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Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by upper and lower motor neuron loss. Numerous genes regulating mitochondrial function have been associated with neurodegeneration. In a pilot whole-exome sequencing study of 27 ALS patients, rare heterozygous variants in the *DHTKD1* gene were recurrently identified, including one nonsense variant. *DHTKD1* encodes a dehydrogenase subunit acting in L-lysine degradation in mitochondria. Pathogenic variants in *DHTKD1* have been described causative of autosomal recessive 2-aminoadipic and 2-oxoadipic aciduria and autosomal dominant Charcot-Marie-Tooth disease type 2. Here, we aimed to investigate the frequency and clinical implications of rare *DHTKD1* variants in an ALS cohort.

Methods: We performed whole-exome ($n = 46$) and targeted ($n = 179$) sequencing of 225 unrelated ALS patients of central European ancestry. All patients were diagnosed according to the El Escorial criteria by a specialized neurologist. Clinical (e.g. age and region of onset, ALS subtype, and disease progression) and diagnostic (e.g. electrophysiological, biochemical) characteristics are being analyzed to discover genotype-phenotype relations.

Results: Five missense and one nonsense variant, all rare (MAF < 0.5%) or novel and predicted to be pathogenic, were identified in 10 of 225 patients. Two of these variants were previously described in patients with 2-aminoadipic and 2-oxoadipic aciduria. Preliminary clinical correlations indicate a slightly later ALS onset in *DHTKD1* variant carriers (median: 71 years) compared to non-carriers (median: 63 years).

Conclusions: We identified rare heterozygous *DHTKD1* variants in 4.4% of central European ALS patients. Potential phenotype-genotype correlations and frequency of rare *DHTKD1* variants in other ALS cohorts are currently being assessed.

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P10.16.B
Dystrophinopathy patients with non-contiguous molecular alterations: diagnosis and characterization of the genetic mechanisms involved

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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 mutations 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. This study was supported by PTC Therapeutics and University of Buenos Aires.

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P10.17.C
Elucidating myopathies with high creatine-kinase- from unsolved cases to common diagnosis

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Background: One of the most common causes for myopathies are related to X-linked DMD gene, including Duchene Muscular Dystrophy(DMD) and Becker Muscular Dystrophy(BMD). Other autosomal recessive myopathies affect males(M) and females(F) consisting of limb-girdle myopathies.

Method: 42 patients with high levels of creatine-kinase addressed the Regional Center of Medical Genetics Timis County, Romania (October 2017-September 2019), either having suggestive symptoms of myodystrophy (23M and 1F) or for verifying carrier (6M and 10F).

Results: DMD was diagnosed in 13M, with hemizygous variants in *DMD* gene (using MPLA) with following exons deletion: 10-11;13-17;22-41;39-43;46-53;51;58,exon 3-7 duplication and variants (using NGS): c.7174dupG; c.8608C>T; c.8688_8689delAG; c.10738delG. BMD was diagnosed by MLPA in 6M: deletions in exons 45-47 and 45-51. Two male cousins had a previously unreported splice site NM_004009.3:c.175-1G>T pathogenic variant in DMD gene. After negative testing for DMD gene, limb-girdle muscular dystrophy was confirmed using NGS in compound heterozygosity in *FKRP* gene in 3 patients, of which 2 were siblings(M/F): c.148A>G/c.985G>A, and one unrelated (c.826C>A/c.935G>C). Pompe disease was diagnosed in two brothers showing pathogenic variants in *GAA* gene (exon 18 deletion) and in one unrelated patient with c.784G>A/?. Carrier status for DMD was identified in 6 out of 10F. Using MLPA for DMD and a NGS panel of 4813 genes, no pathogenic variants were detected in 4M and 1F (persistent extremely elevated CK levels).

Conclusions: This study shows a diagnostic yield of almost 80% and lists the variants associated with myodystrophies, underling the limitation in establishing the diagnosis in patients with increased CK levels.

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P10.21.A
The floppy child syndrome is not only caused by mutations in genes related to neuromuscular disorders.

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Introduction: The, floppy child syndrome" is one of the most unambiguous clinical manifestations. Neonatal or childhood hypotonia can present with other symptoms, like decreased muscle tone, arthrogryposis or developmental delay making a definite clinical diagnosis challenging. We aimed to assess the genetic causes of this condition, with interest in neuromuscular disorders (NMD).

Patients and methods: For 124 patients with excluded common genetic causes of early hypotonia (e.g. SMA, PWS, *MTM1* and *ACTA1* mutations), an exome sequencing