nosed. Trio whole-exome sequencing revealed *PFKFB2* as a novel candidate gene in congenital hypothyroidism.

102. (506) **A NOVEL DEEP INTRONIC *DMD* VARIANT CAUSE DUCHENNE MUSCULAR DYSTROPHY BY PSEUDOEXON ACTIVATION ENCODING A NONSENSE CODON**

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Dystrophinopathies are a group of X-linked recessive neuromuscular disorders caused by pathogenic variants in the *DMD* gene, which include Duchenne muscular dystrophy (DMD), Becker muscular dystrophy, X-linked dilated cardiomyopathy and mild forms of the disease. The spectrum of dystrophin gene pathogenic variants includes large deletions (60%), large duplications (5-10%) and small variants (30%) (missense, nonsense, indels and splicing variants) that are detected by standard diagnostic methods; namely, MLPA and sequencing of the coding regions of the *DMD* gene. However, in a minority group of patients (<1%) deep intronic variants are detected by mRNA analysis from muscle biopsies. The aim of this study is to present the molecular findings in a patient with clinical suspicion of DMD, absence of dystrophin in muscle biopsy and negative molecular studies for deletions, duplications and small variants. In order to search for deep intronic variants, RT-PCR of the mRNA isolated from muscle biopsy was performed and the cDNA of the entire *DMD* gene was amplified into 14 overlapping fragments. Sanger sequencing of these fragments revealed an insertion of 141 bp between exon 8 and 9. This pseudoexon inclusion introduced a premature stop codon at the mRNA level. Sequencing of the pseudoexon and its flanking regions of gDNA was performed to investigate the underlying mechanism causing the insertion. The variant NG_012232.1 (NM_004006.3): c.832-186T>G, which creates a cryptic 5' splicing donor site (T>G substitution at the +1 position) and the pseudoexon activation, was detected. Carrier status was confirmed in the proband's mother. In conclusion, RNA analysis followed by gDNA sequencing allowed us to confirm the genetic cause of the disease. The introduction of a premature stop codon due to the pseudoexon activation correlates with the absence of dystrophin in muscle biopsy. Besides, this study allowed to provide an adequate and timely genetic counselling to the family.

103. (508) **A CUSTOMIZED NEXT-GENERATION SEQUENCING-BASED PANEL APPROACH FOR THE MOLECULAR DIAGNOSIS OF EARLY-ONSET NEUROMUSCULAR DISORDERS IN REFERRAL CENTRE IN ARGENTINA**

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Neuromuscular disorders (NMD) are phenotypically and genetically heterogeneous diseases. To date, 587 genes and 1042 different diseases have been described. Congenital myopathies (CM), congenital muscular dystrophies (CMD), early-onset forms of limb-girdle muscular dystrophy (LGMD) and congenital myasthenic syndromes (SMC) present in the neonatal or childhood period. NGS offers a value tool for the molecular diagnosis of NMD due to the large number of candidate genes, phenotype heterogeneity and overlapping clinical features. **Objective:** to perform the molecular characterization in a pediatric patient cohort with clinical and pathological features of CM, CMD, LGMD and SMC. **Methods:** we included 49 patients divided in four groups according to the initial clinical suspicion: CM (n=27), CMD (n=11), LGMD (n=1), SMC (n=7) and SMC vs MC (n=3). Phenotypes groups were classified according to the clinical signs and symptoms in the neurologic examination, pathological features in muscle biopsy and/or electrophysiological studies. We designed two customized NGS panels, TruSeq Amplicon (Illumina) (n=11) and SureSelect (Agilent) (n=44) to study 28 and 80 related genes respectively. Six patients with TruSeq Amplicon negative results were restudied with the SureSelect panel. **Results:** pathogenic variants were detected in 29 patients (59%); 16 cases in genes associates with CM (9 *RYR1*, 3 *NEB*, 2 *ACTA1*, 1 *TPM2* and 1 *TTN*), 6 cases with CMD (2 *COL6A1*, 1 *COL6A3*, 2 *LAMA2*, 1 *LMNA*), 5 individuals with SMC (2 *RAPSN*, 1 *CHRNB1*, 1 *COLQ*, 1 *DOK7*) and 2 cases with LGMD (1 *CAPN3,* 1 *SGCG*). SureSelect technology gave a diagnostic yield of 59% compared to 27% for TruSeq Amplicon. **Conclusion:** the diagnostic sensibility obtained in this study highlights the advantages in applying an NGS-based panel approach for genetically and phenotypically heterogeneous diseases such as NMD. Besides, it provides information for therapeutic options for treatment conditions and can contribute to the genetic counselling.

104. (526) **THE ROLE OF NM_004827.3:c.421C>A VARIANT OF ABCG2 GENE IN THE TRIGGERING OF PORPHYRIA CUTANEA TARDA IN HIV-INFECTED INDIVIDUALS**

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Genetic variants affect the expression of the ABCG2 transporter, altering the efflux of drugs and heme; NM_004827.3:c.34G>A, NM_004827.3:c.376C>T and NM_004827.3:c.421C>A variants are present in a high frequency. Porphyria Cutanea Tarda (PCT) is caused by a deficiency in Uroporphyrinogen decarboxylase; there are 2 main types of PCT: hereditary and acquired. Xenobiotics, alcohol, abuse drugs and hepatotropic viruses are the main triggering factors of the disease. In our country, 16% of PCT patients are HIV infected individuals. Previously, the influence of *ABCB1* genetic variants, a transporter of the same family as ABCG2, in the onset of PCT in HIV carriers was reported. The aim was to evaluate the role of the NM_004827.3:c.421C>A (rs2231142) variant of ABCG2 gene in the association PCT-HIV. A population of control, HIV, PCT and PCT-HIV individuals was studied. Genotyping was done by PCR-RFLP. The non-wild type allele A was in a very low frequency in all the groups. In PCT-HIV, the frequency of A (0.21) was higher than PCT and HIV values (0.05; $p<0.001$). When analyzed the genotypic frequency, SNV was in a very low frequency in heterozygosis in all the groups with higher values for PCT-HIV group (36%, $p<0.01$) than PCT (10%) and HIV (9%). The AA genotype (3%) was only found in PCT-HIV group. These results, although preliminary, suggest that NM_004827.3:c.421C>A variant in the *ABCG2* gene could be related to the manifestation of this porphyria only in HIV patients. The analysis of the other SNVs (NM_004827.3:c.34G>A, NM_004827.3:c.376C>T) will allow us to establish the presence or absence of risk haplotypes in the manifestation of PCT associated or not with HIV infection. The results of this analysis, together with those previously obtained for *ABCB1* drug transporter gene variants, will enable us to further conclude about the risk haplotype for PCT triggering.

105. (551) **CHEMERIN GENE VARIANTS ASSOCIATION WITH METABOLIC PARAMETERS IN WOMEN WITH POLYCYSTIC OVARY SYNDROME**

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Objective: To evaluate *Chemerin* gene (RARRES2) SNVs rs4721 and rs17173608 association with clinical and biochemical characteristics, also with the metabolic and androgenic condition in women with Polycystic Ovary syndrome (PCOS).
Materials and methods: We analyzed 107 women with PCOS

according to the Rotterdam criteria (17-38 years). PCOS were divided into subgroups by the presence or absence of metabolic syndrome (MS) and hyperandrogenism (HA). Peripheral blood genomic DNA was purified and genotyped by T-ARMS PCR (Tetraamplification refractory mutation system for rs 17173608 (RARRES2 NC_000007.14(NM_002889.4):c.280-494A>C) and PCR-RFLP for rs4721 (RARRES2 NM_002889.4):c.*13A>C). Statistical analyzes were performed with GraphPad Prism and SPSS (t-Student test, ANCOVA with p-values adjusted for age and χ2 analysis).
Results: The PCOS patients showed a genotype distribution of TT genotype (44.9%), followed by TG genotype (43%) and GG (12.1%) for the rs4721; similar to the frequency found in Caucasians (HapMapProject). The population was in Hardy Weinberg equilibrium (x2 = 0.147; p = 0.70). The rs17173608 could not be analyzed because of the low presence of the minor allele (4%). Through ANCOVA analysis age adjusted, it was shown that the presence of rs4721 G allele was associated with higher levels of total cholesterol/HDL (p=0.04), LDL-c (p=0,02) triglycerides (p=0.03), insulin (p=0.02), HOMA (p=0.04), LAP index (Lipid accumulation product, p=0.03), testosterone (p= 0.08), LH/FSH (p=0.03). Also, rs4721 G allele was associated with larger telomere length (p=0.04) and lesser mitochondrial DNA mass (p=0.08).
Conclusion: In women with PCOS, rs4721G allele was associated with worse metabolic and hormonal parameters. In this preliminary study, no association was found between SNV rs4721 *Chemerin* gene and susceptibility to MS and HA in women with PCOS.

HEMATOLOGÍA

106. (62) **ERYTHROPOIETIN AND IRON AVAILABILITY IN THE REGULATION OF HEPCIDIN IN HEPATIC CELLS**
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Maintenance of systemic iron (Fe) levels undergoes regulation by the erythropoietic demand, inflammation and Fe status. Liver hepcidin (Hep), a small peptide which binds the Fe exporter ferroportin thus inducing its internalization and degradation, is a key regulatory target for Fe homeostasis.
We examined the ability of erythropoietin (Epo) to regulate Hep mRNA expression (real-time PCR) in the human hepatic cell line HepG2. Control (C) Hep levels were significantly reduced by Epo (160 ng/mL, 6 h), while its non-erythropoietic, carbamylated derivative cEpo failed to exert this effect (C: 1, *Epo: 0.4±0.2, cEpo: 0.9±0.1; *P<0.05 vs C). Abrogation of the Epo receptor with a specific siRNA or a blocking antibody prevented Hep downregulation in Epo-treated cultures (C: 1; *Epo: 0.4±0.2, siEpoR+Epo: 1.0±0.1; antiEpoR+Epo: 1.0±0.1; *P<0.05 vs C), showing EpoR is required for Epo signalling in this context. The observed decrease in Hep mRNA was followed (24 h) by lower intracellular Fe levels and higher Fe release to the culture media in Epo-exposed cells (ferrozine method).
Regarding the simultaneous regulation of Hep by Epo and different extracellular Fe conditions, Fe chelation by 100 μM deferoxamine reduced Hep mRNA by half compared with untreated cells, while 3 μM Fe-citrate almost doubled it. However, higher Fe-citrate concentrations caused lower Hep levels than in untreated cultures, and only in this condition was Epo unable to suppress Hep (C: 1; *Fe100μM: 0.3±0.1; Fe100μM+Epo: 0.7±0.2, *P<0.05 vs C). Protein levels of transferrin receptor 1 were not affected by Epo, but were decreased by Fe addition. However, no differences were observed between 3 and 100 μM Fe-citrate.
Our findings show Epo can increase Fe availability through Hep downregulation in hepatic cells, though there seems to be a complex interplay between Epo and Fe status. Further research is needed to clarify the role of Epo on Fe availability in different pathological and therapeutical scenarios.

107. (94) **SELECTIVE RESPONSE OF IRON CYCLE PROTEINS THROUGH IRON AND ERYTHROPOIETIN SIGNALS IN MOUSE KIDNEY.**
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Iron overload can be regulated by different mechanisms related to erythropoiesis and kidney tissue.Taking into account the labile nature of iron,its circulation and storage are strictly controlled.Approaches to addressing iron trafficking through the hepcidin regulator and importer(DMT1,ZIP14) and exporter(FPN) proteins may offer new insights.The presence of the erythropoietin receptor(EPO-R)in the kidney suggests various non-erythropoietic functions of EPO.This study was designed to extend our previous studies on the relationship between iron overload and iron protein regulation to another important organ,the kidney.The non-erythropoietic functions of EPO will also be analysed.CF1mice(25±5g;3m)split in(n=4/group):1*)Iron-adequate*(IA);2)*Iron-overload*(IO)(ironsaccharate;-*days0,4,8,12*ip;1800mg/kg);3)*EPO(days17-19ip;20000UI/kg)*;4)*Iron-overload+EPO(IO+EPO)*.Immunohistochemistry:anti-DMT1(divalent-metal-transporter1),anti-ZIP14(Zrt-Irt-likeProtein14),anti-prohepcidin.Iron levels Wiener kit.The Protocol was approved by CICUAE-UNS.
Iron levels showed an increase in *IO/IO+EPO* respect to IA/EPO. Abundant hemosiderin was observed in IO in the proximal tubule S2(PTS2),glomerulus and medulla;it was moderate in *IO+EPO* and scarce in EPO/IA.The DMT1 expression was evident in the PTS2 and medulla in IA/EPO and slight in *IO/IO+EPO*.In IO the ZIP14 expression was intense in PTS2 and medulla and slight in *EPO* being this the predominant signal.The prohepcidin expression was intense in *IO/IO+EPO* and slight in IA/EPO.We can conclude that in iron overload,a coordinated regulation of the iron cycle proteins occurs in the kidney,suggesting a protective mechanism against iron excess due to the reduction of iron uptake according to the following modifications:decrease in both the uptake of DMT1 and the release of FPN,also showing a negative regulation of kidney-DMT1 by hepcidin.The cytoprotective role of EPO in controlling iron storage could be explained by the reduced expression of ZIP14 observed.

108. (207) **INFLAMMATORY RESPONSE MEDIATED BY REACTIVE OXYGEN SPECIES IN NEUTROPHILS OF INMUNOCOMPROMISED NO-HIV INFECTED PATIENTS**
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Immunocompromised patients (IP) with neutropenia (moderate (MN), less than 1500 neutrophils PMN)/mm³ and severe (SN), less than 500/mm³) generate susceptibility to infections. Neutropenia is prevalent in Leukemias (Le) and Lymphomas (Li). In order to analyze the inflammatory response the study was made on non-HIV IP with Le, Li and renal transplanted IP (RT), infected (I, no-HIV) and without infection (WI); it was determined in PMN venous blood from IP (n=49, 48±17 years, hospitalized), and healthy volunteer donors (C, n=30, 35±12 years): oxygen consumption (ΔO_2, Clark electrode, indicates generation of superoxide anion, O_2^-), production of hydrogen peroxide (H_20_2, fluorometry), spontaneous chemiluminescence of PMN (CL, photon counter, measures light emission of singlet oxygen ($^1O_2$)), and C-reactive protein (CRP). Increases were observed in IP with respect to C: ΔO_2 9 fold in WI and 3 in I ($p<0.01$); H_2O_2 3 fold ($p<0.001$) and CRP 33 ($p<0.05$) both in I; CL 73 fold in WI ($p<0.0001$). When analyzing results in MN and SN, ΔO_2 increased