNS) in Pediatric Patients Less Than 5 Years of Age With Mucopolysaccharidosis IV A (Morquio A Syndrome, MPS IVA)

R. Giugliani[1], S. Jones[2], P. Harmatz[3], M. Bialer[4], R. Parini[5], K. Martin[6], P. Farmer[6], P. Slasor[6], and C. Haller[6]

[1]*Department of Genetics/UFRGS and INAGEMP, Medical Genetics Service/HCPA, Porto Alegre, Brazil*
[2]*St Mary's Hospital, CMFT, University of Manchester, MAHSC, United Kingdom*
[3]*Children's Hospital & Research Center, Oakland, California, USA*
[4]*North Shore LIJ Health System, Manhasset, New York, USA*
[5]*Az Ospedaliera S. Gerardo, Monza, Italy*
[6]*BioMarin Pharmaceutical Inc, Novato, California, USA*

Preliminary results after 26 weeks of treatment from an ongoing study evaluating safety and efficacy of RHGALNS in 15 patients with MPS IVA <5 years of age are reported. The mean (range) age was 3.1(0.8-4.9) years. Standing height/length ($n = 15$) was severely affected in many patients; 7 (46.7%) at <third, 3 (20.0%) at >3rd to <10th, 2 (13.3%) at >25th to <50th, and 3 (20.0%) at $\geq$50th percentiles. The most commonly reported adverse events (AEs) were vomiting in 12 (80.0%), pyrexia in 11 (73.3%), and cough in 8 (53.3%) patients. The majority of AEs were mild to moderate with 1 severe event of tonsillar hypertrophy. No patients discontinued due to an AE. The RHGALNS treatment had a similar safety

profile as seen in older children and adults. Normalized urine keratan sulfate (uKS) was increased with a mean (range) of 35.9 (18.8-56.5) μg/mg creatinine (n = 15). In 8 patients with 26 weeks of data, RHGALNS led to a substantial decrease in mean (± standard deviation [SD]) normalized uKS by 30.5% (±15.49%) after 2 weeks and sustained at −35.2% (±15.57%) at 26 weeks. Mean height/length for age *z*-scores didn't demonstrate significant change from baseline (1.8 SD) to week 26 (−2.2 SD) for these 8 patients. Anthropometrics will continue to be assessed in all patients to determine impact of RHGALNS intervention on long-term growth.

100 - Production of an Active Recombinant Human N-Acetylgalactosamine-6-Sulfate Sulfatase Enzyme in *Pichia Pastoris*

A. Rodriguez[1], J. Moreno[1], J. Sanchez [1], D. Diaz[1], L. Beltran[1], A. Espejo[1], C. J. Almeciga-Diaz[1], and L. A. Barrera[1]

[1]Instituto de Errores Innatos del Metabolismo, Facultad de Ciencias, Pontificia Universidad Javeriana, Bogotá DC, Colombia

Introduction: Morquio A disease is produced by the deficiency of N-acetylgalactosamine-6-sulfate sulfatase (GALNS) enzyme. **Objective:** In this study, it was evaluated the production of recombinant GALNS in the yeast *Pichia Pastoris* as an alternative to produce a recombinant GALNS. **Methodology:** Human GALNS complimentary DNA (cDNA), with native or heterologous signal peptides (SP), was subcloned into pPIC9 plasmid and transformed in *P pastoris* GS115. *P pastoris* was also cotransformed with GALNS cDNA and human SUMF1 cDNA. Production was carried out at 0.01, 0.1, and 1.5 L scales. **Results:** Recombinant GALNS was detected only extracellulary. The GALNS activities were up to 0.18 and 0.29 U/mg for the strains with native or heterologous SP, respectively. Coexpression with SUMF1 allowed a 7.5-fold increment in enzyme activity, showing for the first time the advantage of SUMF1 coexpression within a yeast system. Western blot analysis showed the presence of a 120-kDa protein, which was processed to 50- and 30-kDa peptides. **Conclusion:** In summary, these results show the feasibility for the production of an active recombinant GALNS enzyme in *P pastoris*, which could be used in the development of an enzyme replacement therapy for Morquio A.

101 - Progress in the Molecular Characterization of BTD Gene in Argentine Patients With Biotinidase Deficiency: Identification of a Novel Complex Allele

C. J. Angaroni[1], L. P. Hill[1], M. Monasterolo[1], A. J. Badino[1], N. Guelbert[1], M. Nuñez[2], N. B. Specola[2], L. Muschietti[2], G. J. C. Borrajo[3], and R. Dodelson de Kremer[1]

[1]Centro de Estudios de las Metabolopatías Congénitas (CEMECO), Hospital de Niños de la Santísima Trinidad, Facultad de Ciencias Médicas, UNC, Córdoba, Argentina
[2]Hospital de Niños Sor María Ludovica, La Plata, Argentina
[3]Fundación Bioquímica Argentina, La Plata, Argentina

So far, 139 pathogenic variations responsible for profound and partial biotinidase deficiency (BD) have been registered for BTD gene. Of them, 15 were found to be complex alleles and 5 variations are responsible for 60% of the BD, including the p.Asp444His change, which is associated with partial BD. **Objective:** To continue with BTD gene variant identification in Argentinian patients and determine carrier status in members of the families involved. **Patients:** Twelve individuals with BD detected by newborn screening and 15 members of the families involved were included. **Methods:** Determination of plasma biotinidase activity. Molecular analysis of the BTD gene. **Results:** Enzymatic determination identified patients with profound (7) and partial (5) BD. Molecular analysis of BTD gene in patients showed the following variations: c.98_104del7ins3 (41.7%), p.Gln456His (16.6%), p.Asp444His (16.6%), p.[Ala171Thr;Asp444His] (12.5%), c.933delT (4.2%), p.Val199-Met (4.2%), and the novel complex allele p.[Arg209Cys;-Tyr540Cys] (4.2%). Parental transmission of mutant alleles was established in 6 probands. Additionally, 1 heterozygous and 2 normal homozygous were identified in 3 unrelated families. Two nonfunctional polymorphisms were detected in 3 different families, p.Pro391Ser and c.1413T>C. Patients with partial BD were compound heterozygous for p.Asp444His variation. The molecular basis of BD in Argentina has not yet been established. This study, which pretended to initiate a BD database in Argentina, allowed identification of 100% of disease-causing mutations in the studied individuals as well as detection of a new complex allele.

102 - Propionic Aciduria: A Strange Clinical Presentation

R. Uicich[1] and R. Rodriguez[1]

[1]Laboratorio Nutrición y Metabolismo, Hospital de Pediatría "Garrahan" Buenos Aires, Argentina

Propionic aciduria (Propionyl-Coa Carboxylase deficiency), which has a neonatal onset, can present with feeding problems, vomiting, hypotonia, neurological deterioration, metabolic acidosis, and hyperammoniemia. Late onset could present, apart from vomiting with lethargy, with coma. Chronic presentation includes anorexia, vomiting, and neurologic disorders. Here, we expose a female case, 1.2 years, who, from 27 days of life, required hospitalization for various reasons (urinary infections, bronchitis). At 9 months, afebrile seizures with normal electroencephalogram were detected. From 1 year of age, loss of motor patterns acquired and an increment in the number of seizures was observed. She has never presented acidosis, hypoglycemia, hyperlactacidemia, hyperammoniemia, or neutropenia. Organic acids analysis showed elevation in 3-hydroxy-propionic, 3-hydroxy-2- methylbutyric, and methylcitric

and acylcarnitines: C3 elevated (5.07 μmol/L VR <2.5) and C0 low (5.44 μmol/L VR >11) It is assumed as probable propionic acidemia and started treatment. The RMN showed left hippocampus hypotrophy, bifrontotemporal cortical atrophy, thinned corpus callosum, and ventriculomegaly. After 15 days repeat of organic acids analysis showed 3-hydroxyl-propionic and methylcitric and acylcarnitines: C3: 5.88 μmol/L and C0: 41.05 μmol/L. A week after, organic acid analysis showed mild 3-hydroxyl-propionic and absence of methylcitric. This case exemplifies the diversity of clinical spectrum in propionic acidemia, demonstrating the complexity of clinical suspicion and the usefulness of the specific biochemical studies in a patient with progressive neurologic deterioration of uncertain cause.

103 - Psychosis and Hepatitis in an Adolescent With Niemann-Pick Type C Disease

A. Fajardo[1], J. F. Vera-Chamorro[2], G. Casas[3], L. K. Ono[4], R. Milanes[5], and R. Lopez[6]

[1]Neuróloga pediatra, Fundación Santa Fe de Bogotá, Universidad de los Andes, Bogotá DC, Colombia
[2]Gastroenterólogo pediatra, Fundación Santa Fe de Bogotá, Universidad de los Andes, Bogotá DC, Colombia
[3]Psiquiatra infantil. Fundación Santa Fe de Bogotá, Universidad de los Andes, Bogotá DC, Colombia
[4]Pediatra, Pontificia Universidad Javeriana, Bogotá DC, Colombia
[5]Pediatra, Universidad de la Sabana, Bogotá DC, Colombia
[6]Patóloga, Fundación Santa Fe de Bogotá, Universidad de los Andes, Bogotá DC, Colombia

Introduction: Niemann-Pick type C disease (NPC) is an autosomal recessive disorder characterized by accumulation of sphingomyelin and other lipids. There are 8 reported cases of psychosis and NPC, 2 in teenagers. **Objectives:** Report the case of a teenager who presents with neuropsychiatric manifestations and hepatitis. **Materials and Methods:** Case report of the Hospital Universitario Fundación Santa Fe de Bogotá (HU-FSFB). Light microscopy in liver biopsy, Filipin staining in skin biopsy, and molecular study were performed. **Results:** A male patient, 15-year-old, previously healthy and with no family history and a psychotic disorder that occurred a month earlier, hospitalized in psychiatric clinic, where he manifests abdominal pain and by increased aminotransferases, is referred to the HU-FSFB. Physical examination: weight 63.5 kg; height 1.71 cm (−0.2 DS); body mass index 21.7 kg/m^2 (0.6 DS); blood pressure 135/90 mm Hg; behavioral and psychotic changes; limitation of vertical gaze upward and hyperreflexia. Paraclinical tests: aspartate aminotransferase 136 IU/L (<31); alanine transaminase 168 IU/L (<36); total bilirubin 0.76 mg/dL; alkaline phosphatase 90 IU/L; international normalized ratio 1.1; bicarbonate 22.3 mmol/L; ammonium 39 μg/dL; urine copper 30.32 μg/24 hours; lactic acid 0.78 mmol/L; lipid profile, infectious, serologic, autoimmune, toxicological, and heavy metal studies were normal. Liver biopsy reported severe steatosis. Filipin staining in skin biopsy reported intense perinuclear fluorescence in the fibroblasts analyzed, which is associated with the classic form of NPC. He was started on miglustat (Zavesca) 600 mg/d. Molecular study of the NPC2 gene was requested for patient and parents. **Conclusion:** We describe a case of a Colombian teenager with NPC. It should be suspected in liver dysfunction and neuropsychiatric manifestations.

104 - Qualitative Urinary Organic Acid Profile: Difficulties in the Interpretation

O. Y. Echeverri[1], J. M. Guevara[1], N. F. Pulido[2], Y. A. Ardila[2], L. Cabarcas[2], E. Espinosa[3], and L. A. Barrera[1,2]

[1]Instituto de Errores Innatos del Metabolismo. Pontificia Universidad Javeriana, Bogotá, Colombia
[2]Hospital Universitario San Ignacio, Bogotá, Colombia
[3]Hospital Militar Central - Universidad Nueva Granada, Bogotá, Colombia

Introduction: Gas chromatography/mass spectrometry (GC/MS) is the gold standard technique for analysis of organic acid in urine for diagnosis of organic acidurias. The institute for the study of inborn errors of metabolism implemented such analysis more than 15 years ago using a qualitative interpretation scheme. Such scheme is based in abnormal metabolites identification or supraphysiological increases in normal metabolites among a profile including more than 100 metabolites derived from normal human metabolism. Additionally, in urinary organic acid analysis, many compounds derived from diet and medications could be detected. These compounds may interfere with identification and detection of abnormal metabolites. **Objective:** To present main GC-MS qualitative profile interferences identified in our experience. **Methods:** Retrospective analysis of GC-MS profiles of samples submitted for organic acid analysis. **Results:** According to our experience 2 main classes of interference could disrupt the normal profile: the first includes presence of signals along the chromatogram overlapping the real profile, in this group the main interferences occur as a consequence of medication with anticonvulsant drugs, particularly valproic acid and phenytoin, and we also detected minor disruptions of normal profiles due to antibiotic schemes and even additives such as sweeteners. The second group implies alterations in the metabolism triggered by special supplementary diets rich in carbohydrates or lipids resulting in profiles similar to some inborn errors of metabolism, complicating the interpretation of profiles. **Conclusion:** A proper interpretation of the chromatographic profile and the ability to perform an adequate clinical –biochemical correlation requires all the information about the medication and diet of the patient at the time of the test as well as a close communication between the clinician and the laboratory in order to improve the correct diagnosis of the patient.

105 - Rapid Electrophoresis of Glycosaminoglycans in Urine of Patients With Mucopolysaccharidosis

C. E. Jaqueline[1], Ana Carolina Breier[1], and Anice Carneiro Coelho[1]

[1]UFRGS, Porto Alegre-RS, Brazil

Introduction: Mucopolysaccharidoses (MPS) are a group of lysosomal storage diseases caused by deficiency of enzymes that degrade glycosaminoglycans (GAGs) and consequently they are accumulated in the urine of patients. Currently, the screening for this disease is done in urine samples through techniques of quantification and identification of GAGs. This study aimed to establish a rapid electrophoresis of GAGs, separating and identifying the compounds that increased in urine of patients. **Materials and methods:** The GAGs were extracted and purified from urine samples of patients according to Hopwood and Harrison (1982). The samples were applied (0.5 μL) to agarose commercial gel, and the electrophoresis was performed according to Pennock's (1976) method with modifications in a total time of 2 hours. The staining was performed with toluidine blue and the gel was destained in water for 30 minutes. **Results:** We observed that migration of GAGs was excellent because it was possible to clearly observe dermatan, heparan, and chondroitin sulfate bands (MPS I and VI). Through chondroitin curve (0.25-2 mg/mL) it was possible to verify the sensitivity of the technique. **Conclusion:** This fast electrophoresis allows identifying the accumulated GAG profile in a short time, and therefore it is an interesting technique for screening for MPS since GAG excretion profile is critical to direct the enzymatic diagnosis. Moreover, among the advantages of this fast electrophoresis, we emphasize the small volume application in the gel and the low concentrations of GAG detected, which makes this technique very sensitive.

106 - Recombinant Production of Human Lysosomal β-Hexosaminidases Using 2 Strains of Pichia sp as an Expression System

Espejo-Mojica Angela Johana[1], Mosquera-Arevalo Angela Rocio[1], Diaz Sergio Andres[1], Diaz-Dennis Johana[1], Rodriguez-Lopez Edwin Alexander[1], C. J. Almeciga-Diaz[1], and L. A. Barrera[1]

[1]Instituto de Errores Innatos del Metabolismo, Pontificia Universidad Javeriana, Bogotá, Colombia

Introduction: Human lysosomal Î²-hexosaminidases belong to family 20 of glycoside hydrolases and comprises 3 dimeric isoenzymes Hexo A, Hexo B, and Hexo S. These isoenzymes are constituted by Î± and/or Î² subunits expressed by different genes. The activity of these enzymes is decreased in Tay Sachs and Sandhoff diseases. **Objectives:** In this study, the 3 isoenzymes were produced in the methylotrophic yeast *Pichia pastoris* GS115 and *Pichia pink*. **Materials and Methods:** The genes for Î± and Î² subunit without the native signal peptide were optimized and subcloned within plasmid pPIC9K for *P pastoris* and within pPink-HC for *P pink*. **Results:** In *P pastoris*, expression assays at 10, 100, and 200 mL scale were performed obtaining activity values of 1207.65, 112563.36, and 36292.06 U/mg for HexoA, Hexo B, and Hexo S, respectively, at 100 mL scale and 3570.2 U/mg (Hexo A), 40482.1 U/mg (Hexo B), and 138356.81 U/mg (Hexo S) at 200 mL scale. Extracts of 200 mL scale were filtered and concentrated by ultrafiltration processes, and enzyme activities were increased up to 2 (HexoA), 3.9 (HexoB), and 1.3-fold (HexoS), respectively. At 100 mL in *P pink*, values of 1187.17 U/mg (HexoA), 276031.45 U/mg (HexoB), and values below 1 U/mg of specific activity for HexoS were obtained. Samples were assessed by protein electrophoresis (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and Western blot. **Conclusion:** These results show the potential of *P pastoris* as an expression system for the production of lysosomal Î²-hexosaminidases and as an option for development of an enzyme replacement therapy for Tay Sachs and Sandhoff diseases.

107 - Reference Values and Cutoff Levels for Acid β-Glucosidase Activity in Dried Blood Spots on Filter Paper

M. Monaga[1], D. Campos[1], D. Herrera[2], and E. C. Gonzalez[3]

[1]Centro Nacional de Genética Médica, Habana, Cuba
[2]Instituto de Información Científica y Tecnológica, Habana, Cuba
[3]Centro de Inmunoensayo, Habana, Cuba

Objective: To establish the reference values, the cutoff level, and the influence of demographic factors on β-glucosidase activity in dried blood spots in the Cuban population. **Methodology:** Samples from 2602 newborns and 1000 healthy individuals (1-94 years) were collected. For newborn samples, a stratified selection procedure was applied. β-glucosidase activity was determined using an ultramicro-fluorescence assay developed in our laboratory. Effect of sex, birth weight, prematurity, timing age of sample, and geographical area of origin were analyzed. **Results:** Mean value of enzymatic activity was 11.24 μmol/L blood/h (reference values: 2.09-33.08 μmol/L blood/h) for newborns and 6.42 μmol/L blood/h (reference values: 2.00-24.56 μmol/L blood/h) for healthy individuals. Significant differences regarding the geographic area of sample's origin and age were obtained in newborns and healthy individuals, respectively. For healthy individuals, the cutoff value was 1.9 μmol/L blood/h. **Conclusion:** The reference values obtained allow the diagnosis of patients with Gaucher disease, in accordance with our country's specificities. β-glucosidase activity is affected by changes during collection, storage, and transportation of samples. Age effect does not interfere with the established cutoff.

108 - Relationship Between Laringeal Stridor and Biotinidase Deficiency: Case Report

M. Ramirez[1], L. Sua[1], P. Reyes[1], M. Morillo[1], J. Alvarez[1], J. Moreno[1], C. Dominguez[1], and A. Mafhoud[1]

[1]Fundación Instituto de Estudios Avanzados-IDEA, Caracas, Venezuela

Introduction: Generally speaking, seizures, hypotonia, vomiting, and respiratory conditions in infants are associated with serious infectious processes (meningitis, sepsis). However, in other patients, gastrointestinal and respiratory complications represent clinical aspects considered for differential diagnosis. Laryngeal stridor, air occlusion manifested at the level of the vocal cords, may persist without implying severe respiratory failure. This sign, together with complementary biochemical findings as hyperammonemia and metabolic acidosis, is suggestive of inborn errors of metabolism (IEM), which leads to screening tests like biotinidase determination, enzyme catalyzing cleavage of biotin (whose deficiency causes alopecia, rash, and psychomotor retardation among other features). **Objective:** To demonstrate the relationship between respiratory failure and biotinidase deficiency. **Case Description:** Here, we present the case of a patient referred to our laboratory (45 days old) with a global hypotonic disorder, chronic progressive encephalopathy, psychomotor retardation, and bronchopulmonary dysplasia. During IEM screening, a partial biotinidase deficiency (qualitative test) was detected, later confirmed by measuring serum enzyme activity (0.75 nmol/min; normal levels: 7-9.90 nmol/min). Additionally, the urine organic acids profile revealed high levels of typical markers of carboxylase deficit (3 OH isovaleric acid, phenylpyruvic, and 3-methylcrotonylglicine). Oral biotin treatment (10 mg/day) improves clinical manifestations, especially laryngeal stridor end point of patient pathology. **Conclusion:** Biotinidase deficiency should be included in the differential diagnosis of respiratory diseases of unknown etiology, although these last are related to infectious processes.

109 - Report of a Hunter Familial Syndrome

M. X. Ruiz-Flores[1]

[1]Centro de Diagnóstico de Genética Humana, Hospital Metropolitano, Quito, Ecuador

Hunter syndrome or mucopolysaccharidosis type II (MPSII) is inherited in an X-linked recessive manner due to mutations affecting the gene coding for the enzyme irudonate 2 sulfatase (IDS2), located on the long arm of X chromosome (Xq27-28). Chondroitin sulfate B, dermatan sulfate, and heparan sulfate are excreted in urine (McKusick, 1972; Wraith et al., 2008). We report a case of a 3-year-old boy. His parents are currently 35 (mother) and 36 (father) years old without consanguinity or inbreeding, and the boy was born in the fifth pregnancy. The first brother of the patient had macrocephaly, and since 6 years old, he presented with involution and loss of psychomotor skills, apnea, seizures, macroglossia, and gingival hyperplasia. He died at 12 years of age. The second sister is healthy. The third brother presented a picture similar to the first brother and died at 11 years of age. The fourth brother had hydrocephalus and died at the age of 1 year and 8 months after shunt implantation. The patient presented with positive data for Hunter disease such as macrocephaly, macroglossia, coarse facial features, sleep apnea, and delayed psychomotor retardation of language. In his evolutionary history, he had loss of skills, and macrocephaly was corroborated by anthropometric studies. Karyotype (46, XY) was normal. It was not possible to perform additional tests to confirm the diagnosis, because the patient lives in rural area and could not be located.

110 - Report of a New Chromatographic Method for the Characterization of Cystine and Homocysteine: Aplication in High-Risk Population for Metabolic Disorders

Jacqueline Benavides[1], Buitrago Luz[1], España Monica[1], and Uribe Alfredo[1]

[1]Centro de Investigaciones en Bioquímica, Departamento de Ciencias Biológicas, Universidad de Los Andes, Bogotá, Colombia

Introduction: Cystine and homocysteine are amino acids produced by the catabolism of succinyl-CoA and pyruvate, respectively. As a characteristic feature, they have disulfide bonds, which is why they react equally in chemical tests of urine screening—such as sodium nitroprusiate—generating a characteristic purple color. To discriminate the detected metabolite, the sample is evaluated with a variant of the technique that uses silver nitrite, a compound that is highly dependent on the concentration of the sample, analyzed by creatinuria. **Objective/Methods:** Here, we report the standardization of a thin-layer chromatographic method (stationary phase: silica gel, mobile phase: butanol–acetic acid–water [10:5:5], color: nihidrine [0.6%]). This method is sensitive and highly specific in the differentiation of cystine and homocysteine in urine samples. We also illustrate its application in high-risk population for aminoacidopathies, which showed positive results for oxidated *sulfhydryl* groups in a sodium *nitroprusside* test (NP). **Results/Discussion:** This study covered 963 patients, analyzed between June 2010 and 2013. Patients were referred because they had clinical findings suggesting aminoacidopathies. Age range: 30 days to 67 years (82.3% $\leq$10 years). In the test profile, 21 (2.2%) cases showed a positive result for NP. The chromatographic separation elucidated the following patterns of migration: cystine (n = 6), homocysteine (n = 4), no cystine or homocysteine (false positives, n = 9 [43%]), and generalized hyperaminoaciduria (n = 2). The excretion of cystine and homocysteine was quantified, finding a range of 1.6 to 20x above the reference value. **Conclusion:** This chromatographic method allowed the discrimination of cysteine and homocysteine patterns in the 10 detected patients, showing a 100% correlation with quantitative studies.

111 - Report of the First 1000 Patients Identified by the Mucopolysaccharidosis Brazil Network

R. Giugliani[1,2,3], K. Jesuino[1], A. Brites[1], C. Rafaelli[1], M. Burin[1], S. Leistner-Segal[1], U. Matte[1], C. Bittar[1], I. Schwartz[1,2], B. Toralles[4], A. Acosta[4], E. Ribeiro[5], E. Valadares[6], L. C. S. Silva[7], C. Kim[8], C. Lourenço[9], D. Horovitz[10], R. Boy[11], C. Steiner[12], M. Ribeiro[13], and A. Federhen[1]

[1]*Med Genetics Serv/HCPA, Porto Alegre, Brazil*
[2]*Dep Genetics, UFRGS, Porto Alegre, Brazil*
[3]*INAGEMP, Porto Alegre, Brazil*
[4]*Medical Genetics Service, HUPE, Salvador, Brazil*
[5]*Hosp Infantil Albert Sabin, Fortaleza, Brazil*
[6]*Medical School, UFMG, Belo Horizonte, Brazil*
[7]*Lab. Inborn Errors of Metabolism, UFPA, Belém, Brazil*
[8]*Children's Institute, HC-USP, São Paulo, Brazil*
[9]*HCRP, Ribeirão Preto, Brazil*
[10]*IFF-FIOCRUZ, Rio de Janeiro, Brazil*
[11]*HUPE, Rio de Janeiro, Brazil*
[12]*Dep Medical Genetics, UNICAMP, Campinas, Brazil*
[13]*IPPMG, UFRJ, Rio de Janeiro, Brazil*

Objectives: Mucopolysaccharidosis (MPS) Brazil network (MBN) was created to improve diagnosis and management of MPS diseases in Brazil. Since then, physicians from all Brazilian regions and from other countries have requested support for the investigation of patients with suspected MPS. **Methods:** Contact with MBN has been established through the Web site (www.mps.ufrgs.br), by e-mail (mps@ufrgs.br), or by a toll-free helpline (800-510-2030). Informative materials and instructions for sample collection and shipment, as well as educational material about MPS can be downloaded in the Web site. Services from all Brazilian regions and from other countries sent biological samples to MBN headquarters, located at the Medical Genetics Service of HCPA, where laboratory studies for MPS is performed free of charge. **Results:** From April 2004 to December 2012, 1000 patients with MPS were identified, of which 542 were new diagnoses (average 5.2/month). Most frequent type of MPS diagnosed was MPS II, confirmed in 300 (30.0%) of 1000 patients with MPS, followed by MPS VI (23.3%), MPS I (19.4%), and MPS IVA (11.5%). Most (49.6%) patients with MPS I came from South or Southeast regions, while most (49.5%) patients with MPS VI patients came from Northeast region. The MPS III-B and IV-A are also frequent in South with 42% and 31.5% of patients, respectively, coming from this region. **Conclusion:** Easy access to information and to diagnostic tests provided by MBN helped to identify many patients with MPS, making MBN a template for the development of similar initiatives for other rare diseases.

112 - Response to Agalsidase α Treatment in Argentinian Patients With Fabry Disease Registered in Fabry Outcome Survey

P. Neumann[1,2], S. Larroque[3], G. Caceres[2], I. Kisinovsky[2], A. Quarin[2], A. Gastaldi[2], M. Choua[2], R. Ceci[2,4], and P. A. Rozenfeld[2,4]

[1]*LISIN, Facultad de Ciencias Exactas, UNLP, La Plata, Buenos Aires, Argentina*
[2]*Servicio de Nefrología, Hospital Italiano, La Plata, Buenos Aires, Argentina*
[3]*Asociacion Argentina de estudio de enfermedad de Fabry y otras enfermedades lisosomales, Argentina*
[4]*Shire HGT*

Introduction: Fabry Outcome Survey (FOS) is a longitudinal multicenter observational survey of patients with Fabry disease. The objectives of this registry are to monitor natural history and the response to agalsidase α treatment. **Patients and Methods:** A total of 63 Argentinian patients with Fabry disease in enzyme replacement therapy (ERT), 29 women and 34 men, registered in FOS were included in this study. Evaluation of kidney and cardiac response to agalsidase α treatment up to 7 years was assayed by periodical determination of serum creatinine, creatinine clearance, and left ventricular (LV) mass. Pain was analyzed by brief pain inventory (BPI) and quality of life by the use of EQ-5D. Statistical analysis was performed on obtained data using nonparametric analysis of variance. A value of $P < .05$ was regarded as statistically significant. **Results:** Patients were divided into 3 groups, according to renal stages at baseline: stage I (n = 10), stage II (n = 13), and stage III (n = 4). Renal function was not significantly reduced ($P > .05$) in patients on ERT, up to 7 years. Analysis of LV mass changes was carried out by separating the patients into 2 groups: patients with LVH (LV mass >50) or without LVH (LV mass <50) at baseline. The LV mass assessment revealed nonsignificant change ($P > .05$) of LV mass in both groups of patients. Patients showed an improvement in quality of life and a reduced pain on average. **Conclusion:** This study revealed a stabilization of kidney and cardiac disease in patients with Fabry disease as well as improvement in pain and quality of life in patients with Fabry disease treated with agalsidase α up to 7 years.

113 - Results of the Admission of Children With Propionic Acidemia and Methylmalonic Acidemia to the Chilean Food Complementary Program

G. Castro[1], Arias Carolina[1], J. F. Cabello[1], E. Raimann[1], V. Hamilton[1], A. Valiente[1], K. Bett[1], A. D. L. Parra[1], M. Colombo[1], and V. Cornejo[1]

[1]Laboratorio de Genética y Enfermedades Metabólicas, Instituto de Nutrición y Tecnología de los Alimentos-INTA, Universidad de Chile, Santiago, Chile

Introduction: The propionic acidemia (PA) occurs due to a deficiency of propionyl CoA carboxylase enzyme and methylmalonic acidemia (MMA) product of a deficit of methylmalonyl CoA mutase or their cofactors. The treatment is a protein-restricted diet, special formula without methionine, threonine, valine, and isoleucine (MTVI), supplementation with L-carnitine, biotin, and vitamin B12. **Objective:** To determine the impact of receiving formulas without MTVI subsidized by the National Complementary Feeding Program (PNAC). **Methodology:** We compared pre- and post-PNAC data regarding calorie intake, protein, MTVI, calcium, iron, zinc, anthropometry (weight and height), ammonium level, and acylcarnitines. Statistical analysis was performed using SPSS. **Results:** A total of 11 patients were admitted with PA and 4 with MMA, age range between 2 and 18 years. There was a statistically significant difference in the contribution of special formula (average of 1 ± 0.7 g/kg/d increased) and a significant correlation with size and significant differences also regarding supplementation of L-carnitine, MTVI, and minerals. The size increased by 5.6 ± 4.4 cm and weight increased by 3.4 ± 5.4 kg; significant differences were observed when compared with preadmission. **Conclusion:** Admission to the PNAC has positively impacted children with PA and MMA, reflected in the size and weight gain. However long-term evaluations are required.

114 - Severe and Rapid Disease Course in the Natural History of Lysosomal Acid Lipase Deficiency in Infants

S. Jones[1], V. Valayannopoulos[2], D. Bernstein[3], M. Bialer[3], A. Dhawan[4], C. Hendriksz [5], C. B. Whitley[6], M. Banikazemi[7], A. Chan[8], O. Guardamagna[9], J. Raiman[10], I. Gamal[11], Laila Selim[11], S. Cederbaum[12], M. Di Rocco[13], J. Domm[14], G. Enns[15], D. Finegold[16], J. Gargus[17], O. Zaki[18], S. Eckert[19], R. Tripuraneni[19], E. Schneider[19], and A. G. Quinn[19]

[1]Central Manchester and Manchester Children's Hospital NHS Foundation Trust, Manchester, United Kingdom
[2]Ref Centre IEM, Necker-Enf Malades Hospital, Paris, France
[3]North Shore Long Island Jewish Hospital, Manhasset, New York, USA
[4]Kings College Hospital NHS Foundation Trust, London, United Kingdom
[5]Birmingham Children's Hospital NHS Foundation Trust, Birmingham, United Kingdom
[6]University of Minnesota, Minneapolis, Minnesota, USA
[7]New York Presbyterian Hospital, New York, New York, USA
[8]University of Alberta Health Services Edmonton, Alberta, Canada
[9]University of Turin, Turin, Italy
[10]The Hospital for Sick Children, Toronto, ON, Canada
[11]Cairo University Children's Hospital, Cairo, Egypt
[12]University of California-Los Angeles, Los Angeles, California, USA
[13]Istituto Giannina Gaslini-Ospedale Pediatrico IRCCS, Geneva, Italy
[14]Vanderbilt Children's Hospital, Nashville, Tennessee, USA
[15]Stanford University School of Medicine, Stanford, California, USA
[16]Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania, USA
[17]University of California - Irvine Medical Center, Orange, California, USA
[18]Ain Shams Hospital, Cairo, Egypt
[19]Synageva BioPharma Corp, Lexington, Massachusetts, United States

Introduction: Lysosomal acid lipase (LAL) deficiency is an autosomal recessive disorder, resulting in the accumulation of cholesteryl esters and triglycerides. The LAL deficiency in infants, also known as Wolman Disease, manifests in the first few months of life and usually leads to death within the first year of life. These patients develop malabsorption, hepatosplenomegaly, liver failure, adrenal calcifications, cytopenias, and growth failure. This is the first natural history study of a large multinational group of patients with LAL deficiency less than 2 years of age. **Methods:** Demographic and clinical information on patients with LAL deficiency were collected using clinical chart data abstractions and summarized. **Results:** Of the 36 patients, more than 66% developed symptoms before 2 months of life. Median alanine transaminase and aspartate aminotransferase were elevated prior to diagnosis (64 and 109 U/L) and increased further where longitudinal data were available prior to death (144 and 333 U/L). Median bilirubin values were normal at the time of diagnosis (0.9 mg/dL) and increased slightly prior to death (1.3 mg/dL). The median age at symptom onset, at diagnosis, and at death were 1.0 month (0-6.0), 2.6 months (1.0-17.7), and 3.7 months (1.4-46.3), respectively. In all, 10 patients underwent transplantation, 9 underwent Hematopoietic stem cell transplantation (HSCT; 7 died before 9 months of age, 1 at 26.9 months, and 1 at 46.3 months). The 10th patient (HSCT and liver transplant recipient) died at 37.3 months. **Conclusions:** The LAL deficiency in infants has a rapidly progressive clinical course and nearly universal mortality during the first year of life.

115 - Skeletal Dysplasia Due to Congenital Disorders of Glycosylation

C. G. Asteggiano[1,2,3], G. Martinez-Domenech [1], M. A. Delgado[1], M. B. Bistue Millon, G. Matthijs G[5], N. Guelbert[1], R. Dodelson de Kremer[1], S. Balcells[2], and D. Grinberg[2]

[1]Centro de Estudio de las Metabolopatías Congéntias (CEMECO), Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[2]Facultad de Medicina, Universidad Católica de Córdoba, Córdoba, Argentina
[3]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

[4]Depto de Genética, Facultad de Biología, CIBERER, IBUB, Universidad de Barcelona, Barcelona, España
[5]Center for Human Genetics, University of Leuven, Belgium

Defects in N-and O-glycosylation and combined glycosylation pathways have been identified as congenital disorders of glycosylation (CDG). Most of them are autosomal recessive, but multiple osteochondromatosis (EXT1/EXT2-CDG) was described as a dominant disease restricted to the cartilage. A peculiar skeletal phenotype has been described in patients with CDG, and it has gained special relevance over the past few years. In patients exposed to cell hypoglycosylation due to altered glycosylation pathway, numerous extracellular matrix proteins undergo glycosylation defects that lead to skeletal manifestations. The aim of this work is to communicate advances of the first CDG research program in Argentina related to studies over the molecular basis of skeletal dysplasias due to CDG. Here, we report skeletal manifestations due to (1) N-glycosylation disorders in 1 patient with osteopetrosis-like phenotype, type-II transferrin IEF, and altered fucosylation observed by mass spectrometry, in which only a heterozygous variation was found in COG6, p.V631M(c.1891G>A); (2) O-glycosylation disorders in GALNT3-CDG (hyperfosfatemic tumoral calcinosis), LFNG-CDG (spondylocostal dysostosis), SLC35D1-CDG (Schneckenbecken dysplasia), B4GALT7-CDG (progeroid variant Ehlers Danlos), B3GALTL-CDG (Peter Plus syndrome), and EXT1/EXT2-CDG (multiple osteochondromatosis, MO); and (3) advances regarding EXT1/EXT2-CDG (multiple osteochondromatosis, OM). Our results highlight hypoglycosylation effects on skeletal manifestation genesis in these CDG pathologies. From 33 patients (27 MO and 6 con SO), we found the mutant allele in 70% of the patients with MO, 83% with severe phenotype and 7% with condrosarcoma malignization. In 30% of the patients, disease-causing mutations remained unknown. In this sense, the new exome sequence techniques will soon become a diagnostic tool for these pathologies.

116 - Standardization of Spectrophotometric Assay to Quantify the Specific Enzymatic Activity of Mitochondrial Complex I

J. P. Cantillo[1], Morillo Moraima[1], Medina Rafael[1], Dominguez Carmen Luisa[1], A. Mahfoud [1], M. De Lucca[1], and Rodriguez Tania[1]

[1]Laboratorio de Pesquisa Selectiva, Unidad de Errores Innatos del Metabolismo, Edificio de Biociencias, Instituto de Estudios Avanzados (IDEA), Venezuela

Introduction: Oxidative phosphorylation is the metabolic pathway that couples several enzymatic complexes of mitochondrial respiratory chain (MRC) to generate adenosine triphosphate. Disorders on this mechanism cause a variable loss of the activity and function of proteins leading to diseases. **Goal:** In this work, we propose to standardize a rigorous assay for spectrophotometric determination of the specific activity dependent on mitochondrial Complex I. **Methodology:** Spectrophotometric determination was carried out using the protocols already described. Our study was executed in a primary cell culture of healthy human fibroblasts and over several rat skeletal muscle biopsies. **Results:** In human fibroblasts, we found an important reduction in enzymatic activity of complex I, which was in the range of values of 793 and 1691.71 nmol/min/mg protein. These results were different from our previous studies that showed overexpression trends. In rat skeletal muscle, the values ranged between 425.7 $\pm$ 590.06 and 1290.59 $\pm$ 647.54 nmol/min/mg protein. **Conclusion:** The combination of methodologies for determining the enzymatic activity of mitochondrial complex I allowed to rule out extreme values that might suggest the occurrence of an overexpression phenomena of this mitochondrial complex, according to previous experience in our laboratory. Likewise, results in human fibroblast and rat muscle reported here were consistent with those reported in the literature.

117 - Stroke in Patients With Fabry Disease: Natural History Data From Fabry Registry

J. Politei[1]

[1]Fundación para el estudio de las Enfermedades Neurometabólicas, Laboratorio "Dr Chamoles," Buenos Aires, Argentina

Introduction: Fabry disease is caused by α-galactosidase A deficiency. Patients may experience transient ischemic attacks or stroke. A better understanding of natural history can provide valuable information about patients who are at greatest risk of stroke. **Objective:** To characterize untreated patients included in the Fabry Registry who or not experienced stroke prior to initiation of enzyme replacement therapy (ERT). **Methods:** The Fabry Registry is an observational database supported by Genzyme, a Sanofi company that tracks natural history and outcomes of patients with Fabry disease. Demographic data as well as renal and cardiac data were compared in patients who did or did not experience a stroke. **Results:** As of April 2012, the Fabry Registry enrolled 4206 patients, including 237 patients who experienced stroke prior to initiating ERT, 137 men and 100 women. The median age at first stroke was 40.8 years for men and 46.4 years for women. Forty-two patients experienced a stroke before the third decade of life. Patients with stroke were diagnosed at an older age than those without stroke. Of the cases, 72% did not present any renal or cardiac event prior to their first stroke. **Summary:** Although stroke has largely been considered to be a late manifestation associated with serious renal and cardiac dysfunction, we found that most patients did not have renal or cardiac events prior to their first stroke. These findings demonstrate that stroke is a relatively common manifestation of Fabry disease that can also occur at early ages.

118 - Suspected Partial Ornithine Transcarbamylase Deficiency: A Case Report

O. I. Beltran Casas[1,2], L. J. Urbina Riveros[1], and S. D. Sanchez Clavijo[1]

[1]*Universidad Militar Nueva Granada, Facultad de Medicina, Bogotá, Colombia*
[2]*Fundación-Hospital de la Misericordia, Bogotá, Colombia*

Introduction: The deficiency of the enzyme ornithine transcarbamylase (OTC) is considered the most common disorder of the urea cycle (incidence 1 of 14,000), being more common neonatal presentation. The late cases with partial deficiency (enzyme activity about 30%) are estimated more common, but the diagnosis may be delayed. **Objectives:** To describe the case of a 3-year-old child with clinical and metabolic support partial OTC deficiency. **Case study:** Preschool, boy, 3 years old, nononsanguineous parents with no family history of EIM known, delayed psychomotor development from 6 months, and severely compromised expressive language and behavioral alterations. **Results:** Magnetic resonance imaging of the brain showed alterations in frontoparietal white matter and basal ganglia, low seric levels of creatinine and citrulline, high serum ammonia. Orotic acid was detected in urine with normal values for age. Molecular study of OTC gene was requested, not yet authorized. The high clinical suspicion of partial OTC deficiency starts interdisciplinary and specialized nutrition management. **Conclusion:** Patient with symptoms compatible with partial OTC enzyme defect with late presentation. Key findings include low serum citrulline, elevated serum ammonia, and elevated urine orotic acid. With negative family history and challenge tests, inconclusive confirmatory studies should be ordered.

119 - Systemic Primary Carnitine Deficiency

N. B. Specola[1], L. I. Muschietti[2], G. J. C. Borrajo[3], M. Nuñez Miñana[1], M. Collini[2], and M. J. Zelaya[3]

[1]*Unidad de Metabolismo Hospital de Niños "Sor María Ludovica," La Plata, Argentina*
[2]*Laboratorio de Metabolopatías, Hospital de Niios "Sor María Ludovica," La Plata, Argentina*
[3]*Detección de Errores Congénitos, Fundación Bioquímica Argentina, La Plata, Argentina*

Systemic primary carnitine deficiency is a recessive disorder of variable frequency, whose range began to be defined through the expanded newborn screening by Tandem Mass Spectrometry (MS/MS). Clinically, it shows variability with respect to age of onset and symptoms severity, being possible to find different presentations: early infantile hepatic, late infantile cardiac or muscular, and adulthood cardiac or asymptomatic forms. In this work, a patient affected by the early infantile cardiac form, not screened by MS/MS at the neonatal period, is presented. The clinical picture started at fourth month of life with hypertrophic cardiomyopathy and severe arrhythmia during a bronchial infection, elevated muscle enzymes, and lack of hepatic involvement signs. As part of the routine cardiomyopathies exploration, free (3.5 µmol/L) and total (4.0 µmol/L) carnitine and acylcarnitines by MS/MS (C0, C2, C3, C16, C18:2, C18:1 and C18 clearly diminished) were measured, establishing the presumptive diagnosis. The retrospective analysis of the neonatal sample showed similar acylcarnitines pattern. After L-Carnitine supplementation, the cardiac function improved significantly, evidencing hypoxic neurologic sequelae and highlighting the importance to complete the metabolic evaluation of acute cardiomyopathies—including those associated to infectious diseases—to rule out secondary causes of carnitine deficiency and contraindicate the use of drugs causing it, and to begin early treatment with L-Carnitine to avoid multiorganic or cardiac failure. The retrospective analysis of the neonatal card is useful, but it requires to be analyzed together with normal samples of similar age in order to allow a correct interpretation of those analytes affected during storage.

120 - Taliglucerase α 36-Month Clinical Safety and Efficacy: Interim Results in Treatment-Naive Patients With Gaucher Disease From Extension Trial PB-06-003

G. P. Duran[1], A. Mehta[2], P. Giraldo[3], H. Rosenbaum[4], F. Giona[5], D. J. Amato[6], M. Petakov[7], E. T. Muñoz[8], S. E. Solorio-Meza SE[9], and D. Elstein[10]

[1]*Pontificia Universidad Catolica de Chile, Division de Pediatria, Santiago, Chile*
[2]*Royal Free and University College School of Medicine, Department of Haematology, Royal Free Hospital, London, United Kingdom*
[3]*Hospital Universitario Miguel Servet, Servicio de Haematologia CIBER de Enfermedades Raras (CIBERER), Zaragoza, Spain*
[4]*Department of Hematology, Rambam Medical Center, Haifa, Israel*
[5]*"Sapienza" University, Department of Cellular Biotechnologies and Hematology, Hematology Section, Rome, Italy*
[6]*Mount Sinai Hospital, Toronto, Ontario, Canada*
[7]*Clinical Center of Serbia, Institute of Endocrinology, Diabetes and Metabolic Disease, Belgrade, Serbia*
[8]*Centro Médico Nacional Siglo XXI, Servicio de Hematologia, México City, México*
[9]*Hospital de Especialidades No. 1, Unidad de Investigacion, colonia Los Paraisos, Leon Guanajuato, Mexico*
[10]*Gaucher Clinic, Shaare Zedek Medical Center, Hebrew University and Hadassha Medical School, Jerusalem, Israel*

Introduction: Taliglucerase α is a β-glucocerebrosidase enzyme used in enzyme replacement therapy (ERT) approved in the United States and other countries for treatment of Gaucher disease (GD) in adults and is the first approved plant cell-expressed biotherapeutic. **Objective:** To report interim results of efficacy and safety of taliglucerase 30 and 60 U/kg in 26 adult patients with GD following 36-month treatment. **Methods:** Study PB-06-003 is an extension of pivotal study

PB-06-001 and switchover study PB-06-002, wherein taliglucerase α was given every 2 weeks at same doses as in original studies. PB-06-001 was a 9-month, randomized, double-blind, dose-ranging trial to assess safety and efficacy of taliglucerase α 30 and 60U/kg in treatment-naive patients with GD. Efficacy measures included change from baseline in spleen and liver volumes, platelet counts, hemoglobin levels, and chitotriosidase activity. **Results:** At 36 months of treatment, the following changes were observed for taliglucerase α 30 and 60 U/kg, respectively: mean spleen volumes decreased by 50.0% and 64.6%; mean liver volumes decreased by 25.1% and 24.4%; mean hemoglobin concentrations increased by 1.8 and 3.0 g/dL; platelet counts increased by 29.783/mm^3 and 71.700/mm^3; and mean chitotriosidase activities decreased by 73.5% and 83.0%. All treatment-related adverse events (AEs) were mild/moderate and transient. The most common AEs were nasopharyngitis, arthralgia, upper respiratory tract infection, headache, and pain in extremity. **Conclusion:** This interim report of the longest multiple-dose ERT study for GD demonstrated that taliglucerase α is a safe and effective treatment for GD. Trial PB-06-003 is ongoing. This analysis was sponsored by Protalix and Pfizer and completed using a validated database.

121 - Taliglucerase α in Pediatric Patients With Gaucher Disease: Efficacy, Safety, and Exploratory End Points

A. Zimran[1], D. E. Gonzalez-Rodriguez[2], A. Abrahamov[1], D. Elstein[1], A. Paz[3], E. Brill-Almon[3], and R. Chertkoff[3]

[1]Gaucher Clinic, Shaare Zedek Medical Center and the Hadassah Medical School - Hebrew University, Jerusalem, Israel
[2]Instituto Privado de Hematologia e Investigacion Clinica (I.P.H.I.C.), Asuncion, Paraguay
[3]Protalix Biotherapeutics, Carmiel, Israel

Introduction: Taliglucerase α is a novel, plant cell-expressed, β-glucocerebrosidase enzyme used in enzyme replacement therapy (ERT) approved in the United States and other countries for treatment of Gaucher disease (GD) in adults. **Objective:** To assess efficacy and safety of taliglucerase α in pediatric patients with GD. **Methods:** Phase 3B, multicenter study in treatment-naive pediatric patients (aged 2 to <18 years) who received taliglucerase α 30 or 60 U/kg for 12 months. Primary end point was median percentage change from baseline in hemoglobin concentration at month 12. Hematologic, visceral, exploratory growth and development, and quality-of-life measures were also assessed. **Results:** Eleven patients were randomized (n = 6, 30 U/kg; n = 5, 60 U/kg). Median percentage change from baseline in hemoglobin concentration at month 12 was 12.2% and 14.2% with taliglucerase α 30 and 60 U/kg, respectively. For patients with anemia at baseline, median percentage change in hemoglobin was 19.6% and 17.9%, respectively. Improvements were observed in platelet counts, mean spleen volume, mean liver volume, and chitotriosidase activity after 12 months of treatment with taliglucerase α 30 and 60 U/kg. Height increased by 4.2% and 7.6%; weight increased by 9.6% and 14.7%; and mean bone age advanced 1.9 and 1.4 years, respectively. Pubertal status remained stable. Scores on the Child Health Questionnaire revealed parents/guardians felt their children's health improved after treatment, more often to "very good" or "excellent." Most adverse events (AEs) were mild/moderate/transient. One serious AE of gastroenteritis was reported as treatment related. **Conclusion:** Taliglucerase α may be a potential treatment option for children with GD. This study was sponsored by Protalix and Pfizer.

122 - The Same Mutation in 2 Patients With Mucopolysaccharidosis Type VI, Belonging to Different Municipalities of Cauca Department in the Southwest of Colombia

M. A. Acosta-Aragon[1], M. R. Lago-Leston[2], F. Barros-Angueira[2], and A. Carracedo-Alvarez[2,3]

[1]Departamento de Pediatría, Facultad de Ciencias de la Salud, Universidad del Cauca, Popayán, Colombia
[2]Hospital Clínico de Santiago de Compostela, Galicia, España
[3]Departamento de Patología, Facultad de Medicina, Universidad de Santiago de Compostela, Galicia, España

Introduction: Lysosomal diseases, to which Mucopolysaccharidosis type VI or Matoreaux-Lamy syndrome belongs, are caused disorders in synthesis or function of a lysosomal acid hydrolase in this specific case of the enzyme N-acetylgalactosamine 4-sulfatase or arylsulfatase B. **Problem:** We identified a total of 36 patients in Colombia, 16 of which have been reported in the Department of Cauca, corresponding to 45% of total registered cases throughout Colombia. Two of these patients in the municipalities of Totoro and Piendamo in eastern and central region of the Cauca department, respectively, were studied molecularly. **Methods:** In both index cases, DNA extraction, amplification, purification, and sequencing reaction for the genomic DNA of arylsulfatase B in a capillary electrophoresis instrument AB1 3100 were done. The results of genomic DNA were placed on the format and reading sequence alignment "staden package," and differences in sequences were determined with reference to the genome sequence encoding for arylsulfatase B. **Results:** We identified the same homozygous mutation Cys447Phe in the index case of these 2 families, from different municipalities of Cauca Department. **Conclusion:** The finding of the same mutation suggests a possible founder effect which would explain the unusual frequency of Maroteaux-Lamy syndrome for the Department of Cauca.

123 - The Suspension of Amino Acids Iatrogenic, the Effect in 3 Patients With Metabolic Diseases

Y. Cuellar[1] and J. Ospina[1]

[1]Center for Hereditary Metabolic Diseases

Introduction: Metabolic diseases (EM) is a deficit in enzyme or a co-factor in a metabolic pathway of an amino acid (AA), which will increase in the body and transform into toxic compounds. The manipulation consists of restricting the limiting amino acid to prevent the accumulation of the same, without suspending it for being essential. **Objective:** To describe the effects of the suspension or the contribution limitations of essential amino acids in 3 patients with EM for an extended time. **Methods:** Retrospective analysis of 3 patients with EM. **Results:** Three patients, term babies, were included who showed symptoms from 3 to 10 days of life. The symptoms included hyporexia, lethargy, underactivity, crying frequently, peculiar smell, hypoglycemia, and beginning of urine protein restriction. In patient 1, at the month of admission Maple syrup urine disease (MSUD) was confirmed, and nutritional manipulation with contribution of 0.8 g/kg/ protein daily, for 45 days, and formula free of AARC-only for 20 days were started. Patient 2 was suspended of protein supply for 37 days, and organic acids confirmed MSUD. Both have scaly lesions on skin, persistent diarrhea, and decrease in speed of growth weight and height. In patient 3 with diagnosis PA, contribution of proteins is suspended for 5 months, handling with exclusive formula for PA, for 1 month with erythematous-scaly lesions in folds and perioral region. In all patients, nutritionally starting contribution of AARC, achieving recovery from lesions to the skin, and improvement in symptoms and growth was observed. **Conclusion:** The EIM should not discontinue with AA contribution involved in the metabolic pathway for chronic manipulation; its deficiencies can cause associated pathologies.

124 - The Use of U18666A in Rat Primary Astrocytes to Mimic Niemann Pick Type C Disease

D. C. Santos[1] and J. C. Coelho[1]

[1]Departamento de Bioquímica, ICBS, Universidade Federal do Rio Grande do Sul

Niemann-Pick C disease (NPC) is a neurodegenerative genetic disorder caused by storage of lipids, especially cholesterol, in the perinuclear space. Some agents, such as U18666A, can mimic this disease in human fibroblasts. It is an inhibitor of cholesterol transport. In order to determine the influence of this drug in rats' primary astrocytes, doses of 0.1, 0.25, 0.5, 1, and 2 μg/mL were incubated for 24, 48, and 72 hours, to verify whether it mimics NPC disease. The rats were killed by decapitation, and the cerebral cortex was removed. This sample was homogenized with CMF-BSS buffer and placed in 24-well plates in Dulbecco modified Eagle medium with SBF10%. After 4 hours, the medium was changed and so on of 4 in 4 days. The drug was added to cultures on day 15, and these cultures were incubated at 37°C in CO_2 incubators. Before 24 hours of the end of incubation time, 50 μg/LDL was added to each well. For visualization and quantification of the storage of cholesterol, we used the Filippin stain technique and the CellM program in fluorescence microscope. The results were compared by GraphPadPrism 5 program using 2-way analysis of variance. Our results demonstrated that the drug in a dose of 0.25 μg/mL incubated for 48 hours was that best mimics the disease. This model can be used for biochemical studies in NPC. These studies are already underway by our research group.

125 - Translational Program on Neuronal Ceroid Lipofuscinoses in Latin America as a Model Experience on Rare Disease Studies

R. Kohan[1,2], I. A. Cismondi[1,2], N. Guelbert[1], M. N. Carabelos[1], F. Pesaola[1], P. Pons[3], G. Alonso[1], R. Dodelson de Kremer[1], A. M. Oller-Ramirez[1], and I. Noher de Halac[1,4]

[1]Centro de Estudio de las Metabolopatías Congénitas (CEMECO), Hospital de Niños, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[2]Departamento de Biología Oral, Facultad de Odontología, Universidad Nacional de Córdoba, Córdoba, Argentina
[3]Centro de Microscopía Electrónica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Córdoba, Argentina
[4]Consejo Nacional de Investigaciones Científicas y Técnicas, CONICET, Córdoba, Argentina

Introduction: Rare diseases (RDs) are more than 6900 pathologies that affect fewer than 200 000 people in the United States or less than 5 of 10 000 in the European Union. The overall prevalence in Latin America remains unknown. A translational program for the study of Neuronal Ceroid Lipofuscinoses (NCLs) in Latin America has existed for the past 10 years at the Children's Hospital in Córdoba-Argentina. **Methodology:** The key traits for the integrated study of NCLs were implemented, including diagnoses, phenotypes/genotypes, care/palliative medicine, and education/social aspects, at the same time promoting families' advocacy. High-technology inputs were enabled through international collaboration. **Results:** A unified study algorithm allowed effective clinical/morphologic/biochemical and molecular diagnostic studies with CLN1/PPT1 and CLN2/TPP1 enzyme testing in dried blood spots/saliva/leukocytes, morphologic studies, and mutational analysis. In 33 families from 4 countries, 20 novel and 11 known mutations were described. Forty-two individuals were diagnosed for 7 of 14 forms of NCLs. CLN2 was stated as the most frequent form, showing 2 markedly differentiated subtypes, classic late infantile, and variant juvenile. **Conclusion:** The program-approach should be considered as an effective model in Latin America for other RDs. It is conducted to coordinate and support diagnoses and research, with the potential to facilitate development of drug and biologic products development for treatments. Capacity building and implementation of educational programs is still needed in the region.

126 - Use of Array-CGH for the Cytogenomicv Evaluation of Chromosome Rearrangements in the IDS Gene Region

M. Riegel[1,2,3], S. Leistner-Segal[1], P. Rozenfeld[4], and R. Giugliani [1,2,3]

[1]Serviço de Genética Médica, Hospital de Clínicas (HCPA), Porto Alegre, RS, Brazil
[2]Centro de Terapia Gênica, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, RS, Brazil
[3]Programa de Pós-Graduação em Genética e Biologia Molecular, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil
[4]Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina

The majority of cases of mucopolysaccharidosis II (MPS II) are caused by point mutations, small deletions, and insertions in the IDS gene. In 20% of the cases, major structural alterations occur and in ~6% to 8% the disease results in a complete IDS deletion. Our aims were to evaluate the use of high-resolution array-based array comparitive genomic hybridization (CGH) in the determination of genomic rearrangements in the IDS gene region in DNA samples from patients with MPS II. Chromosome microarray analysis was performed on 5 patients with MPS II with known deletions within the IDS gene region. The customized oligonucleotide-based microarray using the 1 million arrays was applied in each of the sample tests and controls in order to better delineate the chromosomal rearrangement in the Xq28 region. The array-CGH data confirmed the previous exon-by-exon IDS polymerase chain reaction results which demonstrated partial or complete deletions. Additionally, the microarray analysis revealed a contiguous duplicated region on Xq28 in one of the samples, encompassing approximately 476 Kb. A comprehensive molecular analysis in patients affected by MPS II, especially in the ones with large deletions, is crucial for the understanding of the molecular mechanisms of rearrangements in the IDS gene region and the related phenotypic traits. Possibly, rearrangements of the IDS gene region occur in greater extent than estimated so far, as part Xq28 chromosome variation, with potential involvement of contiguous genes. The array-based methods have proven to be powerful tools in genome-wide detection of copy number changes in different sizes and gene content in patients with MPS II.

127 - X-Linked Adrenoleukodystrophy Presenting as Acute Adrenal Failure With Atypical Neuroimaging: Case Report

Sanchez Yasmin[1], Espinosa Eugenia[2], Cabarcas Lisseth[3], Pulido Ninna Fernanda[3], Ardila YA[3], Echeverri Olga Yaneth[4], Guevara Johana Maria[4], and L. A. Barrera[3,4]

[1]Hospital San Rafael, Tunja-Colombia
[2]Hospital Militar Central, Universidad Nueva Granada, Bogotá, Colombia
[3]Hospital Universitario San Ignacio, Bogotá, Colombia
[4]Instituto de Errores Innatos del Metabolismo, Pontificia Universidad Javeriana, Bogotá, Colombia

Introduction: X-linked adrenoleukodistrophy (X-ALD) is a peroxisomal disorder that impairs normal very-long-chain fatty acids (VLCFA) oxidation. **Objective:** To present an X-ALD case with atypical clinical findings. **Methods:** Case report. **Results:** Patient normal at birth, without family history of neurologic or systemic disease, first union product, and no consanguinity. At the age of 8 years presented with progressive loss of weight, electrolyte imbalance, hyperpigmentation in skin with diagnosis of acute suprarenal failure during hospitalization period that required corticosteroids treatment, achieving hemodynamic stabilization. At this time, motor skills regression, spasticity, and dystonic painful crisis was detected. During the hospitalization, it was observed partial improvement in motor skills, achieving deambulation with support. The cerebral magnetic resonance showed gangliobasal compromise and anterior white matter alteration; these data lead to diagnosis impression of leukodystrophy versus mitochondrial disease. At 6 months, during a new neurologic evaluation, behavioral and cognitive impairment, sleep disorder, and worsening spasticity and hemydistonia were evident. Plasmatic lactic acid, pyruvic acid, and lactic–pyruvic ratio were normal; neuroimaging analysis showed slight improvement in basal ganglia injury and progression of leukodystrophy with predominant anterior involvement. Studies for metachromatic leukodystrophy and VLCFA were performed; the VLCFA result was compatible with peroxisomal disorder, specifically X-Adrenoleukodystrophy C26:1,52ug/ml (VN: 0, 23 + 0,99ug/ml) C24/C22: 2,134ug/ml (VN: 0, 84 + 0, 1) C26/C22:0,079ug/ml (VN: 0, 01+ 0,004). Nowadays, the patient presents with significant decline in motor, cognitive, and neuropsychiatric functions. A younger brother with abnormal VLCFA compatible with asymptomatic disease is under treatment with Lorenzo's oil. **Conclusion:** Here is presented a case with acute onset and atypical neuroimaging. In all patients acute adrenal failure, without specific etiology presenting with or without neuroimaging alterations in ADL-X should be evaluated as a differential diagnosis.

128 - "Recall Rate" Reduction Using a Stage-Based Analytical Process

A. Guercio[1], N. Villarias[1], V. Lobato[1], R. Gaviola[1], N. Carminati[1], S. Valle[1], and B. Castro[1]

[1]Centro de Prevención de Enfermedades Inaparentes de la Infancia (CE.P.E.I.I.), Hospital Pediátrico Dr Humberto J. Notti, Mendoza, Argentina

Introduction: Recall rate is an important indicator to evaluate Neonatal Screening (NS) Program performance. **Objectives:** To reduce the recall rate by following an analytical algorithm

in stages. **Methods:** (1) First stage: activities of thyroid-stimulating hormone (TSH), Phenylalanine (Phe), 17-OH progesterone (17-OH Prog), total galactose (Gal), and biotinidase (Biot) were determined using Tecnosuma reagents. (2) Second stage: samples with "uncertain or positive" results in the first stage were processed with Perkin Elmer (PE) reagents. It was decided to recall based on "positive" results obtained in the second stage. **Results:** From January to December 2012 there were 18 274 newborn (NB). In first stage, 1175 NB (6.43%) had "uncertain or positive" results: 370 (2.02%) Gal, 318 (1.74%) Phe, 254 (1.39%) 17OHProg, 142 (0.78%) Biot, and 91 (0.50%) TSH. Second stage: 1175 samples were processed and 353 (1,93% of all NB) obtained a positive screening. Recitation: 151 (0.82%) NB for 17-OHProg, 109(0,60%) for Gal, 51(0.28%) for Biot, 26 (0.14%) for TSH, and 16 (0. 09%) for Phe. Diagnostic confirmation: 14 NB with congenital hypothyroidism, 4 with congenital adrenal hyperplasia, and 1 with persistent hyperphenylalaninemia. **Conclusion:** The analytical process in stages allowed us to reduce the total recall rate from 6.43% to 1.93%. We avoid the complexity of tracing an important number of children, 822, in addition to intangible cost benefits such as familial concern and anxiety, the negative impact on the health team and its multiple implications every time a "positive" NS result is informed.

129 - Ten Years of Neonatal Screening Program for Congenital Hypothyroidism in the Instituto Nacional Materno Perinatal, Lima, Peru

R. Zumaeta[1], C. Lujan[1], G. Santiago[1], B. Ayala[2], C. Davila[1], and G. Larrabure[1]

[1]*Instituto Nacional Materno Perinatal (INMP), Lima, Peru*
[2]*Instituto Nacional de Salud, Lima, Peru*

Introduction: The neonatal screening program for congenital hypothyroidism was initiated in 2003 at the Instituto Nacional Materno Perinatal. Mandatory in Peru by Law 29885. **Objective:** To determine the incidence and characteristics of congenital hypothyroidism. **Methodology:** In the period 2003 to 2012, 143 996 dried blood spots samples were collected from newborns and analyzed by enzyme-linked immunosorbent assay. **Method:** Diagnosis of Congenital hypothyroidism was confirmed by blood serum samples showing thyroid-stimulating hormone (TSH) level $\geq$ 10 µIU/mL. **Results:** A total of 143 996 newborns were analyzed and 66 cases were diagnosed. Incidence was 4.6 of 10 000 live births. The findings were TSH average: 82.1 $\pm$ 58.02 µIU/mL (10.27-793.52 µIU/mL), gestational age: 39.14 $\pm$ 1.2 weeks, male: 27.5%, female: 72.5%, birth weight: 3441 $\pm$ 418 g, birth length: 49.9 $\pm$ 2.5 cm, cephalic perimeter: 39.14 $\pm$ 1.19 cm, Apgar 1': 8.2 $\pm$ 0.57, jaundice: 55.07%, umbilical hernia: 50.72%, dry skin: 28.98%, wide fontanelle: 23.28%, macroglossia: 18.8%, macrosomia: 13%, puffy face: 8.69%, and hoarse cry: 5.79%. Coverage by percentage versus cases: 2003: 1478 (31%), 1; 2004:13 596 (67.50%), 4; 2005: 1 629 391 (48%), 9; 2006: 15 416 (93.60%), 7; 2007: 16 326 (95.95%), 6; 2008: 17 684 (89.93%), 8; 2009: 18 033 (98.16%),11; 2010: 16 149 (95.72%), 9; 2011: 14 224 (93.60%), 4; 2012: 14 797 (91.88%), 7. **Conclusion:** Congenital hypothyroidism incidence was 4.6 of 10 000 live births. Jaundice and umbilical hernia were the most frequent neonatal characteristics.

130 - Thirteen Years of Experience of Neonatal Screening Program in Mendoza, Argentina

N. Villarias[1], A. Guercio[1], V. Lobato[1], R. Gaviola[1], B. Castro[1], N. Carminati[1], E. Ayub[1], L. Verdaguer[1], L. Navarta[1], S. Valle[1], L. Colaboradores Cestino[2], M. Pereyra[3], and E. Lentini[4]

[1]*Centro de Prevención de Enfermedades Inaparentes de la Infancia (CE.P.E.I.I.), Mendoza, Argentina*
[2]*Unidad Endocrinología Departamento de Bioquímica, Mendoza, Argentina*
[3]*Servicio de Crecimiento y Desarrollo, Mendoza, Argentina*
[4]*Centro Provincial de Fibrosis Quística. Hospital Pediátrico Dr Humberto J. Notti, Mendoza, Argentina*

Introduction: Early detection and diagnosis of unapparent childhood diseases, treatment, and follow-up of the affected children with systematic and continuous evaluation are the pillars of Neonatal Screening (NS) Program. **Objectives:** To present results and indicators of NS Program. **Methods:** From 1999 to 2009, samples of newborns (NBs) were processed to determine thyroid-stimulating hormone (TSH) and phenylalanine (Phe) using Perkin Elmer (PE) method. Since 2010, processing was done in 2 stages. First stage: samples were processed to determine TSH, Phe, 17-OHProgesterone, total galactose, and biotinidase activity using TecnoSuma method. Second stage: samples with "uncertain or positive" results on the previous stage were processed using PE method. Immunoreactive trypsin (IRT) was determined only by PE. The NS data and indicators were analyzed using specially designed software. **Results:** From 1999 to 2009 and from 2010 to 2012, 200 004 and 67 004 NBs were analyzed. Indicators include child's age (CA) at testing: 3 days; transit time trail: 4 days; CA at screening result report: 9 days; CA at results delivery: 12 days; diagnostic confirmation and treatment initiation: 15 days; samples with incomplete data: 1%; rejected samples: 0.2%; recall rate: 1.93; recalled NB: 93% was located; coverage: 98% from public hospitals and 22% from private; NS Program, 13 years: 122 children with congenital hypothyroidism, 5 phenylketonuria, 10 persistent hyperphenylalaninemia, 9 congenital adrenal hyperplasia, and 6 cystic fibrosis. **Conclusion:** Prevention of disability and other consequences of diseases is possible with strong interdisciplinary work and optimizing NS Program indicators.

131 - Twenty-one Years of Experience in Newborn Screening for Phenylketonuria

G. J. C. Borrajo[1], N. B. Specola[2], L. I. Muschietti[3], M. F. Dietz[1], P. I. Castillo[1], and M. Nuñez Miñana[2]

[1]Detección de Errores Congénitos, Fundación Bioquímica, Argentina
[2]Unidad de Metabolismo, Hospital de Niños "Sor María Ludovica," La Plata, Argentina
[3]Laboratorio de Metabolopatías, Hospital de Niños "Sor María Ludovica," La Plata, Argentina

Newborn screening (NBS) for phenylketonuria (PKU) was started in January 1991 by Fundacion Bioquimica Argentina (FBA) together with the Children's Hospital ''Sor María Ludovica'' (HSML) from La Plata. During the first 4 years of activity, NBS was carried out on request and without a program organization until the ''Diagnostic and Treatment of Congenital Diseases Program'' (PRODyTEC) was implemented by the Ministry of Health of Buenos Aires Province, on April 1995. The functional organization of the Program includes screening testing at the FBA NBS Laboratory and confirmation, diagnostic, treatment, and follow-up at the HSML, giving free of charge coverage to all newborns (NB) born in public hospitals since July 2010. Sample collection is made between 24 hours and 5th day of life, and phenylalanine (Phe) is measured using a home-made fluorometric method (cutoff: 2.5 mg/dL). Until December 2012, 3 242 571 NB were screened with a recall rate of 0.08%. In all, 120 cases of PKU and 139 of persistent hyperphenylalaninemia (HPA) were confirmed, with incidences of 1:27 021 and 1:23 328, respectively. The Phe levels in the first sample were 11.9 $\pm$ 8.3 mg/dL in patients with PKU and 3.8 $\pm$ 1.1 mg/dL in patients with HPA. The treatment was started at 20 $\pm$ 12 days (median = 17) and 36 $\pm$ 25 days (median = 28) for PKU and HPA, respectively. During 2012, coverage for the public sector was >97%. No false-negative result was reported until now. Public follow-up and nutritional treatment is provided to all patients without limit of age.

132 - Advances in Genetic Diagnosis of Cystic Fibrosis in Misiones

N. S. Pires[1], C. Trigo[1], M. M. Tiscornia[1], M. A. Guastavino[2], and A. L. Albrekt[2]

[1]Instituto de Biotecnología Misiones "Dra Maria Ebe Reca," Universidad Nacional de Misiones, Pcia de Misiones, Argentina
[2]Cátedra de Bioquímica Clínica III, Modulo de Bioquímica y Farmacia, Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones, Pcia de Misiones, Argentina
[3]Programa Provincial de Pesquisa Neonatal Misiones, Posadas, Argentina

Cystic fibrosis (CF) is a disease caused by alterations in the CFTR gene. There are more than 1300 mutations, being ΔF508 the most frequent, followed by G542X. A polymorphism of thymidine in the final portion of intron 8 with 3 different alleles designated as 5, 7, or 9 T is also known. The objective was to analyze representative gene regions due to high frequency of mutations and poly-T variants of intron 8 that are particularly important in the diagnosis of individuals with suspected CF. Exons 4 and 11 of the gene were tested using the polymerase chain reaction (PCR)-single-strand conformation polymorphism (SSCP) technique. Study of intron 8 was made and amplification refractory mutation system (ARMS)-PCR optimization technique for G542X mutation. DNA was extracted from a total of 66 samples from normal patients and patients with CF; primers were designed for studied regions and PCR amplicons were run on electrophoretic gels. The amplicons obtained for exons 4 and 11 and intron 8 were run on polyacrylamide gels by SSCP technique; only 1 band pattern was observed for exon 4 and intron 8, while 2 differential patterns were visualized for exon 11. These results were confirmed by sequencing. In conclusion, differential patterns were obtained using the SSCP technique for exon 11, resulting in sequence differences from the same samples, and we standardized ARMS-PCR technique for G542X mutation and PCR-SSCP for intron 8, respectively.

133 - Analytical Performance Verification of a Kit for Acylcarinitines by Mass Spectrometry

C. Castillo[1], F. Smithuis[1], A. Oneto[1], P. Muntaabsky[1], M. Junco[1], C. Aranda[1], and G. Maccallini[1]

[1]Programa de Pesquisa Neonatal, Ministerio de Salud, Gobierno de la Ciudad de Buenos Aires, Buenos Aires, Argentina

Introduction: The introduction of mass spectrometry (MS) allowed expanding newborn screening, increasing diseases detection and improving the quality of life in affected infants. Analytical performance verification is the initial stage prior to its use in the laboratory. **Objective:** To verify analytical performance of an MS acylcarnitine's neonatal screening kit. A nonderivatized kit from Chromsystems for 13 acylcarnitines was used in a MS AB-Sciex, API-3200 model. Three levels of internal quality controls (CDC-Set 1-2013) were analyzed in triplicate for 5 days to assess imprecision and veracity. Experimental repeatability and intermediate precision were compared with those reported by the manufacturer for validation with an API-4000 instrument. For veracity calculation, control's mean results were compared with the Centers for Disease Control and Prevention (CDC) peer group, considering standard deviation data and the number of participants of the group to obtain uncertain. **Results:** Compliance percentages were repeatability: 46%, 46%, and 69%; intermediate precision: 100%, 92% NS, 100%; and veracity: 92%, 85%, and 92%. **Conclusion:** There were good compliance results for intermediate precision and veracity, with a lower acceptance rate for repeatability. This could be because the manufacturer's data were obtained using a more sensitive and precise instrument. This initial assessment allowed us to know the analytical performance prior to its routine use in newborn screening.

134 - Assuring Quality of Newborn Screening Dried Blood Spot Assays Worldwide: The Newborn Screening Quality Assurance Program

V. R. De Jesus[1], J. V. Mei[1], and C. D. Cuthbert[1]

[1]Newborn Screening and Molecular Biology Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

Background: The Newborn Screening Quality Assurance Program (NSQAP) at the Centers for Disease Control and Prevention (CDC) operates quality control (QC) and proficiency testing (PT) programs for state public health and other domestic and international laboratories, ensuring the quality and accuracy of screening tests for millions of babies each year worldwide. The program monitors filter paper blood collection device (FP) performance characteristics and provides certified QC and PT dried blood spot (DBS) materials to over 550 laboratories in 73 countries. The PT and QC data collected by NSQAP can be used to assess newborn screening laboratory and method performance over time for endocrine disorders and several amino acid, organic acid, and fatty acid oxidation metabolic disorders. **Methods:** Blinded PT panels and QC materials were sent to participating laboratories. Quantitative and qualitative PT results were collected, and the results were assessed for false-positive and false-negative errors and bias plots were constructed. Using reported quantitative QC data, dose–response curves, intercepts, and slopes were calculated for individual analytes for method comparisons. **Results:** In 2012, the foreign false-positive rate ranged from 0% to 5.4% and the false-negative rate ranged from 0% to 7.0% for all disorders measured in PT challenges for all methods. Reasonable biases of less than $\pm$ 20% of the expected value were achieved for all analytes. **Conclusion:** Providing QC and PT services for analytical measurements in newborn screening demonstrates the need for surveillance to ensure harmonization and continuous improvements and sustain the high-performance of newborn screening laboratories worldwide.

135 - Biotinidase Deficiency Newborn Screening Experience in the Mexican Social Security Institute, 2005 to 2012

B. Valdes Huezo[1], E. E. Delgado Gonzalez[1], M. A. Burciaga Torres[1], J. L. Gonzalez Guerrero[1], and L. A. Ferrer Arreola[1]

[1]Instituto Mexicano del Seguro Social, Coordinación de Programas Integrados de Salud, División de Salud Reproductiva, México DF, México

Introduction: In July 2005, the Mexican Social Security Institute (IMSS) began screening for biotinidase deficiency (BD) as part of institutional nationwide newborn screening program. **Objective:** To report coverage and accumulated incidence of BD in the IMSS newborns in the 2005 to 2012 period. **Methods:** Biotinidase activity in newborns was determined by a colorimetric assay (UMTEST) in dried blood spots on filter paper. Probable case was defined with biotinidase activity test without color change and confirmed case with a biotinidase activity less than 30% of the average healthy individuals, determined by quantitative tests using ultraviolet/visible light spectrophotometry. All detections were collected and registered in the epidemiological surveillance system and were analyzed to obtain the coverage, positive predictive value (PPV), and accumulated incidence. **Results and Discussion:** A total of 3 444 962 samples were analyzed and coverage was 93.3%. In all, 90 probable cases and 12 confirmed cases (PPV 11.8%) were identified. The incidence was 1:287 070 (0.35 $\times$ 100 000 screened); there were confirmed cases in 6 states: Guerrero 1:18 205 (5.5 $\times$ 100 000 screened), Tabasco 1:21 974 (4.55 $\times$ 100 000 screened), Yucatán 1:24 401 (4.10 $\times$ 100 000 screened), Baja California Sur 1:36 325 (2.75 $\times$ 100 000 screened), Jalisco 1:155 816 (0.64 $\times$ 100 000 screened), and Guanajuato 1:174 647 (0.57 $\times$ 100 000 screened). **Conclusions and/or Implications:** The incidence of BD is lower than international reports; however, the real incidence in México is still unknown. This study supports the need to implement the newborn screening in all health institutions in the country.

136 - Comparison of 2 Strategies of Newborn Screening for Cystic Fibrosis: A Pilot Study

G. Maccallini[1], Smithuis[1], C. Aranda[1], M. Junco[1], A. Odriozola[1], G. Ropelato[1], G. Dratler[1], M. Frusti[1], V. Micenmacher[1], Rodriguez[1], Teper[1], and P. Muntaabsky[1]

[1]Programa de Pesquisa Neonatal, Ministerio de Salud, Gobierno de la Ciudad Autónoma de Buenos Aires, Argentina

Two-tier strategy with immunoreactive trypsin (IRT) is currently used in our cystic fibrosis (CF) newborn screening program. The use of IRT in combination with pancreatitis-associated protein (PAP) all on 1 specimen may improve the screening. **Objective:** To evaluate the IRT/IRT strategy and the IRT/PAP strategy in a prospective pilot study. We evaluated newborns (NBs) of the second half of 2011. The NBs with high IRT results in first sample were tested for PAP on same sample and were resent for a second sample for IRT before 25 days of life. Results were considered pathologic when IRT $\geq$60 ng/mL and PAP $\geq$1.6 ng/mL (for IRT 60-100 ng/mL) and PAP $\geq$0.5ng/mL (for IRT >100 ng/mL). Delfia method from Perkin Elmer was used for IRT and enzyme-linked immunosorbent assay method from Dynabio for PAP. The CF confirmation was done by Sweat Test (ST). Of a total of 15 000 NBs, 105 (0.7%) NBs had pathological IRT in first sample; 83 (87%) NBs attended for retesting for a second IRT sample before 25 days of life, constituting the study population. Of the 83 NBs, 20 (24%) showed a second pathological IRT which was referred to perform ST. In contrast, only 6 (7.2%) of 83 NBs had abnormal PAP in first sample and would be referred

to ST. A single patient had pathological ST (IRT/IRT: 190/147 ng/mL; PAP: 1.95 ng/mL; homozygous DF 508). **Conclusion:** The proposed new strategy (IRT/PAP) would reduce false-positive rate by eliminating a second testing, improving diagnosis and reducing family stress

137 - Congenital Hypothyroidism: Experience of Mexican Social Security Institute 2000 to 2012

L. P. Ferrer Arreola[1], J. L. Gonzalez Guerrero[1], B. Valdes Huezo[1], M. A. Burciaga Torres[1], and E. E. Delgado Gonzalez[1]

[1]IMSS Coordinación de Programas Integrados de Salud; División de Salud Reproductiva, México, DF

Introduction: The Mexican Social Security Institute (IMSS) began neonatal screening of congenital hypothyroidism (CH) in 1994 by thyroid-stimulating hormone (TSH) determination in dried blood spots on filter paper. From 1998 to 2009, samples were obtained from cord blood. Since 2010, samples were taken from heel blood. The epidemiologic surveillance system was strengthened in 2000. **Objective:** To report coverage, nationwide and state incidence of CH during the 2000 to 2012 period. **Methodology:** Information was obtained from the epidemiologic surveillance system. TSH levels were determined by immunoenzymatic assay (ultramicro enzyme-linked immunosorbent assay). The cutoff value of cord blood was >20 mUI/mL (2000-2009) and of heel blood was > 10mUI/mL (2010-2012). Probable cases were confirmed by thyroid profile. Detection coverage, suspect index, positive predictive value (PPV), and national and state incidence were analyzed. **Results:** A total of 6 678 702 children were screened (96.5% coverage), 7621 were probable cases (suspect index 0.11%) and 2532 were confirmed cases (PPV: 33.2%). Incidence in the period 2000 to 2012 was 1:2638 (3.8 × 10 000 screened). Confirmed cases were more frequent in Zacatecas, Nuevo León, Estado de México, San Luis Potosí, Tamaulipas, Puebla, Jalisco, and Guanajuato (5.47-6.72 × 10 000 screened). **Conclusions:** The incidence of CH is similar to national and international reports; however, this study reveals a higher frequency in newborns from northeast, central, and Bajio areas.

138 - Congenital Hypothyroidism: What Happen With Preterm and Twins?

P. Garlo[1], K. Franca[1], and G. Queiruga[1]

[1]Instituto de la Seguridad Social, Montevideo, Uruguay

Introduction: Newborn screening in Uruguay began with the study of congenital hypothyroidism (CH). In a hypotonic preterm patient with normal thyroid-stimulating hormone (TSH), the pediatrician request a repetition of TSH. With 20 days of life the TSH was high. The immaturity of the pituitary gland in preterm causes difficulties in the detection of HC, even if there is a lack of T4. We decide to repeat at 20 days of life in preterm and low weight newborn, and we also incorporate repetition in twins. **Objective:** To present 4 cases of preterm and low birth weight detected in the second sample with 20 days of life, which were normal in the first screening. **Materials and Methods:** Newborns were studied from 2007 to 2013, evaluating the number of preterm and the incidence of HC. Cutoff 25 uUI/mL cord blood and heel cutting point at 40 hours 10 μUI/mL. Premature considered under 36 weeks gestation. We select, from the newborn studied, those infants who were normal in the first sample and positive in the second. **Results:** Between 2007 and 2012, 169 492 babies were studied of which 6779 were preterm. We detected 72 babies with CH, with an incidence of 1 of 2354. Data of 4 patients with HC, which would not be detected if we did not performed the repetition of TSH after 20 days of life, are shown. **Conclusion:** We recommend performing another evaluation of TSH in preterm and twins babies.

139 - Congenital Metabolic Diseases in Chiapas: Results of an Expanded Neonatal Screening Pilot Program

R. Ochoa-Tapia[1], R. Sanchez-Zebadua[2], F. Maldonado-Solis[2], R. Salazar-Escalante[2], I. Matus-Alonso[1], C. Moreno-Graciano[2], and R. Enciso-Montoya[2]

[1]Secretaría de Salud del Estado de Chiapas
[2]TamizMas Chiapas, México

Introduction: In the state of Chiapas (Mexico), epidemiology of congenital metabolic diseases (CMD) and the efficiency indicators of neonatal screening (NS) are poorly understood, thus a pilot program was conducted. **Objective:** To know some of the epidemiologic characteristics of CMD affecting the population of Chiapas and analyze the efficiency indicators of NS. **Methods:** An expanded neonatal screening pilot program was implemented in 28 municipalities of Chiapas from February 7, 2012, to September 19, 2012. A total of 10 000 live newborns (LNB) were studied. Newborn screening for 67 diseases was performed (endocrinopathies, aminoacidopathies, organic acidemias, galactosemia, hemoglobinopathies, and other hematologic disorders) by immunoassays, tandem mass spectrometry, and high-performance liquid chromatography. The state was traveled by 3 routes to pick up the samples. The suspected cases were localized by residence search. **Results:** A total of 19 cases were confirmed (1 in every 526 LNB), 10 with congenital hypothyroidism (1 in every 1000 LNB), 3 organic acidemias, 3 aminoacidopathies, 3 cystic fibrosis, and 1 galactosemia. Twenty-three hemoglobinopathies carriers were detected (1:435 LNB). The average sampling age was 9 days. Of suspected cases 100% were located. **Conclusion:** There is great diversity of CMD in Chiapas; these must be followed in order to reduce morbidity and mortality. High frequency of abnormal hemoglobin (1:435) is a very important finding for regional epidemiology. These results may allow better choices on public health, especially in NS.

140 - Deficiency of Glucose-6-Phosphate Dehydrogenase (G6PD) in Newborn of Anápolis and Goiânia – Goiás, Brazil

Andre, Miguel Antônio Isaac[1], Pedatella, Flaviane Marcilia[1,2], Eliane Pereira dos Santos [1], Tais Pires Terra Araujo[1], and Mauricio Carvalho Suzana[1]

[1]*Associação de Pais e Amigos dos Excepcionais de Anápolis – APAE, Anápolis, Brasil*
[2]*Universidade Estadual de Goiás – UEG, Anápolis, Brasil*

Introduction: Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzymopathy, estimating about 400 million people affected worldwide. **Objective:** To determine the incidence of deficiency of G6PD and its relationship with sex in a sample of newborns from the cities of Anápolis and Goiânia-Goiás in Brazil. **Methodology:** From January to December 2011, we analyzed 520 whole blood samples collected by puncturing the heel of the newborn until the 30th day of life on filter paper S&S903. The samples were analyzed by the fluorimetric method semiautomated for the quantitative determination of G6PD activity. Suspicious samples were confirmed with a second test being considered in individuals with deficiency who exhibited enzymatic activity values $\leq$2.6 U/gHb. **Results:** We analyzed 520 samples of newborns, in which 251 (48.27%) were female and 269 (51.73%) male; 517 (99.42%) samples were normal and 3 (0.58%) had deficiency in G6PD activity. **Conclusion:** The incidence in the sample population of newborns Anápolis and Goiania was 0.58%. The 3 samples with total deficiency are male remembering that the mutation occurs in the gene located on the X chromosome, the disease having 1 recessive sex linked. The test used is very simple and has a low cost, so we suggest its adoption in the service routine neonatal screening since an early diagnosis favors a higher life quality. In this way, our results support the recommendation for early diagnosis and the need for more treatment opportunities in the population with G6PD deficiency.

141 - Demographic Characteristics, Social Inequality, and Inequity in People Affected With Congenital Hypothyroidism in Paraguay

M. Ascurra[1,2], M. Samudio[2], and M. S. Rodriguez[1,2]

[1]*Programa de Prevención de la Fibrosis Quística y del Retardo Mental-MSPyBS*
[2]*Instituto de Investigación de Ciencias de la Salud-UNA, Asunción, Paraguay*

Introduction: Congenital hypothyroidism leads to mental retardation if it is not detected and treated early. Economic and social inequalities are closely related to the health disease process and must be taken into account for the elimination of preventable diseases. Hypothyroidism neonatal screening programs should therefore consider social disparities for effective prevention of mental retardation. In this context, in Paraguay the Cystic Fibrosis and Mental Retardation Prevention Program (PPFQRM) provides free and compulsory diagnosis and treatment. **Objective:** To characterize demographically and identify social disparities in people affected with congenital hypothyroidism (CH) in Paraguay. **Methods:** Observational and descriptive study in patients with CH who attended the PPFQRM. With prior informed consent, 73 relatives of 75 patients were interviewed about their sociodemographic and parent job status. **Results:** Of patients with CH, 68.8% were female, 73.3% between 1 month and 5 years of age, 44.0% were first born, and 40.0% the last child. In relation to mother's information, 45% were under 30 years of age, 65.4% with less than 9 years of education, 41.3% live in rural areas, 50.7% have no income and 21.6% informal jobs, and 74.7% have no health insurance. Demographic and socioeconomic characteristics highlight the economic and social inequality that affects these people; it also highlights the importance of PPFQRM support to prevent mental retardation in this group.

142 - Development of a Protocol for Determination of Recovery of Amino Acids and Acylcarnitines by Tandem Mass Spectrometry

G. J. C. Borrajo[1] and M. J. Zelaya[1]

[1]*Detección de Errores Congénitos, Fundación Bioquímica Argentina, La Plata, Argentina*

One of the most important limitations in the determination of the analytical recovery (AR%) of amino acids (AA) and acylcarnitines (AC) by tandem mass spectrometry is the unavailability of samples with no analytes. In order to solve this difficulty, a protocol has been designed for the enrichment of blood samples with AA and AC labeled with stable isotopes (AA* and AC*; NSK-A and NSK-B, CILs) and its posterior analysis. Whole blood samples at 9 concentration levels were prepared trying to cover all the clinical ranges of interest. The analysis protocol consisted in: (1) to quantify the AA and AC present in the nonenriched blood, using methanol/oxalic acid containing internal standards (IS) as extracting solution and (2) to quantify the AA* and AC* added to the enriched samples working with methanol/oxalic acid without IS, and using the signals of the AA and AC and the concentrations determined in (1) as reference for calculations. The AR% corresponding to the evaluated AA was in the range (64%-99%) except for Arg and Asp (24%-32%), while for AC were between 89% and 124%. Nevertheless, these values must not be considered as representative of the complete range as lower AR% values were observed at higher analytes' concentrations, being of higher magnitude for AC. The developed protocol not only allows objectively determining the AR% of AA and AC but also allows evaluating comparatively the extraction efficiency of different extraction solutions in order to define the better analytical assay conditions.

143 - Development of Fully Automated Multiplex Assay for Measurement of Thyroid-stimulating hormone, T4, 17-OHP, and IRT

R. Veturiano[1], A. H. Luques[1], V. L. Frias Silva[1], G. P. Gil[1], B. Gallo[1], J. S. C. Pires[1], and C. A. Sampaio Filho[1]

[1]INTERCIENTIFICA, Sao Jose dos Campos, SP, Brazil

Introduction: The variation in the incidence of diseases related to newborn screening is an imminent factor for differentiation in the process of implementation of existing programs and priorities. In Brazil, the Ministry of Health has been reinforcing the policy for implementation, in all states of Phase III (Cystic Fibrosis) and the inclusion of Phase IV (Congenital Adrenal Hyperplasia and Biotinidase Deficiency). INTERCIENTIFICA, in order to meet the needs of its market, developed a solution in a fully automated system with multiplex assay through NeoMAP 4 Plex kit (TSH/T4/17-OHP and IRT). **Objective:** To present the performance of NeoMAP 4 Plex product in a fully automated system for Newborn Neonatal Screening of congenital hypothyroidism, congenital adrenal hyperplasia, and cystic fibrosis in direct comparative with fluorimetric product which analyzes each parameter individually. **Methods:** The evaluation assays used samples of newborn screening laboratories with NeoMAP 4plex Kit, associating Nimbus reader equipment to Luminex 200/Magpix®. For direct comparative, kits and fully automated equipment using the fluorimetric method were used. **Results:** The results demonstrate the reproduction of sample results when compared to retrospective results with a high level of concordance for all parameters, in addition to advantages related to the use of multiplex analysis technology. **Conclusion:** The fully automated system associated with the use of the NeoMAP 4 Plex Kit has shown to be best option for Newborn Screening of congenital hypothyroidism, congenital adrenal hyperplasia, and cystic fibrosis.

144 - Diagnosis of a Cystic Fibrosis Case by Neonatal Screening and Genetic Confirmation

Rodriguez Agnes[1], Jimenez Mildred[1], Trejos Rafael[1], and Saborio Manuel[2]

[1]Laboratorio Nacional de Tamizaje Neonatal y Alto Riesgo, San José, Costa Rica

[2]Servicio Genética y Metabolismo, Hospital Nacional de Niños, San José, Costa Rica

Cystic Fibrosis (CF) is an autosomal recessive inherited disorder caused by mutations in the CFTR gene. The most frequent mutation is the deletion deltaF508 in the gene; however, there are more than 1400 mutations associated with this disease. Functional disruption of the CFTR protein causes a disorder in chloride transport within epithelial cells, causing an alteration in the exocrine glands, affecting lung, pancreas, liver, skin, and male reproductive tract. Lung disease remains as the leading cause of morbidity and mortality. This article describes, in the framework of a clinical case, the experience of the Neonatal Screening Program in Costa Rica for detection of CF, by quantifying immunoreactive trypsinogen (IRT) in dried blood samples on filter paper using the method of AutoDELFIA Perkin Elmer Neonatal IRT and genetic confirmation of mutations by Oligonucleotide Ligation Assay (OLA) by Abbott. The clinical case corresponds to a male patient with IRT of 107 ng/mL at 4 days of life, second sample at 16 days of 365 ng/mL, and genotyping revealed that he was compound heterozygote for deltaF508/G245X mutations, and a clinical impression compatible with CF. Early detection allowed that this patient to receive timely specialized evaluation by Pneμmology Unit, with a positive impact on morbidity and mortality and discarding several differential diagnoses that would delay treatment. This work demonstrates the importance of implementing a protocol for CF in neonatal screening programs.

145 - Epidemiologic Overview of Congenital Adrenal Hyperplasia in the Mexican Social Security Institute

M. A. Burciaga Torres[1], J. L. Gonzalez Guerrero[1], B. Valdes Huezo[1], L. P. Ferrer Arreola[1], and E. E. Delgado Gonzalez[1]

[1]IMSS Coordinación de Programas Integrados de Salud, División de Salud Reproductiva, México DF, México

Introduction: The Mexican Social Security Institute (IMSS) began detecting congenital adrenal hyperplasia (CAH) since July 2005 as part of a nationwide newborn screening program. **Problem studied and/or objective:** To report coverage and incidence of CAH in the IMSS newborns in the 2005 to 2012 period. **Methods:** Information was obtained from the epidemiological surveillance system. In all newborns, 17-hydroxyprogesterone levels were determined by immunoenzymatic assay (ultra microenzyme-linked immunosorbent assay) in dried blood spots on filter paper; probable cases were identified on a second sample. Cutoffs: in >2500 g at birth >60 μmol/L and <2500 g >82μmol/L. Confirmed cases were identified by plasmatic levels of 17-hydroxyprogesterone by radioimmunoassay. Detection coverage, positive predictive value (PPV), and accumulated incidence were analyzed. **Results and Discussion:** A total of 3 444 962 patients were screened (93.3% coverage); 8832 probable cases were detected (suspect index 0.25%), 394 were confirmed with an incidence of 1:8744 screened (1.1 × 10 000 screened), and PPV of 4.46%. Only in Zacatecas (39 651 screened) and Tlaxcala (28 007 screened) states, no cases were reported; there was a higher incidence in Nayarit, Sonora, Oaxaca, Baja California Sur, Veracruz, Tamaulipas and Sinaloa states. **Conclusion and/or Implications:** Incidence of CAH was similar to international reports; however this study reveals a higher frequency in newborns from coastal states. This has not been reported previously, suggesting that it might be genetically and environmental factors

related and strengthens the necessity of genotype assessment in Mexican cases for genetic counseling.

146 - Evaluation of 6 Years of Expanded Newborn Screening in a Sector of the Neonatal Population of Chile

A. Valiente[1], K. Betta[1], E. Raimann[1], V. Soto[1], J. F. Cabello[1], and V. Cornejo[1]

[1]Laboratorio de Genética y Enfermedades Metabólicas, INTA, U. de Chile, Santiago, Chile

Since 2007, our laboratory has the technology to screen up to 32 disorders in newborns (NB), in addition to phenylketonuria (PKU) and congenital hypothyroidism (CH). This has allowed us to offer the expanded neonatal screening (ENS) as a service. We evaluated 6 years of implementation of the ENS to a sector of the NB population. The analysis is made in dried blood spots on filter paper card of NB, up to 72 hours of life. From the samples are obtained 3 mm diameter discs to be analyzed. The ENS involves aminoacidopathies, organic acidurias, defects of fatty acid oxidation, congenital adrenal hyperplasia (CAH), cystic fibrosis (CF), HC, biotinidase deficiency, and classic galactosemia. We analyzed a total of 13 192 samples, with an average age of 56.4 hours. In this population, 840 (6.4%) were preterm, defined as less than 37 weeks gestation. The results of analysis were obtained in an average of 3.1 days. The average age of NB at the time of the result was about 5.7 days of age. In all, 307 samples were found abnormal, confirming 4 cases of CH, 2 cases of CAH, and 2 cases of CF. Recall rate was 2.3%, receiving 55.7% of second requested samples. For our ENS, the times for preanalytical, analytical, and postanalytical phases are adequate. The ENS is not mandatory; this explains the rate of second samples not received. Increasing the number of diseases increases the benefit of preventing mental retardation and other risk factors.

147 - Evaluation of Expanded Newborn Screening in Uruguay: 4 Years of Experience

C. Queijo[1], A. Lemes[1], F. Gonzalez[1], B. Segovia[1], and G. Queiruga[1]

[1]Instituto de Seguridad Social (BPS), Montevideo, Uruguay

Introduction: National Newborn Screening System in Uruguay is universal, uncharged, and mandatory. It is centralized in the Newborn Screening Laboratory. Expanded newborn screening began in 2009, studying aminoacidopathies, organic acidemias, and β-oxidation defects in dried blood spots from all the newborns of our country. **Objective:** To evaluate, retrospectively, the evolution of different biochemical markers in relation to cutoff values, repetitions and recalls. **Methods:** Analysis of results was done from data obtained for amino acids and acylcarnitines from 2009 to 2012. **Results:** Cutoff evolution for different markers between 2009 and 2012 shows: C3 (3.15 to 7.67 μmol/L), C5OH (0.60 to 0.45 μmol/L), C5 DC (0.13 to 0.36 μmol/L) and Tyr (73.7 to 156.8 μmol/L). These results correlate with the percentage of recall for each marker, which decreases with higher cutoffs and significantly increases with lower cutoffs. Repetition percentage ranged between 0.7% and 1.6%. Recall percentage results ranged from 57% (2009) to 82% (2012). **Conclusion:** Cutoff values evaluation is very important, at least once a year. Although repetition percentages correspond to the expected, introduction of a second-tier test for propionic and methylmalonic acidurias as well as succinylacetone and protocols for premature infants and for newborns on parenteral nutrition could result in a reduction of false positives, avoiding parental stress. Including a professional for patients' recalls and follow-up had improved the recall percentage.

148 - Evaluation of Laser Printing Effect on Filter Paper Used in Newborn Screening

G. J. C. Borrajo[1] and C. M. Di Carlo[1]

[1]Detección de Errores Congénitos. Fundación Bioquímica Argentina, La Plata, Argentina

The printing process on filter paper (FP) used for newborn screening sample collection can affect its properties and indirectly the test results. Considering that there is no information about the laser printing effect, an evaluation protocol was designed. Several experimental parameters that indirectly show FP absorption capacity were studied: (1) blood spots diameter (volume 70 μL, hematocrit 50%), n = 20; (2) absorption time, n = 5; (3) quantitative analytical measurements in blood samples enriched with Phe (5.0 mg/dL), TSH (55 μU/mL), IRT (100 ng/mL), galactose (Gal; 10.0 mg/dL), and Leu (10.0 mg/dL). Samples were analyzed in quadruplicate in 5 different runs using home-made fluorometric methods for Phe, Gal, and branched chain amino acids (BCAA), and AutoDelfia for TSH and immunoreactive trypsin (IRT). The evaluation was made working with Whatman 903 FP (Lot W-112), unprinted (U/P) and printed, with a Laser HP-1320n printer (LP). The comparative analysis between U/P-FP and LP-FP showed no statistically significant differences in the evaluated parameters (*t* test = 0.05: 1) blood spots diameters mean; 12.90 versus 12.81 mm; (2) absorption time mean: 6.87 versus 6.91 second; (3) analytical measurements means: Phe: 6.2 versus 6.3 mg/dL, TSH: 58.9 versus 60.2 μU/mL, IRT: 116.1 versus 120.6 ng/mL, Gal: 8.0 versus 8.1 mg/dL, BCAA mg/dL: 14.2 versus 13.9 mg/dL. Preliminarily, these results allow establishing that laser printing does not affect FP absorption capacity and does not introduce any interference that could affect the results, at least for the evaluated analytes.

149 - Evaluation of 2 Diagnostic Techniques for Congenital Hypothyroidism

J. Chacin[1,2], A. Costagliola[1,2], L. Ocando[2], C. Villalobos[2], J. Picado[2], C. Zanardo[3], M. C. Delgado[3], B. De Guerrero[3], M. Briceño[4], D. Montilla[4], I. Camacho[4], J. Torres[5], D. Villamizar[5]

[1]*Universidad del Zulia*
[2]*Fundación Hospital de Especialidades Pediátricas*
[3]*Centro de Estudio y Prevención del Retardo Mental y Alteraciones en el Desarrollo (CEPREMAD) Programa del Centro de Desarrollo Infantil de Mérida*
[4]*Fundación Centro de Desarrollo Infantil*
[5]*Sub-proyecto 2 Proyecto FONACIT 2008001053. Maracaibo, Venezuela*

The Zulia Neonatal Screening Program is responsible for processing all samples referred from regional health centers for congenital hypothyroidism (CH) diagnosis. Given this responsibility, it was of interest to evaluate thyroid-stimulating hormone (TSH) levels obtained through 2 diagnostic techniques for HC in screened neonates. A total of 1226 newborn screening samples were quantified by ultramicro enzyme-linked immunosorbent assay (ELISA; UMELISA; Tecnosuma) and resolution time fluoroinmunoassay DELFIA (Perkin Elmer). The TSH concentrations obtained were compared using Wilcoxon test for related samples means comparison, and sensitivity and specificity of both techniques were calculated. Both techniques identified 2 patients with HC. The average for UMELISA TSH was 1.4 ± 2.6 versus 2.9 ± 2.8 for DELFIA ($P \leq .0001$). The percentage of false-positive patients was higher by UMELISA 0.9% versus 0.2% for DELFIA. The sensitivity for both techniques was 100% and the specificity was 99.75% versus 99.11% for DELFIA and UMELISA, respectively. Although both techniques have adequate sensitivity and specificity diagnostic performance, TSH values obtained by UMELISA showed greater dispersion and increased false positives.

150 - Evolution and Redesigning of the Brazilian Newborn Screening Program

Tania Marini de Carvalho[1], Ana Stela Goldbeck[1], Ana Clecia Maria dos Santos Marchi[1], Renata Augusto Martins[1], and Paula Juliana Antoniazzo Zamaro[1]

[1]*Ministry of Health / Secretary of Health Care / Blood and Blood Products management/ Brazilian Newborn Screening Program– MS/SAS/CGSH/PNTN (1) – Brasília/Distrito Federal, Brazil*

In Brazil, the first initiative for the newborn screening (NS) started in January 15, 1992, when the Children and Adolescents Bill of Rights made phenylketonuria (PKU) and congenital hypothyroidism (CH) testing mandatory. In June 06, 2001, the Brazilian Ministry of Health created the National Newborn Screening Program (NNSP), aiming to cover 100% of the Brazilian newborns, expanding diseases screened to include PKU, CH, SCD, and CF and specifying the steps to be followed in the process: testing, active case finding, diagnostic confirmation, and specialized multidisciplinary treatment and follow-up of patients. In addition, the program provided resources and established mechanisms for necessary procedures. In 11 years, the program managed to partially meet its targets and became an extremely relevant program as far as preventive medicine is concerned, covering 85% of the national public health system. This gross index, despite its extent and importance, hid the unwanted truth about the heterogeneity of the regional accessibility and the duration of the process as a whole, revealing that the best indexes were in Southern and Southeastern Brazil, thus jeopardizing the equity, universality and integrity proposed by the country's Unified Health System. Such data, highlighted at an NNSP Situational Diagnosis suggested by the Secretary of Health Care of the Brazilian Ministry of Health (2012), started a broad redesigning of the NNSP, which was considered as a target of the country's administration. The first results of this new political momentum show huge advances in the national newborn screening (NNS) scenario.

151 - Expanded Neonatal Screening Program in the Health Services From Mexican Secretary of the Navy: Implementation and Results

De la Torre Garcia Oliver[1], Vera Olvera Jose Jaime[2], Mar Aldana Roberto[3], and Trigo Madrid Ricardo Max[4]

[1]*Genética y Genómica Médica, Secretaria de Marina Armada de México, México*
[2]*Pediatría y Neonatología, Secretaria de Marina Armada de México, México*
[3]*Hematología Pediátrica, Secretaria de Marina Armada de México, México*
[4]*Salud Publica; Sanidad Naval, Secretaria de Marina Armada de México, México*

Introduction: Currently, neonatal screening (NS) is performed for 1 to 4 diseases in the main social security systems of Mexico. In July 2012, the Naval Health Directorate of the Ministry of the Navy of Mexico (SEMAR for its acronym in Spanish) amended its program of NS, expanding it from 4 to 67 diseases. **General Purpose:** To present results of SEMAR NS new strategy. **Methods:** The NS was performed in all newborns (NB) in SEMAR (14 hospitals, 13 nursing homes and 10 clinics) in 17 coastal states, and the Mexico City. The NB included detection of 5 endocrinopathies, 2 carbohydrate metabolism disorders, 25 organic acidemias, 20 aminoacidopathies and 15 hemoglobinopathies by tandem mass spectrometry, immunoassays, isoelectric focusing and high performance liquid chromatography. **Results:** From July 2012 to July 2013, NS was performed to 2909 children born in the SEMAR (98.7% coverage). The average age at the time of sampling was 5 days ($\pm$ 2). Two cases were confirmed: 1 congenital hypothyroidism and 1 mild glucose-6-phosphate dehydrogenase deficiency. A total of 36 heterozygotes were detected: 2 HbC, 1 HBG-Philadelphia, and 33 HbS. The cases started treatment at the Naval Hospital

of High Specialty. For hemoglobinopathies carriers, family genetic study was performed allowing diagnosis of other affected family members, appropriate genetic counseling was given. **Conclusion:** In our population 1:1455 NB present a birth defect, and 1:485 are hemoglobinopathies carriers, which support the NS implementation for these diseases.

152 - Experience Expanded Newborn Screening Strategy: 4 Markers in the State of Aguascalientes, Mexico

Y. Rodriguez Badillo[1], R. Reyna de Jesus[1], B. Valdes Garcia[1], I. Trinidad Alderete[1], M. Marmolejo de la Rosa[1], S. Hernandez Zavala[1], F. Briones de la Torre[1], P. Martinez Gigena[1], and J. Lopez Valdez[1]

[1]*Instituto de Servicios de Salud del Estado de Aguascalientes, México*

Introduction: Screening for phenylketonuria (PKU), congenital adrenal hyperplasia (CAH) and galactosemia, in public health services in Mexico is conducted since 2011. At the beginning of the program, cutoff values used were obtained from the literature, thus specificity must be evaluated due to the high genetic variability among populations of the country. **Objectives:** To define specific cutoff values for PKU, CAH, and galactosemia screening in Aguascalientes population and perform clinical evaluation of these. **Methodology:** To do this, we performed 3 statistical analyses of the results of biochemical analysis by fluorometric detection of 23 934 samples from Aguascalientes' newborns, integrating the follow-up results from suspect cases. **Results:** The first statistical analysis of 7181 samples showed the low specificity of the cutoff value used to CAH (20 ng/mL), with a positive predictive value of 0.7%, suggesting premature stratify the population. Patients suspected with PKU and galactosemia insufficient to perform the cutoff clinical evaluation, the positive predictive index for these were 50% and 0% respectively. After analyzing more than 23 900 samples, a major number of confirmed positive cases were available, including some positive cases with small biomarker elevations over cutoff value, allowing clinical validation of these. The number of suspected false cases were reduced up to 80%, and values of the positive predictive index reached over 20%. **Conclusion:** The evidence showed the need for specific populations' cutoff values in metabolic newborn screening, being of particular relevance to CAH, PKU, and galactosemia, in order to provide useful information for timely medical care and efficient resources usage.

153 - Experience of Congenital Adrenal Hyperplasia Screening in Uruguay

K. Franca[1], P. Garlo[1], B. Segobia[1], F. Gonzalez[1], L. Corbo[1], M. Machado[1], C. Queijo C[1], and G. Queiruga[1]

[1]*Instituto de la Seguridad Social, Laboratorio de Pesquisa Neonatal; B.P.S., Montevideo, Uruguay*

Introduction: Screening for congenital adrenal hyperplasia (CAH) is mandatory for all newborns (NB) in Uruguay since November 2007. It is done by 17-OH-progesterone quantification in whole blood obtained from heel prick, after 40 hours of life on S & S 903 filter paper. **Objective:** To report the confirmed cases found from January 2008 to June 2013. **Methods:** Hormone quantification was performed by competitive enzyme immunoassay, using Quantase TM 17-OHP Neonatal Screening-BIO-RAD on a CODA Open Microplate System. The cutoff value (CO) was 12.0 ng/mL. Since 2012 other CO calculated for our population have been applied for NB of 34, 35, and 36 weeks of gestation. Twins and premature newborns were asked to repeat the test at 20 days of life. **Results:** From 264 392 samples tested, 1.3% required retesting, of which 75% were from preterm infants. In all, 28 cases of CAH were found, 64% were NB weighing more than 2500 g. In 1 premature NB with CAH, the first sample was lower than the corresponding CO, but he had a higher value at 20 days of life. **Conclusion:** During the studied period the incidence was 1 in 9442, 25% of the cases were full-term male newborns without clinical symptoms at diagnosis. Additionally, it was evidenced the importance of repeating the test in preterm infants.

154 - Extended Neonatal Screening in Rural Areas of Mexico

P. Perez Reyes[1], J. Olvera Alvarez[1], F. Ortiz Garcia[1], and M. Munguia Ramirez[1]

[1]*Mexican Institute of Social Security, Program IMSS Oportunidades, Federal District, Mexico*

Introduction: In Mexico, IMSS (Mexican Institute for Social Security) Oportunidades takes care of rural marginalized population. It carries out neonatal screening in 3668 rural medical units. The screening of congenital hypothyroidism (CH) started in 2000 and of phenylketonuria (PKU), congenital adrenal hyperplasia (CAH), and biotinidase deficiency (BD) in 2007, and of classic galactosemia (CG) in 2012. **Objective:** To identify the frequency of HC, PKU, CAH, DB, and CG in infants taken care of in period 2000 to 2012. **Methods:** Cross-sectional and descriptive study. Information of the screened children and of confirmed cases was collected. Detection was performed by immunoenzymatic test (ultramicro enzyme-linked immunosorbent assay (UMELISA). Blood sample for HC detection was obtained from umbilical cord (newborn) or heel (third or fifth day of life). Samples for PKU, CAH, DB and CG were collected from heel. Diagnoses were confirmed through determination of thyroid profile (HC); 17-hydroxyprogesterone, cortisol, and testosterone by radioimmunoanalysis (CAH), quantification of biotinidase activity by spectrophotometry of visible–ultraviolet light and organic acids by gas chromatography-mass spectrometry. **Results:** In all, 370 cases with HC were confirmed among 1 544 535 screened children, (1:4174 frequency). Extended neonatal screening was carried out in 743 713 children, 25 were confirmed for CAH (1:29,748 frequency), and 2 for DB (1:371,856 frequency), without PKU or CG confirmed

cases. **Conclusion:** The IMSS Oportunidades is conducting extended neonatal screening since 2007, in areas of high geographic dispersion of Mexico, in which follow-up of patients is difficult. Improvement strategies have been established to detect, diagnose and treat affected children.

155 - Extent and Functioning of the Neonate Testing Program During the Term 2010 to 2012

M. C. R. Flores[1] and S. S. Fernandez[2]

[1]Servicio Autónomo Hospital Universitario Antonio Patricio de Alcalá
[2]Instituto Autónomo de Altos estudios Dr. Arnoldo Gabaldón, Cumaná, Venezuela

The neonate screening program (NSP) began in the state of Sucre, Venezuela, around 2005 through 2006, coordinated by the Regional Office of Epidemiology, as part of a pilot program introduced in the country. This research evaluated the range and functioning of the NSP in the state, between 2010 and 2012, with the purpose of optimizing its performance to improve newborns' quality of life. Objectives formulated allowed us to describe NSP as it has been implemented in the state, in order to establish its range, identify participating regions, establish percentage of newborns tested in that period, determine nursing staff, laboratory staff, and newborns' parents or guardians knowledge about newborn screening; check received samples' quality during the year 2012 as well as results' reports. This was a semiquantitative descriptive transversal retrospective field, research. As a result, we observed that in 66.7% of the region sample for testing are collected; percentage of tested newborns reach an average of 67.9%. Regarding people's general knowledge about the test, nursing staff got 43%, lab staff 87%, and the parents or guardians ignore test usefulness, despite having heard of it. In general terms, the NSP presents great strengths in its structure, with some weaknesses in its performance.

156 - False-Negative Frequency to Congenital Hypothyroidism by Cutoff Value

L. Ocando[1], J. Chacin[1,2], C. Villalobos[1], J. Picado[1], and A. Costagliola[1,2]

[1]Pediatric Specialty Hospital Foundation, Maracaibo, Venezuela
[2]University of Zulia, Maracaibo, Venezuela

The strategy to diagnose congenital hypothyroidism (CH) in neonatal screening and the cutoff point for thyroid-stimulating hormone (TSH) must be checked for each population. Venezuela has been using 15 mUI/L as the cutoff point, but there are several cases of HC which have been misdiagnosed, for this reason it is proposed to compare the frequency of HC by decreasing the cutoff point for TSH 15 to 10 mUI/L. The TSH levels of 186 327 children assessed in the Zulia Neonatal Screening Program were quantified by ultramicro enzyme-linked immunosorbent assay (UMELISA; Tecnosuma) using a cutoff point of 15 mUI/L between January 2006 and May 2012; from June 2012 until now, a cutoff of 10 mUI/L is use in the first sample and 5 mUI/L for the second sample. The sensitivity and specificity of the cutoff value were calculated, keeping 15 mUI/L (2006-2013) versus actual experience of the program by reducing the cutoff point to 10 mUI/L. Of 186 327 children tested, 36 (1.9%) were found with HC when the cutoff value was maintained at 15 mUI/L; HC frequency increases to 42 (2.3%) by lowering the cutoff value of 10 mUI/L. The sensitivity was 0.88 and 0.98 for 15 mUI/L and 10 mUI/L, respectively; specificity holds for both cutoff points in 0.99. Decreasing the cutoff point from 15 to 10 increases the sensitivity of the test, generating fewer false-negative individuals.

157 - Familiar Experiences: From Recalling to Treatment

V. Micenmacher[1], A. Chiesa[1], C. Fraga[1], M. Stivel[1], G. Ropelato[1], A. Dure[1], C. Aranda[1], and P. Muntaabski[1]

[1]Ministerio de Salud de GCBA - Programa de Pesquisa Neonatal de Ciudad Autónoma de Buenos Aires, Argentina

Introduction: The families of the recalled newborn in a neonatal screening program (NSP) undergo a process of distress/anxiety. **Objective and Methodology:** In order to assess the experiences of the recall/diagnosis process, a semistructured survey with psychosocial interpretation was administered. Variables such as program knowledge anxiety during the recall procedure and while waiting for diagnostic confirmation and treatment, need of improvement opportunities in psychosocial field and access to treatment supplies, were investigated in 16 families of patients with confirmed pathologies (G1) and 16 families of healthy patients that were recalled but not confirmed (G2) and were used as a control group for the first stages. **Results:** Of the 32 families, 60% were waiting for the screening results. The 69% of G1 versus 25% of G2 did not understand the explanation provided when recalled. Of G1, 50% referred a high anxiety level while waiting for diagnostic confirmation and treatment, needing improvements in the initial information provided and to be heard in a more guided environment in that circumstance. The anxiety was linked to the ignorance of the disease referring that they would have needed an early exchange of information with other families with similar experiences. There was no distress related to treatment supply. **Conclusion:** The recall diagnostic process causes a high level of distress/anxiety that persists in patients with confirmed diagnosis. An environment of contention and listening, sharing with other families and multimedia artwork would help families that undergo this process.

158 - First Validation of Capillary's Neonate Hemoglobin for Newborn Screening for Hemoglobinopathies in Brazil

S. M. Hadachi[1], H. H. Martins[1], L. R. R. G. Garcia[1], G. B. S. Ascari[1], E. M. Silva[1], J. M. Viana[1], T. D. P. Oliveira[1], T. M. Anjos[1], R. S. Luongo[1], M. A. M. Iskandar[1]

[1]APAE DE SÃO PAULO, São Paulo, Brasil

Newborn screening (NS) has a fundamental role in early diagnosis of hemoglobinopathies. The methodologies used are based on the separation of fractions of hemoglobin (Hb) by isoelectric focusing (IEF) and high-pressure liquid chromatography (HPLC). The capillary system uses capillary zone electrophoresis (CE), with good resolution and fast separation. The goal is to perform tests comparing quantitative results between Capillary and IEF/HPLC. We analyzed 224 samples from hemoglobinopathies routine of APAE DE SÃf O PAULO. A total of 130 (58.1%) samples showed a normal Hb profile (FA / FA / FA * / AA), and 94 (41.8%) Hb variants: 50 (22.3%) HbFAC / ACF, 39 (17.4%) HbFAS / ASF, 2 (0.9%) HbFS, and 3 (1.2%) for Hbs FSC, FAD, FC. The mean (standard deviation, SD) concentrations of Hb were concordant in both the methodologies, except for HbF 49.9% (SD: 8.83) in HPLC and 50.2% (SD: 10.67) in EC ($P = .064$). In total, 98.2% of the samples showed agreement between the HPLC and CE methods with Cohen coefficient of 0.971 ($P < .001$). Analysis of 4 (1.8%) samples that showed disagreement between methods, the mean concentrations of HbA 4.2% (SD: 0.34) in HPLC and 4.3% (SD: 0, 53) in the EC, are acceptable considering that is a semiquantitative method. The concentration of 4.5% of Hb â‰ is considered a standard result (Hb × FA) that belongs to an internal security criterion for β thalassemia trait investigation. Both methods showed agreement proving to be effective for hemoglobinopathies detection.

159 - Five Years of Experience in the Neonatal Screening Program for Congenital Hypothyroidism (CH) in La Paz, Bolivia

Jove Alba[1]

[1]Hospital Arco Iris, La Paz

Introduction: Congenital hypothyroidism (CH) is the only disease studied by the neonatal screening program. **Objective:** To evaluate the results and determine CH prevalence in our program. **Materials and Methods:** We included all newborns (NB) delivered between April 2008 and April 2013. Quantification of TSH in blood collected on filter paper was done using a time-resolved immunofluorometric method (DELFIA). Thyroid-stimulating hormone (TSH) values above 10 uUI/mL were considered suspicious and were confirmed by complete thyroid profile (ultrasensitive TSH, T3, T4 and free T4 in serum by DELFIA**). Results:** A total of 64 686 RN were screened, of them 518 were probable and 43 were confirmed, of which 60% were girls. **Conclusion:** The cumulative experience in 5 years of the CH screening program allows us to observe a prevalence of 1:1504. This frequency is higher than reported in other countries of South America and the world. Hence, the importance to continue with the program and do it nationwide.

160 - Galactosemia in the Province of Santa Fe

L. Maggi[1], M. Moscato[1], and A. Argüelles[1]

[1]Provincial Laboratory of Endocrine Metabolic Inborn Error Neonatal Research- Biochemistry and Pharmacy Office, Santa Fe, Argentina

Introduction: Galactosemia is a disorder in carbohydrate metabolism and expression of the genetic disorder is reflected through a galactose metabolism enzyme failure. **Goal:** To analyze the results of the January 1, 2008 to December 31, 2012, period research assessing: (1) number of children studied, (2) number of positive cases, (3) recitation rate, (4) false positives, (5) average yearly population coverage, and (6) incidence in Santa Fe population. **Methodology:** Cross-sectional retrospective study. Dry blood samples were taken in filter paper SS 903 through heel incisions on newborns older than 36 hours. Total galactose was measured through fluorometric enzyme method, cutoff point: 10 mg/dL. Children with values between 10 and 30 mg/dL were requested a new card with a 3-hour fast, and for children with $\geq$ 30 mg/dL 1 Phosphate Uridil Transferase enzyme was determined. Positive cases were confirmed through molecular biology. **Results:** In 131 820 newborns tested with 97.57% of average population coverage, results were: 44 suspects recitation rate: 0,033%, 4 confirmed cases (false positives: 0.03%), 1 classic galactosemia (Incidence: 1/131 820), 2 duarte variant cases (incidence 1/65 910) and 1 heterozygous. **Conclusions:** Results underline the importance of galactosemia screening as it enabled 4 children to be referred to Nutrition and Medical Genetics Centers for evaluation, diagnosis, specific medical treatment, genetics advice and family planning.

161 - Galactosemia: A Case Report

E. Crespo[1], M. De Lucca[1], K. Araujo[1], C. L. Dominguez[1], Y. Guevara[1], A. Mahfoud[1], L. Perez[1], L. Sua[1], M. Ramirez[1], L. Casique[1], I. Arias[1], S. Rodriguez[1], Y. Ceballos[1], and R. Canelon[1]

[1]Unidad de errores innatos del metabolismo (udeim)-fundacion instituto de estudios avanzados (IDEA), Caracas, Venezuela

Galactosemia is an inborn error of carbohydrate metabolism, which is caused by the deficiency of 3 enzymes involved in the catabolism of galactose. The most common cause is the deficiency of galactose 1-P uridil transferase (GalPUT), resulting in the accumulation of galactose, galactose-1-phosphate, galactonate, and galactitol. Infants with galactosemia may have symptoms including hepatocellular dysfunction, sepsis by *Escherichia*

coli, cataracts, neurological depression, weight loss, and shock. We present a 5-day-old girl, referred to the laboratory for neonatal screening. Total galactose value was 24.0 mg/dL (cutoff point of 10.5 mg/dL). In the second sample, the value obtained was 21.9 mg/dL, and as expected the GalPUT activity was completely absent. Following soy milk treatment, 2 successive determinations showed galactose concentrations of 9.79 and 4.3 mg/dL, respectively. The patient had normal temperature and was hydrated, she was active with no evidence of lens opacity or hepatomegaly; however, a hypertonic motor disorder was still evident. Molecular study was performed to determine the phenotype causing mutations in the *GALT* gene, and 2 mutations were detected: p.N314D and p.X380C in exons 10 and 11, respectively. Currently the patient is 2 years of age, showing satisfactory progress. Taking this into account, we can conclude that an invaluable contribution is provided by neonatal screening programs, enzymatic and molecular studies, and treatment as well as timely and appropriate follow-up, on morbidity and mortality of screened diseases.

162 - Girolab Neonatal Research Center in Venezuela Detects Presumptive Positives for Multiple Disorders: Results of the Past 3 Years

P. Martinez[1], R. Rodriguez[1], E. Nuñez[1], and G. Merzon[1]

[1]GIROLAB Centro DE Pesquisa Neonatal C.A

Introduction: In most Latin American countries, neonatal screening is very important, although the percentages of presumptive positives in the population are generally unknown. GIROLAB Neonatal Research Center (Private Reference Center) has provided screening since 2001, testing a total of 107 423 children. It is important to note that in the private sector, GIROLAB is the laboratory that currently processes the highest number of analytes in Venezuela. The results obtained in the last 3 years of screening are presented for congenital hypothyroidism (TSH), phenylketonuria (PKU), galactosemia (GAL), cystic fibrosis (immunoreactive trypsin [IRT]), glucose-6-phosphate dehydrogenase (G6PD) deficiency, biotinidase (BIOT), congenital adrenal hyperplasia (17-α-hydroxyprogesterone [17-α-OHP]), and hemoglobinopathies (Hb). **Methods:** Neonatal thyroid-stimulating hormone (TSH), free thyroxine (FT4), human TSH (hTSH), PKU, GAL, G6PD, IRT, BIOT, Hb, and 17-α-OHP were analyzed using technology, reagents, and kits from PerkinElmer Inc. Upon suspected positive results a new sample was taken on filter paper to conduct a second test. If the result remained altered, the patient was immediately referred to appropriate specialists. **Results and Discussion:** A total of 32 894 children were examined for TSH; 32 164 for PKU; 30 473 for Gal; 19 884 for IRT; 21 122 for G6PD; 16 827 for BIOT; 15 451 for 17-α-OHP; and 5060 for Hb. We identified 89 suspected cases identified as positive for TSH, PKU (1), GALT (8), IRT (5), G6PD (108), BIOT (3), 17-α-OHP (4), and Hb (24). **Conclusion:** Based on the results obtained in the laboratory in the past 3 years, we can conclude that the disorders with the highest percentage of presumptive positives were G6PD and Hb.

163 - Detection of Hemoglobin Variants in Costa Rican Neonatal Population

A. Gabriela[1], N. Marta[2], V. Katia[2], S. Gabriela[2], T. Rafael[1], R. Julio[1], and S. Manuel[1]

[1]Programa Tamizaje Neonatal, Hospital Nacional de Niños
[2]Servicio de Genética Médica y Metabolismo, Hospital Nacional de Niños

A total of 426 418 whole blood samples were collected in filter paper (S&S 903) from January 2006 to December 2012 from newborns in Costa Rica. Samples were analyzed to detect hemoglobin variants with isoelectric focusing technique and then confirmed by cation exchange chromatography and high-performance liquid chromatography (HPLC). Results showed that 8214 cases presented some variant, corresponding to a 1 out of 52 frequency. In all, 62 cases were homozygous for hemoglobin S genotype and 8 cases for double heterozygous genotype SC. Additionally, 14 cases present S + Beta variable genotype. This study showed that S and C hemoglobin variants are distributed throughout the country, even though most of the cases were found in the center, north, and west of the country. Information obtained in the 7 years of study of hemoglobin variants in Costa Rican neonatal population confirms that early detection of cases is necessary. Additionally, a national program of education directed to affected, carrier individuals, their families, and medical personnel is required for a proper genetic counseling and to improve the treatment and reduce morbimortality.

164 - Hemoglobinopathies: Newborn Screening Pilot Study in Uruguay

P. Audicio[1], M. S. Mendez[1], and G. Queiruga[1]

[1]Laboratorio de Pesquisa Neonatal, Instituto de la Seguridad Social, Montevideo, Uruguay

Background: Hemoglobinopathies are a group of autosomic recessive congenital disorders that affect the synthesis and structure of hemoglobin. These diseases are frequent in Mediterranean area and Africa. Many Latin American countries have included hemoglobinopathies in their newborn screening programs. Most Uruguayan population are of European descendant; and according to the most recent national census, 8% are Afro descendant. **Objective:** To determine hemoglobinopathies carrier incidence in newborn population in Uruguay. **Methods:** Dried blood spot samples from Laboratorio de Pesquisa Neonatal between January and April 2013 were studied. Samples were analyzed by high-performance cationic exchange (HPLC Variant nbs; BIORAD). **Results:** A total of 11 200 samples from Uruguay were screened; 84 presented hemoglobinopathy traits: 66 FAS, 15 FAC, and 3 FAD, and

2 probably disease: 1 sickle cell disease and 1 double heterozygote sickle-cell beta thalassemia. These results correspond to the incidences of 1 out of 133 for carriers and 1 out of 5200 for affected. **Conclusion:** National health policies, whose priority is to improve the quality of life and the incidence of hemoglobinopathies carriers presented here, justify the necessity of including hemoglobinopathies in Uruguayan newborn screening program.

165 - Hemoglobinophaties' Detection in Families of Patients Diagnosed During the First Semester of 2010 in Rafael Calvo Maternity Clinic of Cartagena

S. Romero[1], M. Viola-Rhenals[1], C. Alvear[1], C. Moneriz[1], and M. Barboza[1]

[1]Universidad de Cartagena, Cartagena de Indias, Colombia

Introduction: Hemoglobinopathies are inborn hereditary disorders that include a heterogeneous group of anemias. They are the most common diseases in humans, affecting approximately 5% of the world population. Prevention of complications due to hemoglobinopathies is based on the identification of individuals at risk through family history or by screening programs for carriers and providing adequate information about the risks and the possibilities to minimize it. **Objective:** To detect hemoglobinopathies in families of patients diagnosed in the first semester of 2010 at the Maternity Clinic Rafael Calvo of Cartagena. **Methods:** Clinically significant hemoglobinopathies were detected in 23 patients using isoelectric focusing technique. **Results:** There were 12 cases (52%) of family traits of sickle-cell anemia and 11 cases (48%) of hemoglobin C carriers. Importantly, 95.6% of the studied families were unaware of their carrier condition. **Conclusion:** This study allowed the confirmation of hemoglobinopathies in children previously screened and allowed to detect the presence of such diseases in their families. Hence, the introduction of a compulsory neonatal screening program including familiar environment should be a health priority in Cartagena city.

166 - High Incidence of Congenital Hypothyroidism in the State of Tabasco, Mexico

F. Maldonado-Soli[1], M. Maldonado-Solis[2], C. Moreno-Graciano[1], L. Herrera-Perez[1], P. Martinez-Cruz[1], M. Sierra-Salas[1], O. Callejas-Francisco[2], and R. Salazar-Escalante[3]

[1]Laboratorios Químicos Maldonado SA de CV
[2]Laboratorio Tamizaje Plus SA de CV
[3]Tamiz Ampliado Yucatán SA de CV, Dirección del Régimen Estatal de Protección Social en Salud REPSS Tabasco, Departamento de Salud Reproductiva del Estado de Tabasco, Hospital de Alta Especialidad del Niño de Tabasco "Rodolfo Nieto Padrón," Secretaría de Salud del Estado de Tabasco, Tabasco, México

Introduction: Congenital hypothyroidism (CH) is the most common preventable cause of mental retardation. The earlier is the diagnosis and treatment initiation, the lower the neurological damage. The prevalence of CH is estimated at 2.5 to 3.3 cases per 10 000 living newborns (LBs); however, there are significant ethnic and geographic variations. **Methods:** Thyroid-stimulating hormone (TSH) was quantified by time-resolved fluorometry with a cutoff value of 7 μU/mL. A residence search strategy was implemented to find suspected cases, these were confirmed by thyroid ultrasound and thyroid profile. The prevalence rate of CH was calculated as the number of confirmed cases per 10 000 LBs examined in this period. **Results:** Between September 2007 and March 2013, 207 313 newborns were screened; 255 suspected cases were found and 172 were confirmed (8.53 per 10 000 LBs), with predominance of female cases (71%). The average age at treatment onset was less than 16 days. The most frequent cause of CH was thyroid hypoplasia (52%) followed by agenesis (39.4%) and dyshormonogenesis (1.1%). The region with most cases of CH was La Chontalpa (43% of cases) and the least affected region was La Sierra (5% of cases). **Conclusion:** The birth prevalence of CH in Tabasco from 2007 to 2013 was 8.53 × 10 000 LBs. This frequency is higher than that reported in other regions of the country and previous registers in the state.

167 - Homogeneity in the Material for Performance Evaluation in Neonatal TSH

D. B. Robayo[1] and A. J. Bermudez[1]

[1]Instituto Nacional de Salud. Laboratorio Nacional de Referencia, Grupo de genética, Bogotá DC, Colombia

Introduction: Neonatal screening for congenital hypothyroidism measuring thyroid-stimulating hormone (TSH) is evaluated through interlaboratory assays. ISO-IEC.55.1 established that homogeneous material is required for comparisons between laboratories, as shown in split sample from a positive case. It is also valid to use a certified standard to prepare material as formulated product. **Problem:** Each laboratory should receive the same sample, ensuring that measurement errors are not attributable to variations in material for testing. For this purpose, an automated method was designed to ensure a homogeneous sample. **Methods:** Thyroid-stimulating hormone samples with low, medium, and high concentrations relative to the cutoff value (15 mIU/L), with certified Sigma standard, Whatman Paper 903, EP-motion robotic system were used. Several protocols were tested for system programming to set the optimal routine sample application on Whatman paper, considering speed, movements, drop height, and volume. Each protocol was assessed by measuring the TSH homogeneity, repeatability, and reproducibility. **Results:** Initial tests were made with theoretical conditions for microplate assembly, variation in droplet size and TSH concentration were high, as

shown by homogeneity values measured by slope >0.5, expected <0.009. We designed a new device for supporting Whatman paper and optimized conditions to get outstanding routines for each concentration and check repeatability and reproducibility in successive measurements. **Conclusion:** Test material preparation with robotics automation and formulated product ensures the homogeneity required for interlaboratory assays.

168 - Hyperphenylalaninemia Neonatal Screening in Paraguay

L. Ortiz-Paranza[1], M. Ascurra[1,2], S. Rodriguez[1,2], M. Cabral[2], A. Valenzuela[1,2] and I. Dalles[1]

[1]*Cystic Fibrosis and Mental Retardation Prevention Program – MSPyBS*
[2]*Research Institute for Health Sciences-UNA, Asuncion, Paraguay*

Hyperphenylalaninemia (HPA) is an inborn error of metabolism on hepatic conversion of phenylalanine (PA) to tyrosine (TYR). Biochemically, there is an increase in plasma levels of PA >2 mg/dL in fasting. The PA values >2 and <6 mg/dL correspond to an HPA, subjected to periodic PA controls, with the possibility of moving to a phenylketonuria (PKU), with PA values >6 mg/dL, causing severe and irreversible neurologic damage. **Objective:** To evaluate and report the experience of HPA cases detected and monitored in the Cystic Fibrosis and Mental Retardation Prevention Program (PPFQRM). **Methods:** Cross-sectional study of clinical cases diagnosed with HPA detected between October 1999 and July 2013. **Results:** A total of 379 517 newborns were tested and 39 were diagnosed with HPA; 20 female and 19 male; HFA 20/39 and PKU 19/39; classical PKU 2/13 (PA >20 mg/dL at diagnosis); 12/19 PKU followed diet adequately showing PA values within normal range, 3/19 followed diet indication irregularly, and 4/19 parents discontinued the diet. Age at treatment initiation was between 21 and 59 days, with current age ranging from 6 to 67 months. **Conclusion:** Both age of treatment onset and adherence to dietary therapy are critical aspects that require improvements to prevent mental retardation and to improve the quality of life of patients and their families, taking into account that treatment must be performed lifelong.

169 - Hypertension, Congenital Hypothyroidism, and Iodized Salt Consumption During Pregnancy

M. Ascurra[1,2], M. Samudio[2], and F. Blanco[1]

[1]*Cystic Fibrosis and Mental Retardation Prevention Program – MSPyBS*
[2]*Research Institute for Health Sciences-UNA, Asuncion, Paraguay*

In Paraguay, the consumption of iodine occurs mainly through iodized salt. Treatment for hypertension (HT) includes reduction or elimination of salt consumption. Hypertension is a common condition in pregnant women, affecting about 20%. **Objective:** To identify the frequency of low consumption or suppression of iodized salt in pregnant women who gave birth to congenital hypothyroidism children (CH). **Methodology:** Descriptive study of mothers of patients with CH, who attended the Cystic Fibrosis and Mental Retardation Prevention Program (PPFQRM). With prior informed consent, 68 mothers were interviewed about their condition of HT during pregnancy and treatment indication. **Results:** Of the mothers, 32.3% (n = 22) reported having had hypertension during pregnancy, of whom 17 completely stopped salt intake and 5 decreased it. None of them reported having received iodine supplementation, although the recommended daily intake of iodine during pregnancy is twice than usual. A subclinical hypothyroidism and antihypertensive treatment of the mothers may be associated with the development of congenital hypothyroidism in the newborn. This situation must be addressed through unifying concepts, early detection of women at risk and administration of appropriate therapy, taking into account both pathologies.

170 - Immunoreactive Trypsin Stability in Blood Samples on Filter Paper

A. L. Albrekt[1], L. I. Czubarko[1], and P. A. Klein[1]

[1]*Programa de Pesquisa Neonatal Misiones, Laboratorio LACMI, Misiones, Argentina*

Introduction: Immunoreactive trypsin (IRT) in blood samples on filter paper (SFP) is stable between 7 and 15 days and is mainly affected by elevated temperatures. **Objective:** To compare the stability of IRT in SPF preserved at room temperature (RT) and refrigerated (cold temperature [CT]). **Methods:** A total of 198 SFP were collected in sequence in FP; all samples were obtained and transported immediately to the laboratory. Samples were separated into 2 aliquots and stored at RT and CT, respectively; IRT concentration was determined in each at 4, 14, 24, and 33 days. We used a commercial enzyme immunoassay (EIA) kit for IRT, with internal quality controls provided by the manufacturer and external quality control by CEMIC. Room temperature in the study period ranged between 15°C and 35°C. **Results:** Results are shown in Table 1; IRT concentrations in ng/mL at 4, 14, 25, and 33 days of sample collection expressed as medians. Taking the first concentration measured as 100%, the percentage decline CT was 100% (14 days), 96.5% (25 days), and 101.5% (33 days) and at RT 100% (14 days), 76.7% (25 days), 73.3% (33 days). **Conclusion:** A marked decrease in IRT concentration was observed in uncooled samples after 14 days for the environmental temperature range listed above, so we do not recommended processing them when the time elapse between collection and receipt exceeds this term.

171 - Incidence of Inborn Errors of Metabolism Detected by Expanded Newborn Screening in Nuevo León, Mexico: Experience of 10 Years

Torres Rosario[1], E. Martinez Laura[1], Ruiz Consuelo[1], Castillo Erik[1], Lopez Areli[1], Sanchez Alejandra[1], Gonzalez Rogerio[1], Arredondo Patricia[1], and Z. Villarreal Jesus[1]

[1]*Departamento de Genética, Facultad de Medicina y Hospital Universitario, Nuevo Leon, Mexico*

Background: Until 2010, only congenital hypothyroidism (CH) was detected by newborn screening in Mexico, then phenylketonuria (PKU), adrenal hyperplasia, and galactosemia were included. In Nuevo León (state located in Northeast Mexico), a pilot expanded neonatal screening program was initiated 10 years ago. **Objectives:** To know inborn error metabolism (IEM) incidence and to obtain experience in order to implement the program in other states. **Methods:** Blood spots were collected from newborns at 24 hours of life and used to quantify 17-hydroxyprogesterone (17-OHP), immunoreactive trypsin (IRT; AutoDELFIA) biotinidase, glucose-6-phosphate dehydrogenase (G6PD), total galactose (DELFIA), acylcarnitines, and amino acids (mass spectroscopy [MS]/MS). Positive results underwent confirmatory tests and positive cases were followed up. **Results:** From December 2002 to November 2012, 111.287 newborns were screened. A total of 115 cases were confirmed: 51 G6PD deficiency, 22 organic acidurias, 19 amino acidurias, 11 FAO disorders, 5 cases of cystic fibrosis (CF), 4 adrenal hyperplasia, and 3 galactosemias. **Conclusion:** Incidence of IEM depends on the number of markers included in neonatal screening programs. Incidence of IEM found in the present study was 1:1000 live neonates (CH not included). Early intervention of cases reduces infant morbidity and mortality. These data demonstrate the possibility of developing expanded screening programs in Mexico and highlight the importance of including more markers in the analysis.

172 - Integral Program of Expanded Newborn Screening From Yucatan Health Services, Mexico: Results of Its Implementation and Prevalence of Congenital Metabolic Diseases

R. Salazar-Escalante[1], C. Gamboa-Gutierrez[1], J. Mendoza-Mezquita[2], F. Uicab-Alonzo[2], A. Urtecho-Herrera[2], M. Maldonado-Solis[1], and F. Maldonado-Solis[1]

[1]*Tamiz Ampliado de Yucatán, Mexico*
[2]*Servicios de Salud de Yucatán México, Mexico*

Introduction: Congenital metabolic diseases (CMDs) are a heterogeneous group of diseases; within which congenital hypothyroidism and inborn metabolic disorders (IMDs) are the most frequent. In Mexico, there have been few studies about CMDs, with minimal information about its frequency. An integral program of expanded newborn screening has been developed on southeastern Mexico for CMD search. **Objective:** To present the results and CMD prevalence found during the first 5 years since the implementation of Integral Program of Expanded Newborn Screening (PITNA for its acronym in Spanish) in Yucatan, Mexico. **Methods:** A retrospective study was conducted from January 2008 to June 2013. From each newborn (NB), 6 drops of blood were taken from the heel to be deposited on filter paper. Analysis was performed to search 5 groups of CMDs: (1) endocrinopathies, (2) hemoglobinopathies, (3) organic acidemias, (4) aminoacidopathies, and (5) other metabolic diseases. The methodologies used were immunoassay, tandem mass spectrometry and high-performance liquid chromatography (HPLC). Cases with suspected disease were located by a residence search system. **Results:** From a total of 84 990 births, 80 740 were screened (95% coverage). Confirmed cases were congenital hypothyroidism (56), congenital adrenal hyperplasia (8), hemoglobinopathies (8), organic acidemias (3), aminoacidopathies (3), and other metabolic diseases (9). CMD prevalence found was 10.78×10 000 NBs. **Conclusion:** One in 928 NBs in Yucatan has a CMD, it can be early detected by PITNA, and it also allows us to determine the frequency of these conditions in the state.

173 - Immunoreactive Trypsin (IRT/IRT) Evaluation Strategy for Early Detection of Cystic Fibrosis

A. Guercio[1] and N. Villarias[1]

[1]*Centro de Prevención de Enfermedades Inaparentes de la Infancia (CE.P.E.I.I.), Hospital Pediátrico Dr. Humberto J. Notti, Mendoza, Argentina*

Introduction: Different strategies are used in cystic fibrosis (CF) neonatal screening (NS); one of them is the IRT/IRT. **Objectives:** To evaluate screening system indicators and diagnostic performance. **Methods:** IRT/IRT strategy, DELFIA-Perkin Elmer method. Cutoff values: IRT $\geq$ 70.0 ng/mL (newborns [NBs] until 7 days of life); IRT $\geq$ 60.0 ng/mL (8-30 days). General test indicators and performance were evaluated (MedCalc v12.7.0). Positive results were reported by CE.P.E.I.I.'s Social Work Area for recitation, CF Center for clinical evaluation and subsequent referral to the Hospital's Biochemistry Department for diagnosis by sweat test and/or molecular studies. **Results:** June to December 2012: 12 657 evaluated NB CF: 2 children (first/second IRT values: 150/158 ng/mL and 147/226 ng/mL); incidence: 1:6329, first IRT positive: 40 NB, recall rate: 0.32%; child's age: 4 ± 3, mean: 111 ng/mL, median: 97 ng/mL, sensitivity (S) = 100%, specificity = 99.7%, positive predictive value (PV+) = 5%, positive likelihood ratio (LR+) = 333.03, accuracy = 99.70%, receiver–operating characteristic (ROC) curve analysis: cutoff > 139 ng/mL, area under the curve = 0.908, S = 100%, specificity = 89.5%, PV+ = 33.3%. Of 40 NBs with first positive

IRT, 3 died, 1 was not located, 36 NBs attended the NS program: 28 before 1 month of age and 8 after 1 month of age; second IRT (28 NBs): 5 high value IRT, child's age: 20 ± 4; IRT/IRT strategy: S = 100%, specificity = 99.98%, and PV+ = 40%. **Conclusion:** Based on cost–benefit perspective and taking into account the indicators presented and system performance, IRT/IRT is considered a valid strategy for CF NS.

174 - Management Indicators in Neonatal Screening Program Idea Foundation

K. Araujo[1], E. Crespo[1], Y. Guevara[1], L. Perez[1], Y. Ceballos[1], R. Canelon[1], C. L. Dominguez[1], M. De Lucca[1], A. Mahfoud[1], I. Arias[1], L. Casique[1], J. P. Cantillo[1], M. Morillo[1], L. Sua[1], M. Ramirez[1], and P. Reyes[1]

[1]*Fundación Instituto de Estudios Avanzados IDEA, Caracas, Venezuela*

Introduction: Management indicators are key elements in neonatal screening programs, allowing the evaluation of behavior and performance of all components involved in the process. In order to evaluate the efficiency of the IDEA Foundation neonatal screening program, we studied the period from 2008 to 2012. **Objective:** To evaluate the effectiveness and efficiency of IDEA Foundation neonatal screening program, through indicators. **Methods:** Database search of patients analyzed during the period 2008 to 2012, in order to obtain total analyzed per year, suspects, and positive. We calculated the rate of recalls, and coverage and analyzed the causes of loss of follow up of the newborn. **Results:** We observed coverage increase from 8.30% to 14%, taking into account the number of births nationally. So far frequencies found for congenital hypothyroidism was 1/3977, phenylketonuria: 1/35 838, and galactosemia: 1/24 981, recall rate ranged between 0.04 and 0.07. The most common mistakes in terms of sample collection were incomplete data (15%) and inadequate samples (20%). **Conclusion:** It is necessary to implement training and awareness strategies through lectures, workshops, and frequent visits to health centers, particularly to those presenting difficulties in sample collection. For this process, government support for creation of other neonatal screening centers in order to increase national coverage is important.

175 - Maternal 3-Methylcrotonyl-Coenzymea Carboxylase Deficiency in Costa Rica: 4 Case Reports

A. Reuben[1], N. Camacho[1], R. Trejos[1], J. Quesada[2], A. Acosta-Gualandri[2], and M. Saborio[2]

[1]*Laboratorio Nacional de Tamizaje Neonatal y Alto Riesgo*
[2]*Servicio de Genética Médica y Metabolismo, Hospital Nacional de Niños, San José, Costa Rica*

Isolated biotin-resistant 3-methylcrotonyl-coenzymeA carboxylase (3MCC) deficiency is an autosomal recessive disorder of leucine catabolism with considerable phenotypic heterogeneity. It is one of the most common inborn errors of metabolism with an incidence as high as 1 in 36 000 newborns. Women presenting this deficiency have been identified only by detection of abnormal metabolites in newborn screening samples of their healthy babies. We analyzed dried blood spots from mothers of 4 children identified by newborn screening, with triple quadrupole tandem mass spectrometer (MS/MS) and electrospray ion source. Nonderivatized method was used to process samples. Qualitative analysis of organic acids in urine from 4 patients and 2 mothers was performed with gas chromatography/mass spectrometry (GC/MS). Concentration of C4DC + C5OH acylcarnitines in children's samples was shown to gradually decrease and finally normalize at the age of 6 months, which is consistent with maternal 3MCC. Organic acid profile was informative in only 1 child, while elevation of 3-methylcrotonylglycine in urine was found in 2 mothers. All women reported normal pregnancies and remain asymptomatic to date. Maternal 3MCC should be taken into account when assessing a positive newborn screening result for 3-hydroxy-isovaleryl carnitine. When available, enzyme activity in isolated leukocytes or fibroblasts form mother and/or child would confirm the diagnosis. It also poses the question of whether or not to give periodic medical examination to children diagnosed with 3MCC in Costa Rica, since there are clinical studies sustaining that most of these patients remain asymptomatic through adulthood.

176 - Maternal Iodide Deficiency and Neonatal Thyroid-Stimulating Hormone

V. Mendez[1], L. Prieto[1], M. Frusti[1], A. Chiesa[1], and L. Gruñeiro Papendieck[1]

[1]*Fundación de Endocrinología Infantil, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina*

Introduction: Neonatal thyroid-stimulating hormone (nTSH) increases when there is an inadequate supply of iodine from mother to the fetus and has been proposed as another tool for iodine monitoring. Buenos Aires is a mild iodine-deficient area adequately corrected through mandatory salt iodination. In 2002, we studied the iodine supply in our population and found sufficient iodine in urinary excretion (n = 100 scholars); median (M) 143 μg/L (16% <50 μg/L) and goiter prevalence (n = 500): 4.5%. In that study, 2.7% of 1500 nTSH samples were >5 mU/L blood. **Objective:** To supervise the current status of iodine supply. **Materials and Methods:** The content of urinary iodine in randomly collected urine samples of 100 scholars (Sandell Kolthoff-Pino) and the prevalence of nTSH values >5 mU/L blood in 1813 samples (2-7 days of life; IFMA-DELFIA). **Results:** Median urinary iodine content was 220.5 ug/L (1% <50 ug/L). Neonatal TSH >5 mU/L in blood rose to 4.1%. While scholar urinary iodine indicated adequate iodine supply, the shift in nTSH levels revealed a possible mild iodine deficiency in newborns. As this trend might be related to maternal iodine nutrition, urinary iodine of 36 pregnant women

in the third trimester of gestation was assessed, finding median levels of 170 ug/L with 47.2% below 150 ug/L (NV: M >150 ug/L). **Conclusion:** As the recommended daily iodine intake and iodine turnover due to infant requirements during pregnancy are higher, and low salt supply is often indicated in pregnant women, the changes in nTSH may show mild iodine supply in this population.

177 - Maternal Phenylketonuria Syndrome

E. Lecuna[1], E. Monzon Geneyro[1], A. L. Albrekt[2], J. Zappa[3], and C. Lopez[4]

[1]*Programa de Pesquisa Neonatal Corrientes, Corrientes, Argentina*
[2]*Programa de Pesquisa Neonatal Misiones, Corrientes, Argentina*
[3]*Hospital Pediatrico "J Pablo II", Corrientes, Argentina*
[4]*Instituto de Cardiologia de Corrientes, Corrientes, Argentina*

Introduction: Maternal phenylketonuria (PKU) syndrome is an embryopathy observed in children of mothers with hyperphenylalaninemia (HFA) >3mg/dL without dietary treatment before or during pregnancy. Fetus levels of phenylalanine (Phe) are twice those of the mother, and it is a potent teratogen. In addition, phenylalanine hydroxylase activity is not present until 28 weeks of gestation. For this reason and depending on the Phe levels in maternal blood, fetus may present morphological and neurological alterations such as delayed intrauterine growth, severe mental retardation, microcephaly, renal, and cardiac malformations. **Objective:** To present the case of a newborn with congenital heart disease with high Phe at birth, which decreases without treatment. **Case Presentation:** Forty-day-old patient derived from Misiones by cyanotic congenital heart disease present the following values of Phe: 10 days of life (DV): 12.50 mg/dL; 21DV: 3.65 mg/dL; 28 DV: 4.50 mg/dL. Cardiac surgery is performed and after 7 days, the Phe levels were 1.8 mg/dL, discarding metabolic error. His mother was studied confirming HFA with Phe: 8.9 mg/dL. **Conclusion:** Maternal HFA should be suspected in specific embryopathies for genetic counseling. Screening neonatal network work allows patient follow-up until results are reported.

178 - NeoLISA PKU Product Performance in a Fully Automated System

R. Veturiano[1], A. H. Luques[1], V. L. Frias[1], F Silva[1], G. P. Gil[1], B. Gallo[1], J. S. C. Pires[1], and C. A. Sampaio Filho[1]

[1]*Intercientifica, Sao Jose dos Campos, SP, Brazil*

Introduction: The process of continuous improvement of newborn screening programs requires automation and processes that are increasingly safe, accurate, and fast in order to accelerate the release of results of routine samples. In order to meet the demands of continuous improvement, INTERCIENTIFICA developed a fully automated system for the quantification of phenylalanine. This system receives the plates with dried blood spot (DBS) samples punched and performs all the steps up to the release of results to LIS without user interference. **Objective:** To present the results of the performance of Fully Automated System developed by INTERCIENTIFICA associated with the NeoLISA PKU Kit. **Methods:** Control samples, material, and both internal and external controls were used for evaluation in different laboratory routines of small, medium, and large volumes. The fully automated system is the result of the integration of platform Nimbus, Reader MRX, and a range of innovations in hardware and software developed to meet the demands of newborn screening laboratories. **Results:** Present statistics obtained with the different samples, internal and external controls, time required for a total of up to 8 microplates simultaneously for routine, totaling daily processing capacity of 1500 samples, 7500 samples per week (5 days/week), and 30 000 samples/month per equipment. **Conclusion:** The fully automated system associated with the use of the NeoLISA PKU kit is the ideal solution for laboratories performing the various sample volume (small, medium, and large routines) of newborn screening.

179 - Neonatal 17-Ohprogesterone Cutoff Varies According to Newborn's Age at Testing in Congenital Adrenal Hyperplasia Screening

G. Y. Hayashi[1,2], D. F. Carvalho[2], C. Faure[1], C. G. Vallejos[1], V. N. Brito[2], B. B. Mendonça[2], and T. A. S. S. Bachega[2]

[1]*Laboratório do Serviço de Referência em Triagem Neonatal - APAE DE SÃO PAULO, São Paulo, Brasil*
[2]*Laboratório de Hormônios e Genética Molecular–LIM42. Disciplina de Endocrinologia, Hospital das Clínicas, FMUSP, - São Paulo, Brasil*

Introduction: Although congenital adrenal hyperplasia–newborn screening (CAH-NBS) is efficient in detecting affected newborns, a high rate of false-positive result (FPR) is the main concern, mainly related to prematurity. In most Brazilian states, the sample is collected after 72 hours of life; however, in Sao Paulo it is collected between 48 and 72 hours. Moreover, neonatal 17-hydroxyprogesterone (N17-OHP) increases progressively in affected patients, and earlier sample collection tends to increase the false-negative result (FNR) rate. **Objective:** To determine the N17-OHP reference values adjusted to birth weight and age at the time of sampling. **Methods:** The N17-OHP levels, measured by immunofluorimetric, of 205 867 newborns were analyzed, in serum equivalent, and grouped according to age at the time of sampling (G1: 48-<72 hours and G2: ≥72 hours) and to birth weight (BW) groups (BW1: <1500 g, BW2: 1501-2000 g, BW3: 2001-2500 g, and BW4: >2500 g). Newborns with abnormal N17-OHP were submitted to hormonal and molecular confirmatory tests. Nonparametric tests were used. **Results:** Eighteen newborns with CAH (16 salt-wasting) were diagnosed (incidence 1:11 437), their N17-OHP ranged from 31 to 410 and 379 to 480 ng/mL in G1 and G2, respectively, and

CYP21A2 sequencing disclosed classical form genotypes. The 99.8th percentile was the best cutoff to distinguish nonaffected from CAH newborns; FPR rate in G1/G2 was 0.03% and no FNR was reported. In normal newborns, N17-OHP was significantly lower in all BW groups when sampled before 72 hours compared to after 72 hours: G1 (BW1: 52.5; BW2: 54.9; BW3: 37.6; BW4: 19.8 ng/mL) and G2 (BW1: 152.1; BW2: 74.7; BW3: 60.8; BW4: 25.4 ng/mL). **Conclusion:** We demonstrated that N17-OHP should be adjusted according to birth weight but also to newborn's age at sampling, which should be considered in order to optimize CAH-NBS efficacy.

180 - Neonatal Screening for Cystic Fibrosis in Premature Newborns: Do We Need a Specific Protocol?

S. Marino[1], C. Arias[1], R. M. Serviddio[1], G. Zeida[1], V. Rodriguez[2], P. Glikman[2], M. Junco[2], A. Odriozola[2], M. Frusti[2], M. G. Ropelatto[2], A. Chiesa[2], G. Maccallini[2], F. Smithuis[2], C. Aranda[2], P. Muntaabski[2], M. E. Liceda[3], A. Belgorosky[1], N. Dujovne[1], V. Herzovich[1], and G. Dratler[1]

[1]*Hospital de Pediatría SAMIC Dr JP Garrahan, Buenos Aires, Argentina*
[2]*PPN Programa de Pesquisa Neonatal de la Ciudad de Buenos Aires, MS, Argentina*
[3]*Programa Nacional de Fortalecimiento de Pesquisa Neonatal, Direccion de Maternidad e Infancia, MSN, Argentina*

The immunoreactive trypsin (IRT)/IRT protocol provides no special considerations for preterm infant subpopulation. However, the biochemical marker may be subjected to influences related to prematurity. **Objective:** To evaluate the recall rates (RR) and IRT values according to corrected gestational age (cGA) and independence among RR for different neonatal screening (NS) diseases. **Methods:** From 63 884 newborns, RR for cystic fibrosis (CF) was assessed according to cGA. The IRT values (ng/mL; DELFIA, PerkinElmer) evaluated were mean, standard deviation (SD), n, percentiles 50, 90, 95, 97.5, and 99. We also compared the recalled samples for CF with those recalled for other NS diseases. **Results:** The general RR for CF was 0.9%. Both RR and IRT progressively increased according to prematurity degree. Recalled rate for CF was related to the observed for 17-hydroxyprogesterone (17-OHP; χ^2 independence test, $P < .0001$). **Conclusion:** The increasing IRT values and higher dispersion observed in preterm infants could explain high RR observed and highlight the necessity to improve the specificity of NS for preterm population. The statistical relationship between IRT and 17-OHP recalled samples suggest a common influence (perinatal stress?) affecting both tests.

181 - Neonatal Screening Experience of Congenital Hypothyroidism Using Technology in Arauca (Colombia) During the Years 2004 to 2012

H. Robinson[1] and Alix[1,2]

[1]*Health Special Administrative Unit of Arauca (Colombia), Arauca, Colombia*
[2]*Specialized Professional Public Health Laboratory, Arauca, Colombia*

Introduction: In Colombia, according to the public health policy, congenital hypothyroidism (CH) neonatal screening is mandatory for all newborns avoiding sequelae such as mental retardation. Arauca department has made this screening since 2004 until 2012, achieving unaltered children brain. **Objective:** To present the experience of neonatal screening for CH, from 2004 to 2012, in Arauca Department (Colombia). **Methods:** We summarize the results obtained from processing 19 585 samples of umbilical cord blood for neonatal thyroid-stimulating hormone (TSH) measurement, using ULTRAMICROELISA technique (UMELISA SUM) in a Public Health Laboratory. **Results:** A total of 19 585 newborns were screened with 8 confirmed cases (0.04%) for CH, distributed as follows: year 2004, 1576 screened (1 case); 2005, 1219 (1 case); 2006, 2124 (2 cases); 2007, 1501 (0 cases); 2008 1664 (1 case); 2009, 3100 (1 case); year 2010, 3448 (1 case); 2011, 2676 (1 case); and 2012, 2277 (0 cases). Children with CH were treated and monitored timely, avoiding mental retardation. **Conclusion:** Continued implementation of CH neonatal screening for early detection and treatment prevents irreversible consequences. For 9 years, we have used UMELISA technology, obtaining excellent results in quality control programs. To contribute to morbidity, mortality, and childhood disability reduction, it is recommended to include other tests in the newborn screening.

182 - Neonatal Screening for Biotinidase Deficiency: Control-Validation of Analytical Stage

A. Guercio[1] and N. Villarias[1]

[1]*Centro de Prevención de Enfermedades Inaparentes de la Infancia (CE.P.E.I.I.), Hospital Pediátrico Dr. Humberto J. Notti, Mendoza, Argentina*

Introduction: To use a method that evaluates "objectively" the biotinidase enzyme activity (Biot Act) as an important factor to accomplish the "validation" process. **Objectives:** To make statistical analysis of data for methodological validation and to start cutoff adjustment in our newborn (NB) population. **Methodology:** Perkin Elmer fluorometric method was used; initial intra-assay control: samples with different Biot Act

(1U = 1 nmol product/min/dL) were processed by duplicate; between-assay control: during 20 days, we worked with 2 samples, average Biot Act 286 U and 56.1 U and Biot Act was determined in 1000 NB, 48 to 72 hours of life, gestation age ≥37 weeks, weight ≥2200 g. **Results:** Intra-assay control: error-answer relationship = 0.0762, confidence limit = 0.1077, quantification limit = 13.7 U, Imprecision profile, CV% different Biot Act: -14.60%(13.7U) -7.19%(41.7U) -5.88%(136U) -5.38%(186U) -5.15%(262U) -5.66%(380U) •Between-assay control, CV% 2 Biot Act: -9.9%(286U) -13.3%(56.1U) •CV% at 72U: -intra-assay: 6.6% - between-assay: 13% •Biot Act in NB: -mean=164U -median=160U -inferior percentile: -0.1%=68U -0.2%=69U -0.5%=72U -1%=74U -2%=76U -the normal average in Biot Act and the analytical variability established an "equivocal zone" with an upper limit of 72 U and a lower limit of 26 U. **Conclusion:** Taking into account the obtained results, methodology used in neonatal screening achieves the essential requisites to detect biotinidase deficiency in our NB population. Regarding cutoff values, Act Biot >72 U: negative screening 26 to 72 U: repeat determination immediately in a second sample <26 U: positive screening, evaluate Biot Act in a second sample, and confirm diagnosis with another methodology.

183 - Neonatal Screening for Galactosemia in Mexican Social Security Institute

J. L. Gonzalez Guerrero[1], B. Valdes Huezo[1], E. E. Delgado Gonzalez[1], M. A. Burciaga Torres[1], and L. P. Ferrer Arreola[1]

[1]IMSS Coordinación de Programas Integrados de Salud, División de Salud Reproductiva, México

Introduction: Classic galactosemia detection was included in the nationwide newborn screening program in April 2012, which is performed in approximately 450 000 children born annually in medical units of the Mexican Social Security Institute. **Objective:** To assess the coverage and frequency of classic galactosemia in the population covered by the Institute. **Methods:** A cross-sectional study was performed with samples collected and processed between April and December 2012. Total galactose (TG) level was determined by inmunoenzymatic assay (UMTEST GAL, Tecnosuma) in dried blood spots on filter paper. If the first sample shows high results, a second sample is requested and if levels are still high, it is considered as a probable case. A high result corresponds to TG level >10 mg/dL or >0.56 mmol/L. **Results:** A total of 337 604 samples were processed, achieving a 95.17% coverage. Fifty-nine probable cases were identified, 8 cases were confirmed by spectrophotometry and fluorometric assay, and also PCR analysis was performed for common mutations: N314 (Duarte), Q188R, S135L, K285N, and L195P (classic). The frequency of galactosemia was 1 among 44 340 newborns screened. **Conclusion:** The frequency obtained is similar to that reported in other neonatal screening programs, but the real incidence in Mexican population is still unknown. It is necessary to continue the detection of galactosemia in our country and keeping an active epidemiological surveillance system.

184 - Neonatal Screening for Hemoglobinopathies in Southeast Mexico: Experience From a Pioneer Program on 192 183 Newborns

F. Maldonado-Solis[1], M. Maldonado-Solis[2], R. Salazar-Escalante[3], R. Sanchez-Zebadua[4], C. Moreno-Graciano[1], P. Martinez-Cruz[1], L. Herrera-Perez[1], and M. Sierra-Salas[1]

[1]Tamizaje Plus S.A de C.V, Mexico
[2]Químicos Maldonado S.A de C.V, Mexico
[3]TamizMas de Quimicos Maldonado Unidad Yucatan, Mexico
[4]TamizMas de Quimicos Maldonado Unidad Chiapas, Mexico

Hemoglobinopathies are one of the most common genetic diseases, and their high frequency represents a great concern for public health worldwide. Clinical consequences of hemoglobinopathies are anemia, failure to thrive, repeated infections in infancy, vaso-occlusive disorders, severe pain, stroke, and organ failure. There is substantial evidence demonstrating that early identification of affected infants by neonatal screening (NS) and a careful follow-up, coupled with relatively simple interventions, substantially reduce morbidity and mortality. The aim of this study is to present the results of an NS program for hemoglobinopathies in the Southeast of Mexico. **Methods:** From September 2007 to July 2013, a prospective and descriptive study was performed; the study included newborns from the Mexican states of Tabasco, Yucatán, and Chiapas. Heel prick dried blood spots from Guthrie cards were analyzed by isoelectric focusing and/or high-performance liquid chromatography (HPLC) techniques. **Results:** A total of 192 183 newborns were analyzed and 3047 hemoglobin variants (1.59%) were detected. In all, 25 patients were diagnosed with hemoglobinopathies; 19 with sickle-cell disease, 5 with beta-thalassemia, and 1 with alpha-thalassemia minor. HbS is the most frequent variant, accounting for 84.6% of all abnormal hemoglobinopathies found in the South of Mexico, followed by variants Hb (4.2%) and HbC (2.3%). Prevalence at birth of hemoglobinopathies (traits and diseases) is 15.9 per 1000 newborns. **Conclusion:** In Mexico, this is a pioneer NS program for detection of hemoglobinopathies, the high prevalence found by us and the public health impact of early medical intervention in these diseases provide evidence for their inclusion in the national panel of NS.

185 - Neonatal Screening for 21-Hydroxylase Deficiency—Pros and Cons

T. Torresani[1]

[1]Swiss Neonatal Screening, University Children's Hospital, Zurich, Switzerland

Assay methods for the determination of 17-hydroxyprogesterone (17-OHP) from filter paper specimens collected in newborn screening programs have been marred by relatively poor specificity and sensitivity, causing an excessive number of recalls, particularly in premature babies. In several screening programs, strategies have been developed for the introduction of second-tier examinations, with the aim of reducing the number of unwanted recalls. The most effective second-tier examinations, tandem mass spectrometry and DNA analysis, are quite expensive and require sophisticated equipment that is not always available. The question has also been raised whether screening of premature babies should be discontinued. In Switzerland, we have adopted a combination of multiple sampling and of reference values related to gestational age and age at sampling. Because the levels of 17-OHP differ significantly according to the maturity of the baby and the day of sample collection, the use of these specific reference values has resulted in a significant decrease in false positive results, without affecting the detection of real cases of congenital adrenal hyperplasia (CAH). When gestational age and age at sampling are taken into consideration for the interpretation of levels of 17-OHP obtained in newborn screening samples, the number of recalls falls to very acceptable levels.

186 - Neonatal Screening in Mérida Venezuela: An Expanding Program

M. C. Delgado[1], C. Zanardo[1], B. Guerrero[1], D. Montilla[2], I. Camacho[2], M. Briceño[2] J. Torres[3], D. Villamizar[3], and C. Guedes[4],

[1] Centro de Estudio y Prevención del Retardo Mental y Alteraciones en el Desarrollo (CEPREMAD) programa del Centro de Desarrollo Infantil de Mérida, Mérida, Venezuela
[2]Fundación Centro de Desarrollo Infantil, Mérida, Venezuela
[3]Subproyecto 2; Proyecto FONACIT 2008001053, Mérida, Venezuela
[4]Ministerio del Poder Popular para la Salud, Mérida, Venezuela

Introduction: Neonatal Screening Program for congenital hypothyroidism (CH) and phenylketonuria (PKU) started in 1997 at the Child Development Center (CDC) in Mérida state. In 2005, the National Neonatal Screening Program was started at the Public Health Laboratory (PHL) in Mérida, and in 2010 the FONACIT 2008001053 project was carried out by the Centers for Disease Control and Prevention (CDC) significantly strengthening this preventive program going through different stages that have enriched the program experience. However, coverage, timely location of patients, and sample quality are the main hurdles to overcome. **Methods:** Capillary blood samples of newborns between 72 hours and 15 days of age were analyzed on paper SS-903, using time-resolved fluoroinmunoassay (DELFIA) and Ultramicroelisa (UMELISA) assays. Confirmation was performed by determining serum thyroid-stimulating hormone (TSH) and free thyroxine (FT4) by immunofluorescence and/or enzyme-linked immunosorbent assay (ELISA). Phenylalanine was measured semi-quantitatively by thin-layer chromatography and/or quantitatively by UMELISA. **Results:** In all, 36 199 samples of children for neonatal screening of CH and PKU have been analyzed from January 2006 to July 2013. In all, 21 cases of CH, 1 case of subclinical hypothyroidism, and 1 case of hyperphenylalaninemia were confirmed. They received treatment and biochemical and medical monitoring at the CDC. **Conclusion:** It is important to legalize the obligatory nature of neonatal screening in Venezuela in order to improve effective and early detection and to establish timely treatment to prevent mental retardation caused by these diseases.

187 - Neonatal Screening: Twelve Years in the Federal District of Brazil

M. T. O. Cardoso[1], M. Viegas[1], R. Oliveira[1], J. Thomas[1], K. Santille[1], and R. Teixeira[1]

[1]Newborn Screening Laboratory, Genetic Service, Hospital de Apoio de Brasília, Brasilia, Federal District, Brazil

National Newborn Screening Program from Health Ministry is available to the entire population. Its aim is to enable prevention and early diagnosis of congenital metabolic, genetic, and infectious diseases, which may be asymptomatic in the neonatal period, allowing prevention of disease development. In this way, it is possible to have specific and early treatment as well as reduction or avoiding of complications associated with each disease. The National Newborn Screening Program allows the diagnosis of 4 diseases: congenital hypothyroidism, phenylketonuria, hemoglobinopathies, and cystic fibrosis. In the Federal District of Brazil, which was created 8 years ago, the program was expanded to cover 21 diseases, including the 4 standard pathologies of the National Program from the Health Ministry. The purpose of this study is to evaluate the positive results of newborn screening, since its implementation in the Federal District, from 2001 to 2013. This study is based on the data provided by the Newborn Screening Program of the Federal District Health Secretary, from the Genetics Department of the Hospital de Apoio de Brasília. During the last 12 years, an average of 2 cases of phenylketonuria were diagnosed per year (1/20 000 newborns), 10 cases of hemoglobinopathies per year (1/4000 newborns), and 12 cases of congenital hypothyroidism per year (1/3000 newborns). Newborn Screening program of Federal District covers more than 90% of alive newborns including an average of 40 000 patients per year. This program has allowed early diagnosis and treatment of serious and disabling diseases.

188 - New 2013 Edition: Blood Collection on Filter Paper for Newborn Screening Programs: Approved Standard CLSI NBS01-A6 (Formerly LA04-A6)

Hannon W Harry[1] and V. R. De Jesus[2]

[1]Document Development Committee Chair, Clinical and Laboratory Standards Institute (CLSI), Wayne, Pennsylvania USA
[2]Newborn Screening and Molecular Biology Branch, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

For over 30 years, the Clinical and Laboratory Standards Institute (CLSI) has recognized the need for providing and updating instructions to health care professionals who collect and submit dried blood spot (DBS) specimens for newborn screening (NBS) as well as manufacturers who develop kit methodologies, and NBS programs that perform routine testing. NBS01 has existed since 1982 with revisions made every 5-years. The 2013 CLSI Document Development Committee (DDC) for NBS01 (formerly LA04-A6) truly had global representation with participants from Argentina, Canada, Denmark, Finland, Korea, Norway, Philippines, Spain, Trinidad, United Kingdom, and the United States. The DDC was charged to produce an up-to-date, revised standard that will harmonize the techniques for collecting the best possible specimens for use in NBS programs worldwide. Some other specific items revised or added to the standard include (1) coping with less-than ideal specimens; (2) pain management strategies; (3) minimum and optional information captured with the specimen; (4) the handling of blood spots collected on filter paper for DNA/RNA analysis; (5) short- and long-term storage of specimens; and (6) an Appendix was added on patient conditions and treatments affecting NBS results. Extensive efforts were made by the DDC to ensure global applicability of the revised standard. This 2013 edition is now available at CLSI (www.clsi.org).

189 - New *CFTR* Gene Mutation Detected by Newborn Screening

L. Maggi[1], A. Argüelles[1], J. Ruatta[2], M. Wagner[2], and F. Meneghetti[2]

[1]Provincial Laboratory of Endocrine Metabolic Inborn Error Neonatal Research, Santa Fe, Argentina
[2]"Orlando Alassia" Children Hospital, Santa Fe, Argentina

Introduction: In *CFTR*, the gene controlling cystic fibrosis (CF) membrane conductance, 1937 mutations and over 200 polymorphisms have been described. **Goal:** To report on a new mutation detected in a child from Santa Fe through the newborn screening (NS) diagnostic algorithm: (IRT/IRT/2ST/MB). **Methods:** NS was conducted with immunoreactive trypsin (IRT) by enzyme-linked immunosorbent assay (ELISA) method using MP Biomedicals from BACON S.A.I.C. Laboratories, confirmation was done through Sweat test (ST) using Gibson & Cook method. In the first molecular biology (MB) 19 mutation panels were made through polymerase chain reaction (PCR) and reverse hybridization, and in the second MB through a double screening with 2 methods, Luminex and multiple ligation probe amplification (MLPA), subsequently sequencing all exons and 2 intronic regions, 11 and 19. **Results:** Asymptomatic patient with a 42-week gestation, 4400 g in weight, 4 days of life. IRT obtained: 189.58 ng/mL, cut value: 150 ng/mL. It is repeated after 20 days of life with a result of 218.91 ng/mL and a cut value (20-25 days of life) of 100 ng/mL. Confirmation included 2 ST, of 76 mEq/L and 66 mEq/L, reference values, up to 6 months: $\leq$29 mEq/L improbable CF, doubtful 30 to 59 mEq/L and $\geq$59 mEq/L compatible with CF. After a first negative MB, the patient was included in a 1000-mutation study protocol that showed 2 mutated alleles: R1162X//L49P. **Conclusion:** A new *CFTR* gene mutation L49P was discovered.

190 - Newborn Screening for Hemoglobinopathies in Goiãs, Brazil

Araujo Tais Pires Terra[1], Pedatella Flaviane Marcilia[1,2], Santos Eliane Pereira dos[1,3], Andre Miguel Antônio Isaac[1], Suzana Mauricio Carvalho[1], and Bataus Luis Arthur Mendes[3]

[1]Associação de Pais e Amigos dos Excepcionais de Anápolis APAE, Anápolis, Brasil
[2]Universidade Estadual de Goiás UEG, Anápolis, Brasil
[3]Universidade Federal de Goiás UFG, Goiânia, Brasil

Introduction: Hemoglobinopathies, hemoglobin variants, and thalassemia are autosomal recessive diseases caused by structural changes and/or synthesis of the globin chains. **Objective:** This study aimed to estimate the incidence of hemoglobinopathies in the state of Goiãs through laboratory diagnosis of newborns treated in the newborn screening. **Methods:** Blood samples were collected on filter paper S&S 903 by puncture of the capillary in the heel of all newborns treated in the reference service from September 2001 to December 2012. The reference service receives samples of 246 municipalities, corresponding to approximately 6000 newborns per month. Dried blood samples were collected from neonates from the 3rd to the 30th day of life. We used isoelectric focusing (IEF) and high-performance liquid chromatography (HPLC), sickle-cell program, and Î²-thalassemia. **Results:** In this period, the reference service screened 790 285 newborns and diagnosed 38 288 (48.44/1000) neonates with hemoglobinopathies. We diagnosed 37 685 (47/1000) infants with hemoglobin trait and 603 (0.76/1000) ill newborns, and the phenotypes FS, FSC, and FC were found most frequently. The project was approved by Federal University of GoiÃ¡s Ethics Committee. **Conclusion:** According to the population studied, the incidence of hemoglobinopathies estimated ranged between 0.05% and 1.1% in different regions of the world. In Goiãs, newborn screening showed high frequency hemoglobinopathies (0.76/1000) in our environment and thus delineate the epidemiological profile of

hemoglobin population of Goiãs. Hemoglobin S is the most common hemoglobin found in our population followed by hemoglobin C.

191 - Newborn Screening for Inborn Errors of Metabolism by Ms/Ms in Brazil: A Retrospective Study of São Paulo Apae

L. R. R. G. Garcia[1], F. B. Piazzon[1,2], H. H. Martins[1], T. M. Belarmino[1], E. N. Silva[1], M. A. M. Iskandar[1], and S. M. Hadachi[1]

[1]APAE DE SÃO PAULO, São Paulo, Brasil
[2]Unidade Neurometabólica, Universidade de São Paulo, São Paulo, Brasil

Electrospray ionization–tandem mass spectrometry (MS/MS) allows identification of various metabolic diseases involving amino acids, organic acids, and acylcarnitines with a positive impact on morbidity and mortality of these diseases. This technology presents acceptable laboratory costs when associated with benefits such as early diagnosis and presymptomatic treatment. The aim of this study is to determine the overall detection rate, incidence, and prevalence of inborn errors of metabolism (IEMs) in expanded newborn screening by MS/MS of APAE DE SÃO PAULO. This methodology allows the identification of pure organic compounds and mixtures of organic molecules by their breaking when subjected to high-intensity energy beams. From 2010 to 2013, 29 553 newborns were screened for 38 IEMs by MS/MS. The overall performance of the screening program was estimated by sensitivity (94%), specificity (99%), and accuracy (99%). In this sample, 391(1.5%) tests were false positives. In this study, the prevalence was 0.05% and the incidence of screened cases was 1:1970. Fifteen babies had a confirmed IEM. Monitoring of all patients detected was performed until the diagnosis was confirmed. The method showed satisfactory sensitivity, specificity, and accuracy rates with low false-positive rate. This way, neonatal screening by MS/MS drastically increased the detection rate compared with conventional techniques. However, in spite of limited availability in Brazil, diagnosis and treatment before the onset of symptoms may be a model for government programs.

192 - Newborn Screening: Cobalamin Deficiency due to Maternal Cobalamin Deficiency

A. Lemes[1], C. Queijo[1], C. Zabala[1], F. Gonzalez[1], M. Castro[1], L. Fernandez[1], C. Ferolla[1], and G. Queiruga[1]

[1]Instituto de Seguridad Social (BPS)

Introduction: An earlier review focused on cobalamin (Cbl) deficiency in newborns showed that most of cases are consequence of deficiency in the mother. Mothers following vegetarian and other special diets are at particular risk. Poverty and malnutrition are known causes of Cbl deficiency in mothers and children. Up to 20% of normal pregnancies have low serum Cbl levels with elevated total homocysteine (tHcy) and methyl malonic acidemia (MMA) levels. **Objective:** Our aim is to present a case of a newborn with C3 elevation because of maternal Cbl deficiency. **Methods:** Mass spectrometric determination of C3 in dried blood spot samples collected from newborns. Each elevation required an assay of Cbl, tHcy, and MMA. **Results:** In the first sample, we found C3 to be 9.5 (<3.57 μmol/L) and C3/C2 0.41 (<0.21 μmol/L); and in the second sample C3 was found to be 14.11 and C3/C2 0.78. After obtaining those results, the following assays were performed: Cbl 150 (176-800 pg/mL), tHcy 30 (<15 μmol/L), and urine MMA 85 (<5 μg/mg creatinine). After B12 administration: Cbl >2300 pg/ml, tHcy 6.1 μmol/l and urine MMA 3.2 ug/mg creatinine). The results in the mothers were Cbl 205 (200-950 pg/mL) and tHcy 16 (<15 μmol/L). **Conclusion:** C3 elevation in a newborn is caused not only by an inborn error of metabolism (IEM) but also by maternal Cbl deficiency, which is an important cause. There are reports of newborns with severe encephalopathy due to this maternal condition. So it is important to create an awareness among poor women and those with special diets about the risks of this deficiency for the newborn.

193 - Phenotype–Genotype Correlations in Patients with Cystic Fibrosis, in Mendoza, Argentina

L. M. Navarta[1], E. Lentini[1], A. Lopez Millan[1], R. Ibarra[1], and S. Pannocchia[1]

[1]Children's Hospital Humberto Notti, Mendoza, Argentina

Introduction: Cystic fibrosis (CF) is the most common inherited autosomal recessive disease in caucasians. There are more than 1500 mutations involving the *CFTR* gene. It is divided into 6 classes: I, II, III, and VI are related to severe clinical forms and IV-V are related to mild forms. **Objective:** To determine the phenotypic characteristics of patients with CF (from Mendoza), relate them to their genotype and prevent CF complications in children with immunoreactive trypsin (IRT) increased. **Methods:** Thirty-six mutations (INNO-LiPA) were studied on 47 patients. Patients with 1 or 2 mutations were grouped in 5 groups: homozygous for class II (II/II) and for class I (I/I); 3 groups were compound heterozygous (I/any or II/any); (IV/any); and (V/any). We evaluated pulmonary function with spirometry and pancreatic function with Van de Kamer test. **Results:** Mean age was higher in groups (IV/any) and (V/any; 9,7 and 8,4 years, respectively) as opposed to 2.4 in (II/II), 2.7 in (I/I), and 2.3 in (II/any or I/any). Pancreatic insufficiency (PI) was found in 100% of patients (II/II), (I/I), and (I/any or II/any). None of (IV/any) had PI. The lowest prevalence of diabetes mellitus and *Pseudomonas aeruginosa* infections was in (IV/any). Mean forced expiratory volume in 1 second as a percentage of predicted (FEV1%) was higher in (IV/any) and (I/any or II/any). Increased liver enzymes were less

frequent in (IV/any) and (V/any). **Conclusion:** Patients with class IV mutations were pancreatic sufficient, presented milder lung disease, delayed onset of *P aeruginosa* infection, and absence of diabetes mellitus.

194 - Phenylketonuria in the Mexican Social Security Institute 2005 to 2012

E. E. Delgado Gonzalez[1], M. A. Burciaga Torres[1], J. L. Gonzalez Guerrero[1], B. Valdes Huezo[1], and L. P. Ferrer Arreola[1]

[1]IMSS Coordinación de Programas Integrados de Salud. División de Salud Reproductiva, México, DF

Introduction: Mexican Social Security Institute (IMSS) began phenylketonuria (PKU) screening in 2005 as part of an institutional nationwide newborn screening program. **Problem Studied and/or Objective:** To report coverage and accumulative PKU incidence in newborns of IMSS in the 2005-2012 period. **Methodology:** Phenylalanine levels in newborns were measured by a fluorescent method (UMTEST) in dried blood spots collected on filter paper. Probable cases were defined by a phenylalanine (Phe) level >4 mg/dL (240 µmol/L) and cases were confirmed when the Phe level in high-performance liquid chromatography was >7 mg/dL. All detections were registered in the epidemiologic surveillance system and analyzed to obtain coverage of detection, positive predictive value (PPV), and accumulated incidence. **Results and Discussion:** A total of 3 444 962 newborns were screened with an average coverage of 93.3%. We identified 127 probably cases and 32 confirmed cases (PPV = 25.2%). Only 12 states Zacatecas, Guanajuato, Jalisco, Nayarit, Baja California Sur, Tabasco, Distrito Federal, Aguascalientes, Guerrero, Chihuahua, Veracruz, and Estado de Mexico reported cases, and the incidence was 1:19 826 to 1:224 328. **Conclusion and/or Implication:** The incidence observed in Mexico (IMSS) was 1:107 655, which is less than that reported internationally; only 2 states in Mexico (Zacatecas and Guanajuato) had a higher incidence. This is the first report of PKU incidence in Mexico by a nationwide population-based neonatal screening.

195 - Population Comparative Study Between an Enzymatic Fluorometric Method for Measurement of Branched Chain Amino Acids and Tandem Mass Spectrometry

G. J. C. Borrajo[1] and M. J. Zelaya[1]

[1]Detección de Errores Congénitos, Fundación Bioquímica Argentina, La Plata, Argentina

Newborn screening for maple syrup urine disease was not massively implemented internationally until tandem mass spectrometry (MS/MS) was introduced. However, Fundacion Bioquimica Argentina has been doing it since 2001 using an enzymatic fluorometric method (EFM) developed in-house, in which the 3 branched chain amino acids (BCAAs) are simultaneously measured. In this work, the results corresponding to a population comparative study of BCAAs measured by EFM and (Leu/Ile + Val) measured by MS/MS using a homemade nonderivatized method and an UPLC-TQD instrument from Waters are presented. Samples from 5154 newborns were tested using both methods in the period from January to March 2012. The main statistical parameters characterizing the evaluated population were calculated (range, mean [X], CV%, median [M], and percentiles [P] 97.5, 99.0, 99.5, and 99.9, respectively) and population distribution characteristics were analyzed. The results obtained, expressed in µmol/L, were (a) EFM: range (7.62-640.08), X = 200.31, coefficient of variation (CV) = 40.9%, M = 190.50, P97.5 = 403.86, P99.0 = 487.68, P99.5 = 542.81, and P99.9 = 594.36; (b) MS-MS: range (83.01-587.48), X = 208.68, CV = 24.0%, M = 203.11, P97.5 = 326.95, P99.0 = 361.57, P99.5 = 376.98, and P99.9 = 422.74. Population distribution corresponding to EFM showed a wider and shorter curve, the mean comparison study showed significant differences (z test, $P < .001$ [= .05], and the correlation between both methods was not good ($r = .432$). The differences observed in the statistical parameters, histograms, z test, and correlation study provide evidence that both methods do not have equivalent responses, although they are analytically suitable for newborn screening.

196 - Preanalytical Phase Assessment of the Neonatal Research Program at Child Development Center, Mérida

M. Briceño[1], J. Torres[3], D. Villamizar[3], C. Zanardo[1], B. Guerrero[1], D. Montilla[2], C. Guedes[4], I. Camacho[2], and M. C. Delgado[2]

[1]Centro de Estudio y Prevención del Retardo Mental y Alteraciones en el Desarrollo (CEPREMAD) programa del Centro de Desarrollo Infantil de Mérida
[2]Fundación Centro de Desarrollo Infantil
[3]Subproyecto 2; Proyecto FONACIT 2008001053
[4]Ministerio del Poder Popular para la Salud Mérida Venezuela

Introduction: To ensure the effectiveness and efficiency, a national neonatal research program must involve a series of links working in coordination. It should be identified as a multidisciplinary process in which each actor joins effort in order to prevent child population diseases. Thus, diseases can be detected early and treated before they cause an irreparable damage. **Objective:** This study aimed to evaluate the preanalytical phase of the neonatal research program at the Child Development Center in Mérida state, from May 2010 to June 2012. **Design and Results:** Through a retrospective analysis, 16 729 researched newborns of age ranging from 3 to 15 days were evaluated (X = 7.71; ± 5.26). From 355 (2.12%) unsatisfactory samples, 97 (0.58%) were refiltered. The average time of reception of the second sample was 22 days (± 16.6). None of the processed samples in the refiltered process was positive.

Data analysis was performed using Epi Info (TM) 3.5.1 program. **Conclusion:** The experience gained in this study indicates that the percentage of unsatisfactory samples does not correlate to the number of refiltered samples. A continuous and increasing sensitivity education system that strengthens the training of the staff assigned to samples collection and that ensures fast and free samples transportation to laboratories for analysis must be established. Thus, the fundamental objective of the program, the early diagnosis of the innate illnesses of the metabolism, can be reached.

197 - Preanalytical Phase: Evaluation of Corrective Measures' Impact

E. Lecuna[1], E. Monzon Geneyro[1], M. Ortigoza[1], E. Medina[1], and G. Alcaraz[1]

[1]Programa Provincial de Pesquisa Neonatal-Hospital "J R Vidal," *Corrientes, Argentina*

Introduction: Health system of Corrientes province is divided according to regions. Since sample quality cannot be improved after its collection, necessary actions have to be taken to allow for preanalytical optimization. Quality indicators are essential to assess the problems and design corrective measures. **Objective:** To evaluate the impact of implementation of corrective actions on preanalytical stage in region I (capital) and to compare it with other regions. **Methods:** Two phases of the program were compared: initial (2007) and current (2013) according to performance indicators. During this period, the following activities were carried out in region I: training workshops on sample collection (4 times); meetings with health staff in maternity centers (4 times); classes for nursing and biochemistry students about preanalytical stages (2 times); broadcasting by local radio (2 times), local newspaper (2 times), and TV (2 times); and talking to pregnant and postpartum women (2 times). **Results:** Attached table. **Conclusion:** Trainings and dissemination carried out in region I improved indicators. These actions must be implemented in other health regions in order to achieve similar results.

198 - Prevalence of Congenital Hypothyroidism in Premature Newborns in a Sample of Patients of Health District Secretary of Bogota - Colombia

G. Giraldo[1], F. Suarez[1], and J. Prieto[1]

[1]*Pontificia Universidad Javeriana, Bogotá, Colimbia*

Introduction: Preterm infants (<37 weeks of gestation) have low levels of thyroid hormones due to multiple factors. **Objectives:** To determine the prevalence of congenital hypothyroidism in preterm infants in a sample of patients from the Health District Secretary of Bogota (HDSB), to assess whether cord thyroid-stimulation hormone (TSH) level is a valid parameter for screening congenital hypothyroidism in preterm infants, and to establish a correlation between serum TSH and TSH on filter paper. **Methods:** We followed the HDSB protocol of screening for congenital hypothyroidism. When preterm infants completed 37 weeks of gestation, serum TSH, free thyroxine (T4), and TSH from heel were measured. **Results:** A total of 40 infants were rescreened, of which 1 (2.5%) that was born at 34 weeks of gestation had a negative neonatal TSH level and on rescreening the TSH level was 17.32 uUl/mL (normal range 1.36-8.80 uUl/mL). The statistical test of Kolmogorov-Smirnov 2-sample/bilateral was used to compare the neonatal TSH levels of preterm and full-term newborns. It was found that they don't follow the same distribution. Spearman correlation between serum TSH and heel TSH was .739 with P <.0001. **Conclusion:** The 2.5% of the infants rescreened presented high levels of TSH, suggesting the need for rescreening of prematures. Additionally a larger study should be performed to determine the screening cutoff values for preterm infants. There is a direct correlation between serum TSH and heel TSH.

199 - Prevalence of Congenital Hypothyroidism Newborn Screening Service in State Goias in 2011

D. B. Rodrigues[1], K. M. S. Soares[1], J. Furtado[1], J. A. L. Mundin[1], E. P. Santos[2], M. A. I. André[2], F. M. Pedatella[2,3], T. P. T. Araújo[2], M. C. Suzana[2], and J. A. Silva Júnior[1,2]

[1]*Faculdades Anhanguera- Anápolis -Goiás, Brazil*
[2]*Associação de Pais e Amigos dos Excepcionais de Anápolis – APAE, Anápolis- Brasil*
[3]*Universidade Estadual de Goiás – UEG, Anápolis- Brasil*

Introduction: Congenital hypothyroidism (CH) is characterized by insufficient production of thyroid hormones, which is manifested since birth. **Objective:** To determine the prevalence of CH in neonates treated by the neonatal screening laboratory of the Association of Parents and Friends of Exceptional, city of Anapolis–GO, in 2011. **Methods:** Data capture with exclusive access to the physical file of the institution using statistical data to demonstrate results. **Results:** In the year 2011, a total of 74 569 neonates were screened by the laboratory of the Association of Parents and Friends of Exceptional. Only 69 patients were actually suspected of being carriers of the disease, and 44 children are currently in treatment, representing (01) 1 case per 1695. According to this research, CH has higher incidence in males, with higher prevalence in Goiás state than in other states. **Conclusion:** Congenital hypothyroidism in the state of Goiás is higher than expected and was found in 2008 (1 case in 2611), according to the research conducted by the laboratory of the Association of Parents and Friends of Exceptional Children.

200 - Profile of Patients With 17-OH-Progesterone Altered After Initiation of Expanded Newborn Screening in Distrito Federal

K. P. D. Gameleira[1], K. S. P. Malheiros[1], V. G. B. De Araujo[1], J. V. Thomas[2], F. V. S. Canuto[2], M. S. Viegas[1], C. J. F. Lima Junior[2], M. T. O. Cardoso[1], and R. D. T. Teixeira[1]

[1]*Núcleo de Genética, Hospital de Apoio, Brasilia DF, Brazil*
[2]*NAISC, Brasilia DF, Brazil*

Congenital adrenal hyperplasia (CAH) is one of the most common metabolic diseases in newborns. Its prevalence is high, and its diagnosis can be performed early, thereby altering the natural history of the disease. The dose estimation of 17-hydroxyprogesterone (17-OHP) by neonatal screening prevents the early death of these children, through the early institution of treatment. The purpose of this study is to describe the profile of patients diagnosed with CAH using neonatal screening, in Distrito Federal in 2012. Samples were collected on filter paper from 43 897 children born between January and December 2012. We used 20 ng/mL as the cutoff level of 17-OHP, and quantification was performed using a fluoroimmunoassay. Children who maintained altered results were referred to the pediatric endocrinology clinic. Patients were divided into groups according to birth weight and age at the time of sample collection. The estimated incidence of CAH was 1:8.779. A total of 599 (1.36%) abnormal results were detected; after recollecting, 134 (0.3%) remained with altered values, and most patients (92.5%) with abnormal results had low birth weight. These results were confirmed in 5 patients, comprising 2 females and 3 males. The salt-wasting form was the most frequent. Mean age for treatment initiation was 16.5 days. Neonatal screening allows early diagnosis and treatment for CAH, avoiding death. Patients with low birth weight presented high false positive rates and only follow-up allows clarifying a diagnosis for these patients.

201 - Reporting Sickle Cell Carrier Results in Ontario, Canada: A Choice-Based Model

C. Davies[1], S. Kennedy[1], I. Odame[2], R. Hayeems[3], J. Carroll[4], R. Klaassen[5], B. Potter[6], J. Milburn[1], and P. Chakraborty[1]

[1]*Newborn Screening Ontario, Ottawa, Canada*
[2]*The Hospital for Sick Children, Toronto, Canada*
[3]*The University of Toronto, Toronto, Canada*
[4]*Mount Sinai Hospital, Toronto, Canada*
[5]*The Children's Hospital of Eastern Ontario, Ottawa, Canada*
[6]*The University of Ottawa, Ottawa, Canada*

Newborn Screening Ontario (NSO) has offered universal newborn screening (NBS) for sickle-cell disease (SCD) to 140 000 infants, every year since 2006. Sickle-cell disease is an inherited blood disorder caused by mutations in the β-globin gene. The prevalence in Ontario, Canada, is 1:2644. Affected individuals are at risk for multisystemic health problems. Early diagnosis and treatment decreases the risk of bacterial sepsis in children with SCD. The technology used for SCD NBS will also identify carriers of hemoglobin S, C, D, and E. On average, 1900 carriers are identified every year. When SCD NBS began in Ontario, carrier results were not initially disclosed because they are incidental to the primary goals of NBS. To determine the best way to manage these results, the Ontario Ministry of Health and Long-Term Care sponsored a study examining this issue. The results guided development of a choice-based model: carrier results for hemoglobinopathies will be available to families, but it is optional for families to receive them. Since this program was implemented, 107 requests were received. In all, 58 (54.2%) came via health care providers and 48 (44.9%) came directly from parents. Most requests (69.2%) came from families self-identified to be at high risk for a hemoglobinopathy. Of those reported, 25 were carriers (26.3% of requests). This is higher than the carrier rate in the population, suggesting that, appropriately, families at risk are requesting these results; however, the number of requests is less than anticipated. The Ontario model, 2-year experience, and the successes and challenges to date will be discussed.

202 - Results Obtained From the Use of a New Fully Automated System for Newborn Screening, Using Intercientifica Products

R. Veturiano[1], A. H. Luques[1], V. L. Frias F Silva[1], G. P. Gil[1], B. Gallo[1], J. S. C. Pires[1], and C. A. Sampaio Filho[1]

[1]*Intercientifica, Sao Jose dos Campos, SP, Brazil*

Introduction: The development of the neonatal screening, with the inclusion of new diseases and larger volumes, triggers the need for a plan to minimize impacts on the quality and processing time. With the concept of reverse innovation, INTERCIENTÍFICA has developed a new fully automated system to meet the needs of different laboratories. **Objective:** To compare current protocols available in the market with the new system using the products of NeoLISA and NeoMAP line. **Methods:** In order to develop the new system, Nimbus equipment, associated with the MRX reader and LUMINEX 200/Magpix, as well as kits from NeoLISA and NeoMAP lines were used. For direct comparative kits, semi- and fully automated equipments using the fluorimetric method were used. **Results:** The new fully automated system can reduce up to 4 times the processing time by using multiplex format of NeoMAP line and reduced time of colorimetric enzymatic assays of NeoLISA line, when compared to the fluorimetric method option available in the market and also when compared to the random access system. **Conclusion:** Despite the use and concept of standardization, each population, laboratory, and newborn screening program has specific needs that require customized solutions. The new fully automated system defines a concept

developed to meet these needs. The system, fully configurable, promotes safety and improvement in processing time, while maintaining the expected quality for the results and, especially, minimizes the impacts of the inclusion of new diseases.

203 - Review of the Thyroid-stimulating hormone Cutoff Value for the Neonatal Screening of Congenital Hypothyroidism

P. Glikman[1], M. Junco[1], M. Frusti[1], A. Odriozola[1], G. Ropelato[1], G. Dratler[1], S. Marino[1], A. Oneto[1], G. Maccallini[1], F. Smithuis[1], A. Chiesa[1], C. Aranda[1], and P. Muntaabski[1]

[1]*Programa de Pesquisa Neonatal, Dirección de Redes de Servicios de Salud, Ministerio de Salud, Gobierno de la Ciudad de Buenos Aires, Buenos Aires, Argentina*

Introduction: Thyroid-stimulating hormone (TSH) cutoff value (CO) for congenital hypothyroidism (CH) in neonatal screening (NS) is a current subject of debate. The CO selection is based on normal population analyte distribution. The CO for TSH in Buenos Aires City Government CH Program is 10 μUI/mL in whole blood. **Objectives:** Our aim was to review neoTSH CO that are currently used by studying distribution of this analyte in our population of normal newborn babies (NBs) and to analyze the impact of an eventually new CO on recall rate (RR). **Methods:** We analyzed retrospectively neoTSH data from 17 411 samples from term NBs, with weight according to gestational age, not identical twins, excluding CH and medications that might alter the thyroid axis. Samples of between 2 and 3 days old were obtained using S&S903 paper and measured with IFMA (DELFIA, PerkinElmer), from January 2010 to December 2012. **Statistical Analysis:** We calculated the median (M) and percentiles (P) 95, 97.5, 99, and 99.5 (Microsoft Excel 2010). Recall rate was calculated using the current CO and neoTSH P99.5. **Results:** The distribution of neoTSH (mg/dL blood) was M = 2.0, P95 = 5.0, P97.5 = 6.0, P99 = 7.0, and P99.5 = 8.0. We recalled 18 NBs (RR = 0.1%). With P99.5 CO, we would recall 53 NBs (RR = 0.3%). **Conclusion:** We propose to lower the neoTSH CO to 8 μUI/mL. Although using P99.5 the RR rises up to 3 times, it remains low and acceptable for an NS program. Prospective studies with this new CO will provide information of detected patients that will help us to validate this analytical decision.

204 - Situational Analysis to Implement Expanded Screening in Colombia

A. J. Bermudez[1], D. B. Robayo[1], M. J. Rosero[1], D. A. Valera[1], and Y. Gonzalez[1]

[1]*National Institute of Health, Bogota DC, Colombia*

Introduction: Since 2000, newborn screening for congenital hypothyroidism began including integrated management and monitoring. Since the initial government act mandated screening implementation, there have been changes in regulations and technology development for diagnosis and clinical management of inborn errors of metabolism (IEM). **Problem:** In Colombia, neonatal screening is a public health program operated by health care providers and funded by health insurers. This multiplicity of institutions determines various logistical mechanisms and different guidelines for screening, causing inequities and inequalities. It is necessary to study conditions required for establishing expanded screening. **Methods:** Review of regulations, screening strategies, and impact of (IEM) on public health. **Results:** Law 1098/2006 considering congenital problems stated that neonatal screening is a right of children. Law 1392/2010 recognizes orphan diseases, implies that children with (IEM), have planned treatment. In Colombia, 130 laboratories are registered for congenital hypothyroidism screening, few of them (almost 10) offers expanded screening, exporting samples to North America for mass spectrometry analysis (MS/MS). In phenylketonuria, the burden of years of life prevented (YLP) is 40 years/case, with 95 (% I) and 31.8 years of potential life lost (YPLL). Considering the prevalence and all IEM screened, hundreds of thousands of years YPLL are avoided. **Conclusion:** Regulations enable expanded screening; however, it is necessary to initiate a pilot study and also organize screening laboratory networks and health provider networks to take care of children with (IEM).

205 - Solvent Extraction of 17-OH-Progesterone Interferents in Neonatal Screening

A. L. Albrekt[1] and N. L. Baumann[1]

[1]*Programa de Pesquisa Neonatal Misiones, Laboratorio LACMI, Misiones, Argentina*

Measurement of 17-hydroxyprogesterone (17-OHP) in congenital adrenal hyperplasia (CAH) neonatal screening has the drawback of falsely elevated values (FP) due to the presence of interfering substances, mainly in preterm and/or low-weight newborns (P/LWNB). The aim of this work is to quantify 17-OHP directly (DIR) and after removal of interference (ARI) by extraction with solvents in samples with high concentration of 17-OHP. Serial samples of 70 P/LWNB were processed till the completion of 37 weeks of gestational age and in the samples of 2 confirmed newborns with CAH. A competitive enzyme immunoassay UMELISA was used for 17-OHP quantification; the solvent used was diethyl ether. **Results:** CAH diagnosis was discarded in all P/LWNBs by 17-OHP DIR value normalization at the completion of gestational age; 17-OHP ARI were significantly lower and normal in all serial samples. In patients with confirmed CAH, the values remained elevated after extracting interferents for the cutoff used (55 nmol/L). **Conclusion:** Extraction of interferents with solvents can be a useful tool to reduce false positives and is an alternative to serial sampling, which is faster, cheaper, and beneficial to the patient, reducing the diagnostic time and starting treatment earlier in true positive cases.

206 - Standardization of Reference Values for Newborn Screening in Michoacán State, Mexico

C. B. R Elizabeth[1], F. A. Gloria Alicia[1], G. A. Carlos[2], C. Martinez Fabiola[1], F. V. J. Efrain[1], G. C. Anahi Viridiana[1], J. G. Irvin Eduardo[1], T. Mejia Jorge[1], A. R. Maria Milagros[1], and B. Botello[1]

[1]*Laboratorio Estatal de Salud Publica de Michoacán Servicios de Salud de Michoacán en Morelia México*
[2]*IMSS Centro de Investigación Biomédica de Michoacán Morelia México*

Introduction: Newborn screening in Mexico has allowed early detection of inborn errors of metabolism. Thus, it is necessary to establish reference values for Mexican population in order to identify and discriminate affected individuals from healthy ones. **Objective:** To establish reference values for metabolic screening of phenylketonuria, congenital adrenal hyperplasia, congenital hypothyroidism, and galactosemia using a population of newborns in Michoacan, Mexico. **Methods:** Retrospective descriptive study of samples analyzed in L.E.S.P. Michoacan. Percentiles, histograms, confirmatory R2, and medical evaluation were obtained from January 2011 to February 2013 for 17 α-hydroxyprogesterone (17-OHP) and phenylalanine, and from January to June 2011 for total galactose and thyroid-stimulating hormone (TSH). Dry blood samples in filter paper drawn from newborn's heel after 48 hours of birth from 8 health districts of the interior of state. **Results:** Of the 11 330 samples analyzed, TSH was confirmed in 9 cases, using a cutoff value of 9.2 μUI/L (98th percentile). Of the 48 188 samples analyzed, 17-OHP was confirmed in 7 newborns, using a cutoff value of 30.0 ng/dL (99.4 percentile). In Phenylalanine: From 53 484 samples analyzed for phenylalanine, 23 cases were suspected and then discarded by confirmatory test and medical evaluation, cutoff values being 3.2 mg/dL (99.93 percentile). For total galactose, of the 11 184 samples analyzed, the initial value was below the 98th percentile, so the value was raised and the cutoff value was established at 18.0 mg/dL. **Conclusion:** Standardization of reference value allows in decreasing the number of suspected cases and costs, avoiding trauma to healthy newborn and parents' anxiety.

207 - Statistical Analysis Preanalytical Stage for the Sampling Process for Newborn Screening in Costa Rica

S. Obando[1], R. Trejos[2], and M. Saborio[3]

[1]*Asociación Costarricense para el Tamizaje y la Prevención de Discapacidades en el Niño, Sao Jose, Costa Rica*
[2]*Laboratorio Nacional de Tamizaje Neonatal y Alto Riesgo (Lab-PNT, Sao Jose, Costa Rica)*
[3]*Servicio de Genética Médica y Metabolismo, Hospital Nacional de Niños, San José, Costa Rica*

Introduction: Neonatal screening program in Costa Rica was implemented over 20 years ago, which currently contributes to the detection of 25 diseases by running 6 different assays. In 2012, the national coverage reached 98.4%. It receives the active participation of 938 sample collection centers across the country (public health centers of the social security 90.9% and private 9.1%). **Objective:** To present the results obtained by statistical analysis focused on the preanalysis, corresponding to the sampling process for newborn screening, in child population of Costa Rica, during the period of May 2012 to May 2013. **Methods:** The statistical study comprised 78 127 population samples that were collected within the period from May 2012 to May 2013. We used the database of the laboratory computer system from STARLIMS v10.1, and the following indicators were determined: age at sampling, time spend in the transportation of the samples, age of babies in days at the time of receiving the sample in the laboratory. The following statistical calculations were performed: mean and percentiles 25-50-75-95-99.5. The percentage of unsatisfactory samples was calculated. **Results:** The average time for age at sampling, time spend in the transportation of the, and age of babies at the time of receiving the sample in the laboratory was 5.1, 3.7, and 8.8 days, respectively. The percentage of unsatisfactory samples was 1.48%. **Conclusion:** It is of utmost importance in frequent monitoring of quality indicator in a neonatal screening program because it allows timely implement of corrective and preventive actions that promote continuous, daily improvements.

208 - Total Automation in the Quality Assessment of UMELISA Assays Used for Neonatal Screening

A. Rego[1], H. Perez[1], N. Carlos[1], and L. Lopez[1]

[1]*Centro de InmunoEnsayo, La Habana, Cuba*

Objective: To implement the automated quality assessment in the laboratory network of neonatal screening with SUMMA technology. **Methods:** Three systems were developed and installed in different levels of the quality assessment program: (1) diagnostic laboratories, (2) laboratory of surveillance destined laboratories network control, and (3) a mail server at the Center of Immunoassay for message reception, assessment, and delivery of results. **Results:** The use of late-generation computational tools proved that total automation (information gathering, evaluation, and results delivery) has boosted quality control process in the neonatal screening network, reducing significantly the time devoted to implement corrective measures. In addition, the introduction of new algorithms that facilitate the use of the internal control has drastically reduced the time spent in detection and correction of undesirable deviations, thus improving quality control evaluation. **Conclusion:** Total automation in quality assessment guarantees the performance of Cuban health programs and has proved its importance for laboratory error detection and in the reduction of false positive and false negative results.

209 - TSH, 17-OHP, Phe, and Galactose Levels in Preterm Newborns and/or Seriouslly Ill Neonates

E. C. Gonzalez[1], R. Coto[2], A. Frometa[1], I. Almeida[1], E. M. Castells[1], Y. Tejeda[1], P. Almenares[1], L. Del Rio[1], M. T. Segura[1], and P. L. Perez[1]

[1]*Centro de Inmunoensayo, Habana, Cuba*
[2]*Hospital de Ginecologia y Obstetrícia "Ramón González Coro", Habana, Cuba*

Objective: To evaluate thyroid-stimulating hormone (TSH), 17-α-hydroxyprogesterone (17-OHP), phenylalanine (Phe), and galactose (Gal) levels in dried blood samples (DBS) on filter paper from preterm newborns and/or seriously ill neonates and its influence on the false positive rate (FPR). **Methodology:** From January 2012 to May 2013, these analytes were evaluated in 2 groups, GI: 840 DBS from seriously ill neonates collected in Gineco-Obstetric Hospitals neonatology rooms and GII: 1816 DBS from 7 Integral Active Screening Centers in Havana. SUMA technology ultramicroassays were used. Mann-Whitney test was used for comparison between groups. A P value of <.05 was considered statistically significant. **Results:** There was significant difference ($P < .01$) in birth weight (GI: 2920.4 $\pm$ 775.4; GII: 3380.1 $\pm$ 461.5 g) and gestational age (GI: 37.6 $\pm$ 2.8; GII: 39.5 $\pm$ 1.3 weeks) between groups; 28.1% and 2.0% were premature newborns in GI and GII, respectively. The age of sample in the GI was 5.6 $\pm$ 3.4 days and in GII 6.3 $\pm$ 3.7 days. Significant differences for average 17-OHP (GI: 30.2; GII: 20.5 nmol/L; $P < .01$), Phe (GI: 115; GII: 122 μmol/L; $P = .04$), and Gal (GI: 2.7; GII: 3.2 mg/dL; $P < .01$) levels were obtained. Mean TSH concentrations were similar in both groups (2.4 mUI/L, $P = .21$); FPR was influenced by prematurity and the newborn condition being more marked for 17-OHP, where the FPR in GI was 7.1% and in GII 0.5%. **Conclusion:** Prematurity and stress due to neonatal illnesses affect the FPR, which indicates the necessity to implement different neonatal screening strategies for preterm and seriously ill newborns, in order to improve the program efficiency.

210 - Which Is the Ideal Cutoff Levels for Detecting Congenital Hypothyroidism Through Screening Test?

Marcia Giusti[1], Giselle Hayashi[1], Lilian Sadeck[2], Geny Yao[2], and Athenee Mauro[2]

[1]*SRTN/APAE DE São Paulo, Brazil*
[2]*Área Técnica da Saúde da Criança e do Adolescente da Secretaria Municipal de Saúde, Município de São Paulo, São Paulo, Brazil*

Introduction: The National Newborn Screening (NS) manual suggests 20 mIU/mL serum equivalents as cutoff level for neonatal thyroid-stimulating hormone (NTSH), but recent studies recommended reducing the cutoff values. **Objective:** To evaluate the incidence of congenital hypothyroidism (CH) in 2011 and compare them through the NS, using 2 cutoff levels 15.0 and 20.0 mIU/mL in serum equivalents to NTSH in filter paper. **Methods:** Retrospective study (January 1, 2011 to December 31, 2011), including 80 321 NS tests in newborns from São Paulo. They were subdivided according to the first TSH: group A (TSH >15 mIU/mL) and group B (TSH >20 mIU/mL), and the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. **Results:** A total of 1231 children presented the first TSH >15 mIU/mL: group A (N = 910/73.9%) and group B (N = 321/26.1%). Of these children, 133 performed confirmatory diagnosis (free thyroxine [FT4] and TSH): 68 (51%) with first TSH between 15 and 20 mUI/mL, and 65 (49%) with the first TSH >20mUI/mL confirmed CH. Considering group A (first TSH >15 mIU/mL): sensitivity 100% (range 97.3%-100.0%), specificity 98.6% (range 98.6%-98.7%), PPV 10.8% (range, 9.1%-12.7%), and NPV 100% (range 100.0%-100.0%); and group B (first TSH >20 mIU/mL): sensitivity 48.9% (range 40.1%-57.7%), specificity 99.7% (range 99.6%-99.7%), PPV 20.3% (range 16.0%-25.1%), and NPV 99.9% (range 99.9%-99.9%). **Conclusion:** These data showed that more than half of the patients with CH would be missed if the cutoff value was >20 mIU/mL. Cutoff for the first TSH >15 mIU/mL showed higher sensitivity and NPV. Early diagnosis and timely treatment improves life prognosis of these children.

Indice por autores

Funding

This supplement was supported by educational grants from the Latin American Society of Inborn Errors of Metabolism - SLEIMPN and from Instituto Genética para Todos.