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Association between CYP450 polymorphisms and the use of complementary medicine among patients with drug-resistant epilepsy in Puerto Rico

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OBJECTIVES/SPECIFIC AIMS: Patients with epilepsy often combine their antiepileptic drugs (AEDs) with complementary medicine (CM). They use CM to treat their symptoms of comorbidities disorder, to reduce the side effect of the AEDs or trying to achieve better control of their seizures. However, the inconsistent patterns of the use of CM among countries have been attributed to cultural and socio-economic factors and limited studies have explored biological factors. The aim of this study is to determine whether or not there is an association between having genetic polymorphisms on candidate pharmacogenes for drug-metabolizing enzymes cytochrome P450 (CYP) and the use of CM among patients with drug-resistant epilepsy (DRE). **METHODS/STUDY POPULATION:** In this cross-sectional study, patients will be recruited in the Epilepsy Clinic in the Medical Science Campus of University of Puerto Rico and in private Neurology clinics. To participate in this study, patients need to have both parents of Puerto Rican origin to be defined as Puerto Rican and have a diagnosis of DRE, defined as persistent seizures after at least 2 good trials of the proper drugs at the right dose. After the patient sign, the informed consent, a buccal swap will be collected, and the patient will complete a questionnaire. In the questionnaire, the patient will do a self-report about the use of CM (including natural products, meditation, yoga, and others), frequency of use and socio-economic information. Polymorphisms for CYP 2D6, 2C9, 2C19, or 1A2 will be determined using TaqMan[®] SNP Genotyping Assays. Data analysis will include descriptive statistical, χ^2 and ANOVA test. **RESULTS/ANTICIPATED RESULTS:** We expected to determine the frequency distribution of functional polymorphisms on CYPs among patients with DRE who are either using CM and AEDs or standard care (AEDs). Quantified the use of CM and ascertain if there is an association with the CYPs polymorphisms. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This study is novel, because we will use an objective test, pharmacogenetics approach to rule out biological factors associated with the use of Complementary Medicine by patients' DRE. The study will provide evidence for prospective study using specific Complementary Medicine guiding by genotyping.

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hnRNP K overexpression drives acute myeloid leukemia emergence and progression

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OBJECTIVES/SPECIFIC AIMS: Acute myeloid leukemia (AML) is a devastating hematologic malignancy wherein <20% of patients will survive 5 years after diagnosis. In an effort to understand alterations that drive AML development and progression, The Cancer Genome Atlas detailed the most common recurrent mutations. One gene of interest identified here was HNRNPK, supporting our clinical observations that suggest altered expression levels of HNRNPK and its corresponding protein (hnRNP K) may impact AML. Here, we aim to elucidate the impact of hnRNP K overexpression in AML by utilizing AML cell lines and mouse models reflective of the human disease. **METHODS/STUDY POPULATION:** We utilized fluorescence in situ hybridization (FISH), qRT-PCR, and reverse phase protein array (RPPA) to evaluate HNRNPK copy number and expression levels in AML patient samples compared with CD34+ cells from healthy human donor bone marrow. Kaplan-Meier survival analyses were performed using clinical data from 415 AML patients at MD Anderson Cancer Center and stratified based on hnRNP K protein expression as evaluated by RPPA. To directly evaluate the impact of hnRNP K overexpression *in vivo*, we created 2 distinct lines of Hnrnpk transgenic mice (HnrnpkTg). Phenotypic differences in the hematologic compartments of these mice were evaluated via flow cytometry, immunohistochemistry, and transplantation assays. Molecular pathways have been evaluated in mice and cell lines using immunoblotting, qRT-PCR, and RNA-immunoprecipitation. The drug JQ1 was used *in vitro* with both OCI-AML3 cell lines and with primary bone marrow and splenocytes from HnrnpkTg mice. **RESULTS/ANTICIPATED RESULTS:** FISH analyses demonstrated that a large proportion of AML cases had amplification of HNRNPK that corresponded with upregulation of HNRNPK at the RNA and protein levels. Indeed, patients with high levels of hnRNP K had decreased overall survival compared with those expressing lower hnRNP K levels. In line

with these clinical observations, we observed altered myelopoiesis in HnrnpkTg mice. These mice demonstrate increased CD11b+Gr1+ populations in the bone marrow and peripheral blood. Indeed, these mice develop myeloid leukemia, indicated by >20% of circulating white blood cells harboring markers of immature stem cells in conjunction with positive myeloperoxidase staining and blast-appearing morphology. RPPA revealed expression of c-Myc positively correlated with increased hnRNP K levels. In HnrnpkTg mice, c-Myc protein was increased, yet MYC RNA was invariably decreased compared to wildtype. To decipher a mechanism by which this may occur, we demonstrated a post-transcriptional interaction between hnRNP K and c-Myc *in vivo*. JQ1, a BRD4 inhibitor, that epigenetically decreases c-Myc expression showed preferential activity against myeloid cells expressing high levels of hnRNP K both *in vitro* and *in vivo*. **DISCUSSION/SIGNIFICANCE OF IMPACT:** These preliminary studies demonstrate that hnRNP K overexpression causes myeloid malignancies in both mouse and man. We have determined that c-Myc contributes in part to hnRNP K-mediated leukemogenesis, and that targeting c-Myc may be an effective strategy for hnRNP K-overexpressing AML. We are currently validating other potential targets for interaction with hnRNP K by performing RNA-Seq and hnRNP K immunoprecipitation followed by mass spectrometry. Fortunately, several of our putative targets are druggable—allowing for viable translational outputs to these mechanistic studies.

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Implanted multijoint functional electrical stimulation assistance improves walking efficiency after stroke: A case report

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OBJECTIVES/SPECIFIC AIMS: Evaluate the effect of multijoint functional electrical stimulation (FES) on energy consumption during post-stroke walking. **METHODS/STUDY POPULATION:** A 67-year-old male with chronic stroke was implanted with an 8-channel implanted pulse generator to stimulate flexor and extensor muscles of the hip, knee, and ankle. Oxygen consumption was measured with a k2b4 portable pulmonary gas analyzer during walking with and without FES assistance. Data were analyzed during steady state oxygen consumption within the last 2 minutes of a 5 minute walk. Distance and walking speed were also measured. **RESULTS/ANTICIPATED RESULTS:** Electrical stimulation increased walking speed from 0.29 to 0.64 minute/second. Faster walking corresponded with increased oxygen consumption from 10.1 to 14.4 mL O₂/kg per minute. Energy cost, consumption as a function of distance, decreased from 3.7 to 2.9 mL O₂/kg per minute walking with stimulation compared with without. **DISCUSSION/SIGNIFICANCE OF IMPACT:** These preliminary data suggest improvements in walking speed with FES are accompanied by increased energy consumption and decreased energy cost. Oxygen consumption during FES assisted walking was <50% of the peak for able bodied individuals of similar age; patients may successfully use the system for community ambulation.

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Targeting MELK in acute lymphoblastic leukemia, new therapeutic approach

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OBJECTIVES/SPECIFIC AIMS: Unlike the high cure rates (90%) of children with acute lymphoblastic leukemia (ALL), that of adults is still lagging behind and better therapies are needed. Maternal embryonic leucine-zipper kinase (MELK) is aberrantly upregulated in cancer, and implicated in cancer stem cell survival. A recent study has identified FOXM1, a MELK substrate, as a therapeutic target in B cell ALL (B-ALL). Thus, we hypothesized that MELK may act as a therapeutic target in ALL via targeting FOXM1 activity. **METHODS/STUDY POPULATION:** Western blot and qPCR were used to assess MELK expression in 14 ALL cell lines. Knock-down and kinase inhibition approaches targeting MELK expression and function, followed by CCK-8 and Annexin V (flow cytometry) assays to measure cell viability and apoptosis, respectively. **RESULTS/ANTICIPATED RESULTS:** MELK was significantly upregulated in patients with ALL (oncomine data analysis). MELK was also significantly higher in B-ALL and T-ALL cell lines compared with that in blood cells of healthy donors. MELK knock-down significantly decreased cell viability (40%–70%, $p < 0.05$, Fig. 1) in ALL cells, and induced apoptosis (~40%). OTS167, a potent MELK inhibitor exhibited cytotoxic activities in both B and T-ALL cells. The IC₅₀ of OTS167 ranged from 20 to 60 nM; we also found a significant increase in apoptosis

($p < 0.05$). Mechanistically, MELK inhibition resulted in decrease of FOXM1 protein levels 3 hours post-treatment. **DISCUSSION/SIGNIFICANCE OF IMPACT:** MELK is highly expressed in ALL and represents a novel therapeutic target likely via modulating FOXM1 activity. Functional and mechanistic studies will complement and ensure the success of the ongoing Phase I/II clinical trial of OTS167 in patients with refractory or relapsed AML, ALL, and other advanced hematologic malignancies.

2210

Anatomical substrates of cognitive fatigue in aging and in Parkinson's disease

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OBJECTIVES/SPECIFIC AIMS: Identify objective neurological substrates of cognitive fatigue in Parkinson's disease and in aging. **METHODS/STUDY POPULATION:** Structural and diffusion MRI. Behavioral assessments for aged adults and Parkinson's disease. **RESULTS/ANTICIPATED RESULTS:** Gray and white matter deficits that correlate with deficits in the basal ganglia for fatigued Parkinson's disease patients Versus anterior cingulate cortex in healthy aged adults with fatigue. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Over 50% of patients with Parkinson's disease and 38% of healthy older adults suffer from cognitive fatigue. However, diagnostics are limited to subjective surveys and there are no treatments for either population. Therefore, objective measures are greatly needed for better diagnosis and development of treatment targets.

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Investigating the correlation between rheumatoid arthritis and *Prevotella copri*

Hannah Fehlner-Peach

OBJECTIVES/SPECIFIC AIMS: Rheumatoid arthritis (RA) is one of the most prevalent systemic autoimmune diseases. It is caused by a combination of genetic and environmental factors. In humans, the intestinal microbe *Prevotella copri* strongly correlates with RA in previously untreated new-onset rheumatoid arthritis (NORA) patients. Metagenomic assembly of *P. copri* from NORA patients and healthy controls suggests genetic differences between *P. copri* from each group. In order to test the hypothesis that genetic differences in *P. copri* from arthritis patients promote arthritis, I am performing genomic comparison of primary *P. copri* isolates from NORA patients and healthy controls, and analysis of the immune response to *P. copri* in mice. Mice colonized with *P. copri* have increased susceptibility to DSS-induced weight loss and death compared with uncolonized controls. Future experiments will assess the local and systemic immune response in *P. copri*-colonized, DSS-treated mice. If this work is successful, then it may be possible to exploit genetic variation in *P. copri*. This could lead to new biomarkers for human disease or even insight into drug metabolism. **METHODS/STUDY POPULATION:** To validate a strategy to screen for the presence of *P. copri* in feces, qPCR primers were designed to amplify 8 regions across the 3.5 Mb *P. copri* reference genome using NCBI PrimerBlast. Primers were validated with DNA from feces for which *P. copri* abundance was previously determined by 16S rDNA sequencing. *P. copri* genome-specific primers were used to screen bacterial isolates from NORA patients and healthy controls. The 16S V3-V4 region was sequenced and compared with the *P. copri* reference 16S sequence to confirm >97% similarity. Genomes of 2 NORA patient isolates were sequenced on Illumina MiSeq, and sequences were compared with the reference genome. A strategy was developed to colonize mice with *P. copri*: 3-week-old C57BL/6 mice were treated with antibiotics in drinking water for 2 weeks, then switched to water for 2 days before oral gavage with *P. copri*; 6–7 days after inoculation, *P. copri* colonization was assessed by plating feces from inoculated mice, and by qPCR of fecal DNA with *P. copri*-specific primers. A systemic immune response to *P. copri* was assessed by microbe-specific ELISA for IgG and IgA in the sera of colonized mice. **RESULTS/ANTICIPATED RESULTS:** *P. copri* was detected in the stool of 20% of healthy individuals and 50% of NORA patients. *P. copri* was isolated from 4 healthy individuals and 6 NORA patients. Whole genomes of 96 primary isolates from NORA patients and healthy controls will be sequenced on the Illumina HiSeq platform, and their genomes will be assembled and compared using Spades software. For 2 *P. copri* isolates for a NORA patient, 89% of 250 bp reads aligned >95% to the *P. copri* reference genome. Mice can be colonized with *P. copri* gavage at > 106 CFU. *P. copri*-specific IgG and IgA were detected in the sera of colonized mice. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Several primary isolates of *P. copri* have been collected from healthy controls and NORA patients, which will enable whole genome comparison of these isolates. For the 2 *P. copri* isolates sequenced, 89% of 250 bp reads aligned

>95% to the *P. copri* reference genome, indicating variability between NORA patient *P. copri* strains and the *P. copri* reference genome. The establishment of colonization of mice with *P. copri* will allow further characterization of the immune response to *P. copri* at steady state and under pro-inflammatory conditions. Further, the systemic immune response to *P. copri* indicates that this microbe may have potential to play a role in systemic disease.

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Amyotrophic lateral sclerosis, stem cells and TALENed technology

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OBJECTIVES/SPECIFIC AIMS: The current treatment for amyotrophic lateral sclerosis (ALS) includes systemic delivery of neurotrophic factors (NTFs). Although this approach may seem theoretically sound, NTF efficacy within the central nervous system (CNS) is largely limited by the blood-brain barrier. Thus, a cell-based approach, which allows for targeted delivery of molecular therapies locally from the CNS, could lead to a paradigm shift in the field. **METHODS/STUDY POPULATION:** The Windebank and Staff group at Mayo Clinic completed a Phase I dose-escalation safety trial of autologous, adipose-derived mesenchymal stem cells (adMSCs) in an effort to move toward personalized medical treatment of ALS. The adMSCs were injected into the intrathecal space by lumbar puncture in 27 patients and the results showed an excellent safety profile across a range of doses. The team is moving forward with this idea by using gene-editing technology to develop clinical-grade, genetically modified autologous MSCs. The patient-derived adMSCs are modified at defined "safe-harbor" regions of the human genome through transcription activator-like effector nuclease (TALEN) technology. **RESULTS/ANTICIPATED RESULTS:** Our results show that electroporating adMSCs with plasmid DNA leads to efficient GFP or TALEN transgene expression, but yields low cell survival and a low rate of genetic modification. **DISCUSSION/SIGNIFICANCE OF IMPACT:** It can be concluded that: (1) TALEN technology may be used to target safe harbor loci for gene integration to produce therapeutic adMSC for ALS. (2) Primary barriers to adMSC modification are inefficient TALEN and donor template uptake, low cutting efficiency, and poor cell survival after electroporation. Future directions include optimizing the protocol to obtain 48 base pairs in the homology arms and increasing transfection efficiency.

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Gene expression signatures of acute respiratory syncytial virus infection in pediatric patients reveals insight into clinical pathogenesis

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OBJECTIVES/SPECIFIC AIMS: Respiratory viruses cause enormous medical burden, yet many of the specific virus and host genetic factors that impact pathogenesis are still largely unknown or poorly understood. To better understand the drivers of both acute clinical pathogenesis and long-term impacts, such as the development of asthma, we investigated the host response to respiratory syncytial virus (RSV) infections in pediatric patients. **METHODS/STUDY POPULATION:** We collected nasopharyngeal swabs from 32 pediatric patients with acute RSV infection. The swabs represented a mixed cell population including epithelial and immune cells at the active site of infection. Unbiased RNA sequencing with ribosomal RNA depletion allowed the simultaneous detection of host gene expression and RSV infection. We sequenced samples 2 × 75 bp on an Illumina NextSeq 500. Sequences were mapped to the human genome using the TopHat 2 aligner and FPKM estimation of reference genes and transcripts and assembly of novel transcripts were conducted with Cufflinks 2. **RESULTS/ANTICIPATED RESULTS:** During acute RSV infection we identified 7343 genes that were significantly expressed. Pathway analysis using KEGG revealed significant upregulation of pathways involved in innate immune response infection, ribosome function, oxidative phosphorylation, spliceosome and autoimmune disorders. We found high levels of innate immune response genes including CXCL8, IFITM1, IFITM2, IFITM3, IL1RN, and ISG15. In comparing RSV subtype A to RSV B we found significant differential expression of multiple noncoding RNAs. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Examination of the host gene response during acute RSV infections, yielded important insight into the mechanisms that cause clinical pathogenesis and may provide understanding of the mechanisms that lead to known long-term impacts, such as the development of asthma. Together, this data may be used to guide clinical treatment and management decisions for children with severe RSV infections.