factors, 6 in damage repair and 2 in cohesins). Seven MDS patients presented 10 affected genes accumulating 17 nucleotide variants with a media of 2 (1-5) mutated genes and 2 (1-7) sequence variants/patient. CMML patients presented 27 variants in 14 genes with a media of 3 (2-4) affected genes and 4 (2-5) variants/patient. The AML cohort had a media of 2 (1-5) mutated genes and 3 (1-6) variants/patient, adding 55 variants in 20 genes. TET2 was the most compromised in MDS (4/17) and CMML (11/27), while in AML it was DNMT3A (7/55). The median VAF was 0.48 in CMML, 0.44 in AML and 0.30 in MDS.
Both panels comprised the most relevant genes and allowed the identification of pathogenic variants in 84% of patients with myeloid malignancies. CMML displayed a higher media of variants with a higher VAF and a prevalence of TET2, while the number of affected genes was increased in AML. Sequence-based genetic tests provide useful information, not only at clinical level, but to improve the description of altered genes and pathways in myeloid diseases.

99. (447) **ANALYSIS OF COOPERATING PATHOGENIC GENE VARIANTS IN PATIENTS WITH MYELOFIBROSIS**

Camacho MF[1], Heller P[2], Enrico A[3], Moiraghi B[4], Castro Rios M[5], Sackmann F[6], Bendek G[7], Vallejo V[8], Varela A[4], De Luca G[2], Belli C[1], Gutiérrez M[9], Larripa I[1]
1. Laboratorio de Genética Hematológica, Instituto de Medicina Experimental (IMEX)-CONICET, Academia Nacional de Medicina (ANM); 2. Instituto Lanari, Universidad de Buenos Aires-CONICET, Instituto de Investigaciones Médicas (IDIM); 3. Hospital Italiano de la Plata; 4. Hospital Ramos Mejía; 5. Centro Hematológico San Isidro; 6. Fundaleu/Centro de Hematología Pavlovsky (CHP); 7. Hospital Italiano de Buenos Aires; 8. Instituto Cardiovascular de Buenos Aires; 9. Laboratorio Stamboulian

The myelofibrosis (MF) is a myeloproliferative neoplasm derived from a clonal hematopoietic stem-cell associated with bone marrow fibrosis. The Dynamic International Prognostic Scoring System (DIPSS) enables prognosis assessment at any point during clinical disease follow-up. This model considers the age, hemoglobin level, leukocyte count, circulating blasts and constitutional symptoms to predict survival. Most of patient present one driver mutations in JAK2, CALR or MPL genes, and the majority acquire others affecting epigenetic (ASXL1, IDH1/2) and splicing (SRSF2) genes, often in multiple combinations.
In the current study, we screened hot-spot regions of ASXL1, IDH1/2 and SRSF2 to identify pathogenic variants and to describe their prevalence in the context of the DIPSS classification.
The series included 67 patients (61% females) with MF diagnosed according to the 2016 WHO criteria. At the time of testing, the median age was 65 years old (range 20–88) and laboratory characteristics included a median hemoglobin level of 10 g/dL (3-16), leukocyte counts of $11x10^9$/L (16-124) and circulating blast of 0% (0-15). The distribution according to the DIPSS was: 18% low, 18% intermediate-1, 30% intermediate-2, and 34% high risk. Driver mutational status revealed 49% JAK2, 30% CALR, 9% MPL and 12% triple-negatives.
Genomic DNA samples was amplified using allele-specific-primers for IDH1/2 (exon 4), Sanger sequencing for ASXL1 (exon 12-13) and high-resolution melting confirmed by Sanger sequencing for SRSF2 (exon 1). Fifteen patients (22%) presented pathogenic variants in ASXL1, 2 (3%) in IDH2, 1 (1.5%) in SRSF2 and 6 (9%) combined two of them. Overall mutational frequencies according to the DIPSS were 3% for low, 6% intermediate-1, 9% intermediate-2 and 18% high risk patients.
Of all the subclonal cooperating pathogenic variants found, 50% were identified in the DIPSS high risk patients associated with a more aggressive disease with clinical and therapeutic implications.

100. (482) **NEXT GENERATION SEQUENCING TECHNOLOGIES APPLIED TO THE MOLECULAR DIAGNOSIS OF CONGENITAL HYPOTHYROIDISM**

Bernal Barquero CE[1,2], Martín M[1,2], Geysels RC[1,2], Peyret V[1,2], Papendieck P[3], Chiesa AE[3], Signorino M[4], Testa G[4], Miras MB[4], Masini-Repiso AM[1,2], Nicola JP[1,2].
1 Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba.
2 Centro de Investigaciones en Bioquímica Clínica e Inmunología (CIBICI-CONICET)
3 Hospital de Niños Dr. Ricardo Gutiérrez.
4 Hospital de Niños de la Santísima Trinidad.

Congenital hypothyroidism is the most frequent endocrine disorder in pediatric patients. Over thirty monogenic forms of the disease have been reported. A meta-analysis demonstrated that only 5-10% of patients with thyroid dysgenesis and 45-88% of patients with thyroid dyshormonogenesis are diagnosed using single-gene sequencing. Here, we used single-gene and next generation sequencing to investigate the etiology of the disease.
Ten patients (p1-10) with dyshormonogenesis showing a defined iodide transport defect phenotype were studied by single-gene analysis. Nine patients (p11-19) with thyroid dysgenesis (n=2) or dyshormonogenesis (n=7) were explored by targeted next generation sequencing. Patients 1 and 17 were studied by trio whole-exome sequencing.
SLC5A5 gene sequencing analysis (p1-10) revealed four compound heterozygous variants (c.970-3C>A/p.D369V; p.G543K/p.L562M) and three in homozygous state (c.1973C> T; c.1673A> C; p.G561E). Multiple gene sequence analysis (p11-19) revealed three heterozygous variants (p.F1542V*fs**20; p.Y2563C; p.S523P) and two compound heterozygous variants (p.Q29*/c.177-2A>C) in *TG* gene. Moreover, the analysis revealed heterozygous variants in *DUOX2* (p.E1496D*fs**51) and *FOXE1* (p.P203R) genes. Finally, we deepened the study of two patients (p1 and p17), without mutations in putative candidate genes and, in the case of p17, with an unusual autosomal dominant inheritance pattern using trio whole-exome sequencing. Heterozygous variants were identified in TG (p.G653D) and *PFKFB2* [c.741-60_741-61insG(2)TG(8)] genes, the latter has not previously been associated with the disease. All identified variants were predicted pathogenic.
Next-generation sequencing constitutes an attractive alternative to systematically explore congenital hypothyroidism. However, we evidenced that a considerable proportion of patients remain undiagnosed. Trio whole-exome sequencing revealed *PFKFB2* as a novel candidate gene in congenital hypothyroidism.

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

Foncuberta ME, Lubieniecki F, Medina A, Campos BC, Monges S, Gravina LP
Hospital de Pediatría S.A.M.I.C. “Prof. Dr. Juan P. Garrahan”

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