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LXVIII REUNIÓN ANUAL DE LA
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10-13 de noviembre de 2020

EDITORES RESPONSABLES
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ANNUAL MEETING OF BIOSCIENCE SOCIETIES 2020

LXV ANNUAL MEETING OF
SOCIEDAD ARGENTINA DE INVESTIGACIÓN CLÍNICA (SAIC)

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RESPONSIBLE EDITORS
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Maria Cecilia Larocca

Introduction: More than 2200 *LDLR* variants have been described in clinical Familial Hypercholesterolemia. Since reporting variants without full knowledge of their pathogenicity represents a risk for patients and their family group, establishment of functional studies for them is of utmost importance for Familial Hypercholesterolemia diagnosis. Somatic genome editing, using CRISPR-Cas9 technology, has a tremendous potential for human gene therapy of lipid disorders.
Objective: we aimed at designing an *in silico* strategy for generating a knockout cell line in *LDLR* gene, using a CRISPR-Cas9 system, to set the basis for a future knock-in stage for variants whose functional pathogenicity must be demonstrated.
Material and Methods: Guide crRNAs for *LDLR* gene were designed using the Chop-Chop and CRISPOR platforms. We investigated the structure of *LDLR* gene and protein by analysing their functional domains using bioinformatics tools like ELM and SMART. The efficiency scores were calculated by Doench JG, *et al* (2016) and Moreno-Mateos M, *et al* (2015).
Results: We obtained a battery of 6 crRNAs which were ranked by their genomic position, efficiency, number and localization of off targets and frameshift frequency. Although several crRNAs were obtained, we selected those ones targeted at exons 1 and 2 of the *LDLR* gene in order to have higher performance in the knockout process. The efficiency score ranged from 46 to 66%, with a maximum of 7 off targets with 3 missmatches. The off targets did not interact or participate in the *LDLR* gene metabolic pathway.
Conclusion: Our strategy provides a battery of 6 crRNAs, targeted at exon 1 and 2 of the *LDLR* gene affecting the translation start and ligand binding domain, respectively. Given these results, we will try to prove these crRNAs in HepG-2 cell line in order to resemble the *in vivo* lipid metabolism to set the basis for functional categorization of *LDLR* gene variants associated with Familial Hypercholesterolemia.

90. (322) **MOLECULAR CHARACTERIZATION OF THYROGLOBULIN VARIANTS IDENTIFIED IN PATIENTS WITH GOITROUS HYPOTHYROIDISM. ANALYSIS OF THE SPLICING MECHANISM.**

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Thyroglobulin (TG) is a homodimeric glycoprotein synthesized by the thyroid gland. To date, two hundred twenty-seven variations of the TG gene have been identified in humans. Thyroid dyshormonogenesis due to TG gene mutations have an estimated incidence of approximately 1 in 100,000 newborns. The clinical spectrum ranges from euthyroid to mild or severe hypothyroidism. Splicing mutations represent a major cause of human disease, between 15–50% of all human disease. Variants at the level of the splice site lminate in important defects at the level of the pre-mRNA splicing process. The splicing process is quite complex whose molecular bases and interactions with underlying elements are still not entirely clear, resulting as we show in the present work, a rare phenotype involving mechanisms of such processing of those pre-RNAS from a variant founded for our group in a hypothyroid patient.Thepurpose of the present studywas to identifyand characterizenew variants in the TG gene. We report an Argentine patient with congenital hypothyroidism, enlarged thyroid gland and low levels of serum TG. Sequencing of DNA, expression of chimeric minigenes as well as bioinformatics analysis were performed.
DNA sequencing identified the presence of compound heterozygous variant in the TG gene: the maternal mutation consists of a c.3001+5G>A, whereas the paternal mutation consists of p.R296*. Minigen analysis of the variant c.3001+5G>A performed in HeLa, CV1 and Hek93T cell lines, shows a total miss of transcript expression. So, in order to validate that the lack of expressionwas caused by such variation, site-directed mutagenesis was performed on the mutated clone,who had a pSPL3 vector change, to give rise to a wild-type clone c.3001+5G and to indorsing that the mutation c.3001+5G>A is the cause of the total lack of expression. These results open up new perspectives in the knowledge of the mechanism of splicingforthe TG pre-mRNA.

91. (385) **A RARE POU1F1 SPLICING VARIANT AS A CAUSE FOR ANTERIOR HYPOPITUITARISM**

Martínez Mayer JJ[1], Smith C[2]., Vishnopolska S[1], Braslavsky D[3], Keselman A[3], Bergadá I[3], Marti M[4], Camper SA[2], Kitzman JO[2], Perez Millan MI[1]
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POU1F1 is a signature pituitary transcription factor that directly regulates the transcription of growth hormone (*Gh)*, prolactin (*Prl*), and both the alpha (*Cga*) and beta subunits of thyroid stimulating hormone (*Tshb*). Multiple missense mutations in *POU1F1* have been reported to cause combined pituitary hormone deficiency and/or isolated growth hormone deficiency (IGHD). Alternative splicing in this gene results in two isoforms: the predominant transcriptional activator alpha and the minor isoform beta that acts as a transcriptional repressor. The POU1F1 beta isoform transcript is created by utilization of an upstream splice acceptor sequence in exon 2 which results in a protein with insertion of 26 amino acids that encode an ETS1 binding domain inserted in the transactivation domain. All the reported mutations are in domains shared by the alpha and beta isoforms of POU1F1 and were functionally tested using the alpha isoform only. We performed whole exome sequencing (WES) in a familial case with IGHD and found a heterozygous and synonymous variant (c.150T>G, p.Ser50Ser50) in *POU1F1* presented on the affected father and son. Interestedly, this variant affects POU1F1 splicing without changing the amino acid sequence. By a high throughput reporter assay we found that this variant shifts splicing to favor the POU1F1 beta isoform almost exclusively, while retaining its transcriptional repressor activity on the *POU1F1* enhancer. Therefore, we conclude that this mutation is causative of the patient's phenotype, highlighting the importance of a detailed analysis of sequencing results, particularly of synonymous mutations near splicing sites, which are often overlooked.

92. (405) **VARIANTS OF UNCERTAIN SIGNIFICANCE (VUS) MODEL SHOW**

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Muscular Dystrophies (MD) are a group of rare inherited diseases that cause weakness and progressive degeneration of muscle tissue. The clinical symptoms of these pathologies overlap, hindering differential diagnosis, which is of paramount importance to establish the standard of care. Among them, Dystrophinopathies are the most prevalent type of MD and are caused by mutations in the DMD gene. Genetic or molecular studies are the gold standard for reach-

ing a MD differential diagnosis, for which molecular alterations in MD associated genes can be detected by Whole Exome Sequencing (WES). One of the major challenges of the Next Generation Sequencing (NGS) data interpretation is the occurrence of Variants of Uncertain Significance (VUS). The present work aims to provide a thorough strategy to analyze the effect of VUS, applying different predictive software, conservation/evolutionary and protein modeling tools. A cohort of 141 patients with presumptive clinical diagnosis of dystrophinopathy and negative MLPA result was analyzed by WES. We deepened the screening to all the MD associated genes included in the Gene Table of Neuromuscular Disorders. In a subset of 6 individuals, we detected VUS in the following genes: DMD (2/6), FKRP (2/6) and POMT2 (2/6). We implemented several predictive software to analyze the effect of VUS, and UCSF ChimeraX for protein modeling. Also, in one case, we could do a segregation analysis of the variants. The implemented strategy provided new insights to predict more accurately the effect of the identified sequence variants and even reclassified them. Finally, this work provides alternative approaches for the analysis of sequence variants, especially when functional studies are not possible to be carried out, to determine the effect of VUS.

93. (406) **BEYOND CLASSIC MOLECULAR ALTERATIONS: NON-CONTIGUOUS MUTATIONS IN DMD GENE**

Leonela Luce[1,2], Micaela Carcione[1,2], Chiara Mazzanti[1,2], Irene Szijan[1], Sebastian Menazzi[3], Liliana Francipane[3], Julián Nevado[4,5], Pablo Lapunzina[4,5], Liliana Rossetti[6] , Pamela Radic[6], Martin Abelleyro[6], Carlos De Brasi[6], Florencia Giliberto[1,2]

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Introduction: Dystrophinopathies are neuromuscular X-linked recessive diseases caused by DMD mutations. Molecular alterations in this gene are large deletions/duplications in 80% of cases and small variants in the remaining. Several authors reported the occurrence of non-contiguous rearrangements within the same DMD allele, with frequencies up to 4%. The present work aims to characterize the incidence of complex rearrangements in an Argentinian dystrophinopathy cohort and unravel the causing molecular mechanisms.
Materials and Methods: We analyzed 437 boys with clinical diagnosis of Dystrophinopathy. The following techniques were implemented: MLPA, WES, WGS, PCR-Sanger Sequencing, CGH Array and HUMARA assay. In 2 cases, breakpoints were precisely determined, so we performed a bioinformatic screening of microhomologies, interspersed repeats, secondary structures and recombinogenic motifs 50pb surrounding each breakpoint.
Results: We detected 6 patients carrying complex rearrangements in DMD: 2 deletions-duplications, 3 non-contiguous duplications and 1 large deletion plus a 20pb insertion. These accounted for 1.4% of our cohort. In a deletion-duplication case, familial segregation and bioinformatics analysis suggested that the duplication was the first mutagenic event caused by Fork Stalling and Template Switching (FoSTeS), while the deletion occurred secondly by Non-homologous end joining. Furthermore, bioinformatic screening of the deletion plus insertion propose that the deletion was due to Microhomology-mediated end joining, while the insertion arose by FoSTeS.
Conclusions: Our findings widen the understanding of the molecular events that may take place in DMD and characterize the occurrence of complex rearrangements in our dystrophinopathy cohort.

94. (407) **IDENTIFICATION OF LIKELY PATHOGENIC VARIANTS IN NOVEL CANDIDATE GENES FOR HYPOPITUITARISM IN ARGENTINIAN CHILDREN**

Camilletti MA[1,2], Vishnopolska SA[1,2], Mercogliano MF[1,2], Braslavsky D[3], Keselman A[3], Bergadá I[3], Marino R[4], Ramírez P[4], Pérez Garrido N[4], Patiño Mejia H[4], Ciaccio M[4], Di Palma MI[4], Belgorosky A[4], Marti MA[2], Kitzman JO[5], Camper SA[5] and Perez-Millan MI[1]

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Congenital hypopituitarism(CH) comprises of a spectrum of disorders that range in severity from isolated growth hormone deficiency(IGHD) to combined pituitary hormone deficiency(CPHD) when two or more pituitary hormones are deficient. The clinical spectrum varies widely and can present in isolation or with other birth defects. We conducted target panel genetic screening using single-molecule molecular inversion probes sequencing to assess the frequency of mutations in known hypopituitarism genes and new candidates. We captured genomic DNA from 170 pediatric patients with CH, either alone or with other abnormalities. We identified novel pathogenic, likely pathogenic(LP) or variants with uncertain significance in 26 cases. Interestingly, we found that the prevalence of known variants in transcription factor genes involved in pituitary development like PROP1, and POU1F1 was quite low in our cohort. A significant number of disease-causing variants in known causative genes(*LHX3, LHX4, GLI2, OTX2 and HESX1)* were found, and for LHX3 and LHX4 variants, both in silico and functional in vitro testing using luciferase assays were performed. One important novelty from our study is the identification of pathogenic variants in novel genes recently discovered in the etiology of CPHD. We found two heterozygous variants in *FOXA2*(p.R228S and p.R229*) which may affect the DNA binding ability of the coding protein in patients with IGHD and CPHD, respectively, and a missense *PNPLA6* variant(p.T1115P) in a patient with CPHD, retinitis pigmentosa and neurodevelopmental delay. In this work we were able to expand our knowledge of pituitary target genes for genetic diagnosis for CH. Identifying population-specific pathogenic variants will improve the capacity of genetic data to predict eventual clinical outcomes for better diagnosis and treatment for the patients.

95. (412) **IMPLEMENTATION OF MOLECULAR DIAGNOSIS FOR PAH RELATED DISORDERS IN A PUBLIC HOSPITAL**

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Introduction: Hyperphenylalaninemia (HPA) is a biochemical phenotype mainly due to variants in PAH. Its spectrum ranges from classical phenylketonuria (PKU) to persistent benign hyperphenylalaninemia (PHPA). Genotyping has become a useful tool to either design the diet accurately or to consider other treatment options now available. Aim: to efficiently implement the molecular diagnosis for PAH related