Keywords: *F8*, haemophilia, cost-effective diagnosis

(1443) **GENETIC DIAGNOSIS OF DYSTROPHINOPATHIES USING NEXT-GENERATION SEQUENCING. CHARACTERIZATION OF TWO NOVEL SPLICING MUTATIONS IN *DMD* GENE**

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Dystrophinopathies constitute a group of neuromuscular diseases caused by pathogenic variants in *DMD* gene. Large deletions/duplications account for 70% of mutations and are detected by MLPA. In the remaining 30% of patients, small mutations are observed. *DMD* gene is composed by 79 exons; therefore sequencing with Sanger method is costly, time-consuming and laborious. Next-generation sequencing (NGS) enables detection of small mutations in a more efficient and cost-effective approach. Objective: to detect small mutations in *DMD* gene using NGS technology. Methods: whole-exome sequencing was performed by Macrogen service in 15 male patients with clinical/biopsy suspicion of dystrophinopathies in whom deletions/duplications had been previously rule out. Quality control, mapping and alignment of the sequences were reviewed in our laboratory. Only variants in the *DMD* gene were analysed. In order to classify the identified variants dbSNP, 1000 Genomes, Exac, ClinVar, Leiden and HGMD databases were consulted. To predict the effect of the variants, SIFT, PolyPhen-2, Mutation Taster and Human Splicing Finder software were used. Total RNA was isolated from muscle biopsy to analyse the effect of two novel splicing mutations. Results: Pathogenic variants were identified in 14 of the 15 patients analysed: 5 nonsense mutations, 5 small deletions, 3 splicing mutations and 1 duplication. mRNA analysis of two novel splice site mutations was performed. Mutation c.3786+5G>C (exon 27) revealed three different transcripts: wild-type, skipping of exon 27 and skipping of exon 27 to 29. Mutation c.9650-1delG (exon 67), produce one transcript skipping exon 67 and two other transcripts with different cryptic acceptor sites in exon 67. Conclusion: the NGS approach used proved to be a highly sensitive tool to detect small mutations in *DMD* gene. This study shows the importance of transcript analysis to determine the consequences of splicing mutations.

Keywords: *DMD* gene, next-generation sequencing, splicing mutation, dystrophinopathies.

(1630) **MOLECULAR DIAGNOSIS OF THE 21-HYDROXYLASE DEFICIENCY**

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Congenital adrenal hyperplasia, the most frequent inborn metabolism error, is caused in 90-95% of the cases by mutations in the 21-hydroxylase gene (*CYP21A2*). The deficiency can present as severe or classical form (C), and nonclassical (NC). About 70% of haplotypes have a bimodular arrangement, with one module carrying *CYP21A2* and the other the homologous pseudogene *CYP21A1P*. Due to the high sequence identity, most of the disease causing mutations are the consequence of non-homologous recombination or gene conversion events between the gene and its pseudogene. Nevertheless, an increasing number of mutations have been found in the last years. The aim of our group is to study the genetic defects that contribute to the phenotypic expression of the pathology. In the present work, we describe our molecular diagnostic algorithm and an update of the mutations found in our population. A total of 1253 samples were studied: 675 patients (517 NC and 158 C) diagnosed as 21-hydroxylase deficient, 411 relatives and 167 partners. Disease causing mutations were analyzed by allele-specific/RFLP PCRs and/or by direct sequencing. When necessary, gene dosage was analyzed by MLPA and the regulatory regions of the gene were sequenced. At least 120 different genotypes were identified in our cohort, including 19 rare or less frequent and 11 novel mutations. The frequency of all mutations was determined. Among the 411 relatives, 387 presented at least one mutated allele. 28/167 partners were classified as carriers. *CYP21A2* gene is located in one of the most complex regions of the genome. The application of different methodologies is necessary for the complete study of the gene. Our current molecular diagnostic algorithm allows us to fully genotype patients, in order to complete genetic counseling.

Keywords: 21-hydroxylase deficiency, *CYP21A2* gene, molecular diagnosis

(1837) **MOLECULAR DIAGNOSIS OF MCADD PATIENTS: CHARACTERIZATION OF THREE NOVEL MUTATIONS IN THE *ACADM* GENE**

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Medium-chain acyl-CoA dehydrogenase deficiency (MCADD) is the most frequent inherited defect of fatty acid oxidation. It is characterized by hypoglycemic crisis under fasting or stress conditions, leading to lethargy, seizures and even death. MCADD is recessively inherited, caused by mutations in the *ACADM* gene. The most common mutation is c.985A>G. In cases detected by newborn screening, the frequency of the prevalent mutation is lower than that observed in clinically affected patients, and variants that have never seen before in those patients, are identified. The aim of this study was to describe the molecular characterization of *ACADM* gene in MCADD patients and newborns with positive screening result. DNA was extracted from peripheral blood or dried blood spot from 12 patients. c.985A>G mutation was studied by PCR-RFLP. Samples negative or heterozygous for c.985A>G were sequenced by analysis of the 12 exons and the exon-intron boundaries of the *ACADM* gene. Taking into account the 9 unrelated patients, c.985A>G appeared in 5/8 alleles of the 4 symptomatic patients and in 4/10 alleles of the 5 screening-positive newborns. 8 different sequence variations were identified, including 3 novel: c.119-12A>G was detected in two siblings with c.985A>G. The older brother presented with hypoglycemia and seizures, his sister was asymptomatic. *In silico* tools predicted a possible alteration of splicing; c.608T>C (p.L203S) was found with c.985A>G in a patient presented with hypoglycemia and fever. Several *in silico* tools predicted this variant as deleterious; c.1012C>T (p.Q338X) was detected with c.985A>G in a newborn with an abnormal screening result. This variant is predicted as deleterious as it is a nonsense mutation. In this cohort, mutational spectrum differed between clinically diagnosed patients and those detected by newborn screening, as previously reported in other studies. Molecular and *in silico* analysis proved to be important to confirm the MCADD diagnosis.

(1838) **ALGORITHM FOR MOLECULAR DIAGNOSIS OF NEUROFIBROMATOSIS NF1, NF2 AND NF3 (SCHWANNOMATOSIS)**

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Neuropathological evaluation of CNS tumors is increasingly dependent on molecular genetic tests for proper classification, pre-

diction of biological behavior and patient management. The neurofibromatoses (NFs) consist of at least three autosomal dominant inherited disorders: neurofibromatosis type 1 (NF1), type 2 (NF2), and schwannomatosis (NF3). The molecular diagnosis is still difficult due to: 1) absence of hotspots in *NF1/NF2* genes, 2) ≥50% of sporadic cases for NF1/NF2, 3) *NF1* gene large size and the existence of several pseudogenes. NF3 the newly recognized form is poorly understood: 1) only 15% of cases are inherited, 2) is caused by concomitant loss of several tumor suppressor genes by a single mutational event, 3) the 2 predisposition genes (*SMARCB1* and *LZTR1*) identified do not explain all cases. Our aim is to show the diagnostic algorithms for molecular genetic testing for the NFs. We have used segregation analysis of STRs, mutational screening by DNA sequencing and exome sequencing (WES).The analysis of a family with NF1 numerous patients revealed the at-risk haplotype in one on the unaffected probands and a recombination event in two individuals (one affected and one asymptomatic). In four out of 11 NF2 patients three small novel germinal mutations (2 frameshift and 1 splice-site) and one partial deletion of the maternal *NF2* copy were identified, as well as a loss of heterozygosity (LOH) in the fifth patient. Molecular analysis of four patients with NF3 showed no mutations in *SMARCB1* gene. One of the patients with family history studied by WES, did not show alterations in the predisposing genes**.** Analysis of four of this patient´s tumors did not display the frequently observed LOH. Evaluation of abnormalities in these genes was performed using a diagnostic algorithm which depends on the type of NF, family history and sample availability.

Keywords: Neurofibromatosis 1, Neurofibromatosis 2, Schwannomatosis/NF3, complex TSGs inactivation, exome sequencing

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(159) **PROBIOTICS INCREASE ANTIMICROBIAL ACTIVITY OF PANETH CELLS IN ELDER MICE.**

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The number of bacteria is very low in the small intestinal tract. This seems to be, at least in part, to the abundant constitutive antimicrobial peptides (AMPs) which are expressed by crypt Paneth cells. Our aim, using mice as experimental model, was to explore whether probiotics can strengthen intestinal barrier through the life of the animals, by increasing the microbicidal activity.

BALB/c mice from 21, 28, 35, 42, 54, 61 and 180 days old, received *L. casei* CRL 431 (Lc 431), *L. paracasei* CNCM I-1518 (Lp 1518) or water (control), upon 7 and 5 days, respectively. After, mice were sacrificed and intestinal fluids, small and large intestine sections were taken.

Forty two days old mice, fed with Lc 431 or Lp 1518, increased the number of positive Paneth cells (64.29 ± 3.58 and 61.80± 5.31%, respectively), respect to control (40.10 ± 3.9%) (Mean ±SEM). Weaning mice (21 days old) that received Lc 431 or Lp 1518 did not show a significant microbicidal activity in the intestinal fluids. In contrast, an important reduction in the CFU of *S. aureus* and S.Typhimurium were observed at 35, 42, 54 and 61 days old ($p<0.05$) as well as in elder mice (180 days old) fed with the lactobacilli. By electron microscopy (EM), pathogens co-incubated with the intestinal fluids from probiotics fed mice, displayed severe disruption of the walls cells and fragmentation.

Interestingly, intestinal fluids of mice from different ages fed with the probiotics showed microbicidal activity against Lc 431 and Lp 1518. These results show the regular consumption of probiotics, do not induce overgrown of them, nor cause adverse effect on the microbiota. Moreover, oral administration of Lc431 or Lp 1518 did not influence the population of total anaerobic bacteria, lactobacilli, and enterobacteria in the colon, at any of age analyzed.

Probiotics appear as effective tools, in young as well as in elder mice, to enhance AMPs in order to face pathogens, without deregulate commensal bacteria and gut homeostasis.

Keywords: Probiotics, antimicrobial peptides, Paneth cells.

(370) **MYELOID-DERIVED SUPPRESSOR CELLS EXPANDED IN INTESTINAL MUCOSA AND ESPLEEN AFTER ORAL INFECTION WITH YERSINIA ENTEROCOLITICA: ROLE OF VIRULENCE FACTORS**

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immature and immunesupressive myeloid cells. These cells are characterized by expression of CD11b and Gr-1, and are divided into two major subsets: granulocytic and monocytic MDSCs. *Yersinia enterocolitica* (Ye) are Gram-negative bacteria that cause food-borne acute or chronic gastrointestinal diseases. The role of MDSCs in Ye infection has not been determined. The purpose was to elucidate whether oral Ye infection induces the expansion of MDSCs and to define the role of these cells in this infection. Therefore, C57BL/6 mice were infected with Ye WAP-314 serotype O:8. On days 5 post-infection (p.i), cell infiltration in mesenteric lymph nodes (MLN), Peyer´s patches (PP) and spleen was analyzed. Suppressive activity was determined by MTT assay. Moreover, the role of Ye outer protein P (YopP) or H (YopH) in MDSC expansion was explored using for infection Ye deficient in YopP or YopH, respectively. We found that Ye-infected mice presented an increase in the frequencies of $CD11b^{+}Gr\text{-}1^{+}$ cells in PP, MLN and spleen on days 5 p.i, compared to uninfected mice ($p<0.05$). In PP and spleen, granulocytic subset was expanded while in MLN both granulocytic and monocytic subsets were detected. In addition, splenocytes and MLN cells obtained from MDSC-depleted mice and stimulated with specific antigen showed increased proliferation compared to non-depleted mice ($p<0.01$). Moreover, we observed that Ye deficient in YopP induced an increase of MDSCs frequency in intestinal mucosa compared to mice infected with Ye WAP ($p<0.01$). Interestingly, MDSC depletion induced a significant increase in the bacterial load in PP, MLN and spleen in comparison with non-depleted mice ($p<0.05$). We conclude that oral Ye infection induces expansion of MDSCs, which could directly or indirectly promote the elimination of bacteria. MDSC accumulation in intestinal mucosa may be modulated by YopP.

Keywords: *Yersinia enterocolitica*; Infection; MDSCs

(473) **NEW PROTEOME SCREANING METHODS USEFUL TO SENSITIVILY MONITOR NOVELSPECIFIC BIOMARKERS OF TLR-SPECIFIC SIGNALING WITH POTENTIAL IN DIAGNOSTIC AND DRUG TARGETING OF INFECTIOUS DISEASES.**

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Abstract: There is a worrying paucity of research for the accurate identification of intracellular protein biomarkers specific for the ligation of an individual receptor involved in immune and/or inflammatory responses. Finding them is complicated and failed so far due to the intense crosstalk between the different inflammatory or immune receptor pathways which share a plethora of adapter, scaffold and signalling proteins, at multiple levels in several cell types. They also share PTMs between proteins. Thus, we developed novel, potent procedures to sensitively and systematically screen for proteome alterations in human macrophages infected with different bacteria or stimulated with specific TLR2/4 ligands. We found many cytosolic proteins affected in their levels. One was a novel post-translationally generated, anionic form of a chaperone. It was always up-regulated ($p=0.01$, $n=33$) in the treatments and by 9-fold ($p=0.05$) in the peaking time-point. Its level and temporal profile were reproducible after bacterial infection or specific ligation of one TLR type but not the others. The results strongly suggest that its increment is not influenced by the ligand structure but only by the ligation act and by the ligand(s) concentration/half life. It was coreceptor-independent. Inter-