Abstract/Resumen: Acute Intermittent Porphyria (AIP) is a hepatic pathology characterized by the accumulation of porphyrin precursors. It has sudden neurovisceral manifestations, commonly known as acute attacks or crises, which are triggered by exposure to different factors (fasting, stress, hormones and commonly used medications). Many hypotheses try to explain the variability in the prediction of the porphyrinogenicity of drugs, among which are the genetic polymorphisms of the CYP-450 enzymes. In this study, we wanted to verify if the difference in the triggering of an acute crisis was given by some of the most frequent and clinical significant polymorphisms of the enzymes of the CYP3A family, or even by a difference in their expression, given by the AKR1D1, an enzyme that regulates the expression of various CYPs and whose polymorphisms would augment CYPs expression. Peripheral blood samples of AIP patients (n= 50) and no AIP patients were analyzed (n= 74) by PCR-RFLP and PCR-sequencing. The variants analyzed were CYP3A4*22, CYP3A5*3, and AKR1D1 rs1872929 and rs1872930. Both allelic and genotypic frequencies found in the AIP group are very similar to those of the non-AIP group. So, when they were statistically analyzed with the X^2 test ($p < 0.05$), it was not a surprise to find out that there is no trend of the AIP population to any SNP. Therefore, the genetic polymorphisms analyzed do not explain the interindividual variability that exists in these patients when an acute attack arises. Another interesting conclusion obtained from this study is that there is a significant amount of extensive drug metabolizers compared to poor drug metabolizers in both groups. This is important for adjusting the dose of numerous drugs that are metabolized by this path. If these results are maintained with a higher number of patient in the Argentinian population, it will mean that doses of the drugs affected by these CYPs need to be adjusted, contributing to the safety and efficacy of therapeutics.

0572 - . GENOMIC DIAGNOSIS IMPLEMENTATION IN A PEDIATRIC HOSPITAL. PRELIMINARY RESULTS

Paula Alejandra SCAGLIA (1) | María ESNAOLA AZCOITI(1) | Bárbara CASALI(1) | Laura VALINOTTO(2) | Solange ROSENBROCK(1) | Florencia VILLEGAS(3) | Celeste ARGUELLES(3) | Ariel BERENSTEIN(4) | Agustín IZQUIERDO(1) | Nora SANGUINETI(1) | Débora BRASLAVSKY(1) | Marina SZLAGO(3) | María Del Carmen FERNÁNDEZ(3) | Romina ARMANDO(3) | Franco BRUNELLO(1) | Gabriela SANSO(1) | Ignacio BERGADÁ(1) | Claudia ARBERAS(3) | Marcelo MARTÍ(5) | María Gabriela ROPELATO(1) | Rodolfo Alberto REY(1)

CENTRO DE INVESTIGACIONES ENDOCRINOLÓGICAS "DR. CÉSAR BERGADÁ" (CEDIE) - CONICET - FEI (1); CONICET (2); HOSPITAL DE NIÑOS "RICARDO GUTIERREZ" (3); INSTITUTO MULTIDISCIPLINARIO DE INVESTIGACIONES EN PATOLOGÍAS PEDIÁTRICAS (IMIPP), CONICET-GCBA (4); DEPARTAMENTO DE QUÍMICA BIOLÓGICA, FACULTAD DE CIENCIAS EXACTAS Y NATURALES, UBA. (5)

Abstract/Resumen: The access to new technologies, like Next Generation Sequencing (NGS) and microarray, has allowed the development of effective high-performance diagnosis algorithms for genetic pediatric diseases. The aim of this work was to establish standardized procedures for genomic diagnosis of genetic pediatric diseases in a pediatric hospital. Patients with presumptive diagnoses of genetic diseases (intellectual disability, metabolic, hematological or immune diseases or delay of growth and puberty) were included. DNA from peripheral blood was obtained from the patients and their parents. Genomic diagnosis procedures were performed by NGS (Clinical exome, TruSight One, NextSeq 500 Illumina) and microarray studies (8x60K Platform, Agilent). NGS results were analyzed by own designed bioinformatic pipelines, and B platform (Bitgenia) was used to prioritize variants. All variants found (sequence changes or Copy Number Variations) were classified according to American College of Medical Genetics and Genomics recommendations. This study was approved by the hospital ethical review board. Diagnostic flowchart was implemented according to designed operative protocols. Patients referred by specialized pediatricians were evaluated by the interdisciplinary team to agree on the best diagnostic pathway. From March 2018 to August 2019, 200 probands were included (86 with delay of growth and puberty, 12 hematologic, 4 immunologic and 55 metabolic disorders and 43 with intellectual disability). Among the 36 cases studied by microarray, 5 pathogenic variants (13.9 %), and 3 variants of uncertain significance were found. In 24 of the 60 patients (40 %) studied by NGS, genic variants related to patient's phenotype were found. Conclusion: Interdisciplinary team work has enabled the successful implementation of these new genomic diagnosis techniques in the hospital. Diagnosis efficiency achieved agrees with international standards.

0588 - ASSESSMENT OF F9 GENOTYPE SPECIFIC INHIBITOR RISKS ASSOCIATED WITH A LARGE SERIES OF ARGENTINE PATIENTS WITH HEMOPHILIA B

Betiana Michelle ZIEGLER (1) | Vanina MARCHIONE(1) | Karen WAISMAN(1) | Martín ABELLEYRO(1) | Daniela NEME(2) | Carlos D. DE BRASI(3) | Pamela RADIC(1) | Liliana Carmen ROSSETTI(1)

INSTITUTO DE MEDICINA EXPERIMENTAL (IMEX), CONICET-ACADEMIA NACIONAL DE MEDICINA (ANM) (1); FUNDACIÓN DE LA HEMOFILIA (2); INSTITUTO DE MEDICINA EXPERIMENTAL (IMEX), CONICET- IIHEMA - ACADEMIA NACIONAL DE MEDICINA (ANM) (3)

Abstract/Resumen: Hemophilia B (HB) is an X-linked disorder caused by pathogenic variations in the coagulation factor IX gene (F9). Currently, HB is successfully treated by substitution of the deficient FIX. Development of inhibitory antibodies (INH) against the therapeutic FIX represents a major complication affecting patients and the public health economy. INH development in HB is typically associated with allergic and/or anaphylactic reactions. The objective was to estimate the global and partial F9 genotype risks of INH in a large series of Argentine HB patients (about 1/3 of all HB patients registered in Argentina (WFH Global Survey 2018)). We characterized the HB causative variation in 98 out of 104 studied patients (94 % of efficiency) by application of an in-house developed algorithm including 12 PCR-amplifications allowing gross deletion detection in hemizygous probands, small-mutation screening by CSGE (conformation sensitive gel electrophoresis), and Sanger DNA-sequencing of the suspected region. The case (INH+)/control(INH-) study included 10 cases and 94 controls (n= 104) assessing a global absolute INH-prevalence (GAIP) of 9.6 %. Absolute and relative risks of each F9-genotype are presented as INH-prevalence, AIP and odds ratios, OR (CI95%), respectively. Large F9-deletions showed increased risks, AIP of 50 % (6/12) and an OR of 22 (4.8 - 99.7) p=0.0001; and considering entire F9-deletions, an AIP of 71 % (5/7) and OR of 46 (7.1 - 298) p<0.0001. F9-nonsense variations showed non-significant INH risks 3/17 (18 %) OR of 2.4 (0.5 - 10) p= 0.2 as well as F9-splicing defects, 1/7 (14 %) OR of 1.6 (0.2 - 15) p= 0.5. On the other hand F9-missense showed the lowest risks, 0/49 of AIP and an OR of 0.043 (0.002 - 0.7) p= 0.001. Our GAIP is placed on the upper limit as compared with other international series (9.6 % vs. range 3 – 11 %). Our findings about F9-genotype associated INH risks in Argentina may help hemophilia therapists in designing a case-specific treatment and properly fitted follow-up regimes.

0589 - GENOTYPING PARTIAL F8 DELETIONS CAUSING SEVERE HAEMOPHILIA A IN HEMIZYGOUS AND HETEROZYGOUS STATE: NEW APPLICATIONS OF INVERSE-PCR

Karen WAISMAN (1) | Betiana Michelle ZIEGLER(1) | Vanina MARCHIONE(1) | Pamela RADIC(1) | Carlos DE BRASI(2) | Martín ABELLEYRO(1) | Liliana Carmen ROSSETTI(1)

INSTITUTO DE MEDICINA EXPERIMENTAL (IMEX), CONICET-ACADEMIA NACIONAL DE MEDICINA (ANM) (1); INSTITUTO DE MEDICINA EXPERIMENTAL (IMEX), CONICET- IIHEMA - ACADEMIA NACIONAL DE MEDICINA (ANM) (2)

Abstract/Resumen: Large F8 deletions are responsible for approximately 8 – 15 % cases with severe haemophilia A (sHA) and predispose to the development of FVIII therapeutic inhibitors. This work presents two practical approaches for genotyping large deletions both based on inverse-PCR that permitted resolving the cases of two unrelated families with sHA. Family 1 includes an affected patient with a deletion of F8-exon 24–26 whilst family 2 is composed by a family proband with a F8-exon 5-6 deletion and two female relatives (his mother and sister). The objective was to develop cost-effective approaches to diagnose large F8 deletions in hemizygous patients and their potential heterozygous female carriers. We designed and developed a protocol of inverse-PCR (iPCR) combined with long distance-PCR (LD-PCR) to analyse and characterise the breakpoint junctions in family 1, and the approach of inverse shifting-PCR (IS-PCR) to detect the presence/absence of the specific F8-deletion of family 2 in the proband and their female relatives. Based on the BclI restriction map, a LD-iPCR amplification system was designed to discriminate the normal variant (3.5 kb) and the deletion variant associated with 4.2 kb using primers on F8-intron 22 (IVS22-lo) and on F8-intron 23 (24A). Standard-size IS-PCR discrimination system was designed to detect and recognize the 1 kb normal allele (primers Bup1B and IVS6M-up) and the deleted variant (product size of 1.3 kb) obtained from primers IVS65-lo and IVS6M-up. As it was expected, in both families, deletion-specific LD-iPCR or IS-PCR amplification products were obtained in familiar probands and not-observed in normal control samples. Carrier diagnosis in family 2 indicated that both the mother and the sister resulted heterozygous for the deletion. Our findings points the utility to apply cost-effective and reliable approaches such as LD-iPCR and IS-PCR to allow detection and diagnosis of large deletions on X-linked genes, like the F8, to provide valuable information for carrier detection and prenatal diagnosis in families with X-linked disorders like HA.

0615 - GENETIC STUDY OF 367 PATIENTS WITH MULTIPLE CONGENITAL ANOMALIES (MCA) AND ISOLATED CONGENITAL HEART DISEASE (CHD)

Marisol DELEA (1) | Soledad MASSARA(2) | Lucia ESPECHE(1) | Ma. Paz BIDONDO(1) | Jaen OLIVERI(2) | Paloma BRUN(2) | Pablo BARBERO(1) | Celeste MARTINOLI(3) | Verónica CAZAYOUS(2) | Lilian FURFORO(1) | Viviana COSENTINO(4) | Mónica RITTLER(5) | Sandra ROZENTAL(1) | Liliana DAIN(1)

CENTRO NACIONAL DE GENÉTICA MÉDICA- ANLIS (1); HOSPITAL DE ALTA COMPLEJIDAD EN RED EL CRUCE – SAMIC (2); HOSPITAL SOR MARIA LUDOVICA (3); HOSPITAL INTERZONAL GENERAL DE AGUDOS LUISA CRAVENNA DE GANDULFO (4); HOSPITAL MATERNO INFANTIL RAMÓN SARDÁ (5)

Abstract/Resumen: Congenital anomalies (CA) are morphological and/or functional disorders that originate before birth. Affecting 3 to 5 % of newborns, they represent the second leading cause of infant mortality in Argentina, after perinatal conditions. In approximately 50 % of the patients, the underlying causes are unknown. Cases with MCA are those with 2 or more unrelated birth defects. MCA are present in 2.26 / 1000 births. CHD are the most frequent CA, with a prevalence of 4.06 / 1000 births. The goal of this work was to identify the genetic causes of MCA and isolated CHD cases from our population. We studied 367 patients (169 MCA and 198 isolated CHD) born between June 2015 and August 2017 in 13 public hospitals participating in the National Network of Congenital Anomalies of Argentina (RENAC). Peripheral blood and DNA was obtained from all patients and a karyotype was performed in MCA patients. Patients with conotruncal CHD or DiGeorge phenotype (n= 126) were studied by MLPA. Array-CGH was performed in 77 MCA selected patients. A total of 15 CHD patients were analyzed by a Next Generation Sequencing (NGS) gene panel or by Exome Sequencing. one hundred and seventeen MCA patients displayed a normal karyotype, 12/129 presented cytogenetic anomalies: a trisomy 13, 5 trisomy 18, a 47,XXX/47,XX,+14, 2 translocations a (t(1;2)(q25;q21)) and t(11;17)(p10;p10), a del(15)(q11.2q13) and 2 supernumerary marker chromosome. The karyotype could not be performed in 40 patients due to culture failure. Among 126 cCHD patients, 21 presented a typical 22q11 deletion, three 22q11 short deletion, one 22q11 duplication, and one TBX1 gene deletion. We found that 13/77 MCA patients had a causal or potentially causal CNVs. After NGS analysis, five patients presented 5 different nucleotide variants with possible impact on protein function. Using this algorithm that combines a technical and clinical strategy, 20 % of the patients analyzed were diagnosed.

0626 - MOLECULAR GENETIC STUDIES IN A LARGE ARGENTINEAN COHORT (1152) OF 21-HYDROXYLASE DEFICIENT PATIENTS AND RELATED INDIVIDUALS

Melisa TABOAS (1) | Cecilia FERNANDEZ(1) | Carlos David BRUQUE(1) | Belen BENAVIDES-MORI(1) | Aldana CLAPS(1) | Susana BELLI(2) | Mirta STIVEL(2) | Titania PASQUALINI(3) | Liliana ALBA(1) | Marisol DELEA(1) | Lucia ESPECHE(1) | Eduardo CHARREAU(4) | Noemi BUZZALINO(1) | Liliana DAIN(1)

CENTRO NACIONAL DE GENÉTICA MÉDICA- ANLIS (1); HOSPITAL DURAND (2); HOSPITAL ITALIANO DE BUENOS AIRES (3); IBYME-CONICET (4)

Abstract/Resumen: Congenital Adrenal Hyperplasia (CAH) is an autosomal recessive disease produced in 95 % of cases due to 21-hydroxylase deficiency. CAH is presented in 3 clinical forms, 2 severe or classical: salt wasting (SW) or simple virilizing (SV) and a mild or non-classical form (NC). The gene encoding 21-hydroxylase, CYP21A2, shares 98 % sequence identity with the pseudogen CYP21A1P. Our objective was the molecular characterization of 21-hydroxylase deficiency in patients of our population. In this work, we analyzed 1,152 individuals from our populations: 628 21-hydroxylase deficient patients (78SW, 90SV, and 460NC), 398 relatives and 126 partners. All were recruited between 1996 and 2018. Until 2011, the 10 most frequent derived-pseudogene point mutations in the CYP21A2 gene were screened by allele-specific PCR or PCR-RFLP. For those samples with at least one non-determined allele, as well as for those recruited from 2011 to 2018 (n= 343), direct sequencing was performed. Deletions/duplications were analyzed by MLPA SALSA P050-C1 CAH MLPA kit. The most frequently mutated allele in NC patients was the p.V282L. In classical patients were c.293-13C>G and p.I173N. Patients presenting c.293-13C>G or p.I173N showed more than one possible phenotype. In NC patient, 86.9 % of the alleles presented mutations. A total of 60 alleles disclosed novel or rare mutations. From these, 11 mutations were found for the first time and published by our group in recent years. In addition, 3 novel mutations, p.(S166F); p.(P189R) and p.(R436L) are being described in this study. From the 330 parents analyzed, all but one were carriers. One of the probands disclosed a de novo mutation. Interestingly, 5 fathers, 3 brothers, 1 sister and 2 mothers presented both alleles with a mutation but without clinical signs. By last, 105 of the 126 partners were non-carriers. Thus, several techniques and molecular approaches need to be applied for a comprehensive characterization of the diseased alleles. In that sense, our work represents one of the larger and complete genetic characterization of 21-hydroxylase deficient patients from our region.

0628 - ATYPICAL MOLECULAR CAUSE OF FRAGILE X SYNDROME: A CASE REPORT

Melisa TABOAS (1) | Celeste MATINOLI(2) | Laura ORELLANO(2) | Tania CASTO(1) | Agustín BECKER(2) | Noma CECOTTI(2) | Stella CASTRO(2) | Liliana DAIN(1) | Lucía ESPECHE(1)

CENTRO NACIONAL DE GENÉTICA MÉDICA- ANLIS (1); HOSPITAL SOR MARIA LUDOVICA (2)