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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 patters 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 determinate whether or not there is an association between having genetic polymorphisms on candidate pharmaco-genes for drug-metabolizing enzymes cytochrome P450 (CYP) and the use 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 inform 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 specifics 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 O_2/kg per minute. Energy cost, consumption as a function of distance, decreased from 3.7 to 2.9 mL O_2/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 POPULA-TION: 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