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Gene Therapy for Rare Diseases: Promise and Challenges

February 23, 2018 / 11:00 AM-2:30 PM
Graduate Hotel
615 Washington Ave SE, Minneapolis, MN 55414

SCHEDULE:
11:00-12:00 Poster Session
12:00-1:30 Lunch and Keynote
1:30-2:30 Networking

REGISTER: z.umn.edu/RareDiseaseDay2018

MODERATOR:
Jakub Tolar, MD, PhD
Dean, Medical School, University of Minnesota

KEYNOTE PRESENTATIONS:
Advancing Next Generation Gene & Cell Therapies for Epidermolysis Bullosa
Beau Webber, PhD
Assistant Professor, Pediatric Hematology and Oncology, University of Minnesota

Hematopoietic Lineage Cell Engineering for Rare Diseases
Mark Osborn, PhD
Assistant Professor, Department of Pediatrics, University of Minnesota

Passion, Perspective, and Progress: One Community’s Ongoing Drive to Develop a Potential Gene Therapy for a Rare Pediatric Disease
Michelle Berg, Vice President, Patient Affairs and Community Engagement, Abeona Therapeutics

CLOSING REMARKS:
James Cloyd III, PharmD
Professor, Department of Experimental and Clinical Pharmacology (ECP), University of Minnesota

Co-sponsored by: Center for Orphan Drug Research, University of Minnesota & Stem Cell Institute, University of Minnesota
Alphabetical List of Patient Advocacy Groups & Sponsors

**Patient Advocacy Groups:**
Chloe's Fight Rare Disease Foundation
Crohn's and Colitis Foundation – MN/SD Chapter
Cystinosis Research Network
Epilepsy Foundation - Rare Epilepsy Network
Epilepsy Foundation of MN
Families SCN2A Foundation
Family Voices of MN
Foundation for Sarcoidosis Research
Gavin Flying for a Cure
Huntington's Disease – MN Chapter
I Refuse EB
Legacy of Angels
Midwest Rett Syndrome Foundation
Minnesota Department of Health – Newborn Screening
Muscular Dystrophy Association of Minnesota/Dakotas
National Ataxia Foundation
Organic Acidemia Association
Pulmonary Fibrosis Support Group of Minnesota
Rare Action Network – NORD
Tuberous Sclerosis Alliance
Turner Syndrome Society – MN Chapter
United Mitochondrial Disease Foundation

**Sponsors:**
Abeona Therapeutics
Aldevron
Fairview Pharmacy Services
Pairnomix
Takeda
Upsher-Smith
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KCNQ2 epileptic encephalopathy (EE) arises due to mutations in the Kv7.2 voltage-gated potassium (K+) channel. Mutations conferring either a gain or loss of function in KCNQ2 often lead to early infantile EE that is usually refractory to conventional anti-epileptic drugs (AEDs). A wild-type or R201C mutant KCNQ2 gene, along with a copy of the wild-type KCNQ3 gene, were transfected into CHO cells to generate cell models capable of producing an M-like K+ current. Patch clamp evaluation demonstrated a voltage-dependent increase in outward K+ current for both cell lines. However, the R201C mutation conferred a significant leftward shift in the voltage-current relationship with activation (opening) at lower voltages and a significantly slower deactivation rate compared to wild-type.

Using a 86Rb+ efflux assay (a proxy ion for K+), wild-type and R201C cell lines were screened against the Prestwick library, a collection of 1,280 drugs that are clinically approved; a panel of AEDs and known ion channel modulators were also included. Of the 1,320 compounds screened at 10 μM, 26 significantly inhibited the wild-type channel and 36 significantly inhibited efflux from the R201C mutant cell line. Interestingly, only two compounds significantly inhibited 86Rb+ efflux from both cell lines. The drug with the greatest inhibitory activity against the mutant cell line was paroxetine (SSRI antidepressant; IC50= 6.90 µM; maximum inhibition = 90%); other drugs with potent and significant inhibition of 86Rb+ efflux included acitretin (retinoid for psoriasis), norgestimate (steroidal) and hexestrol (a synthetic estrogen). This study demonstrates the utility of precision genetic modeling by replicating an underlying genetic mutation of EE in a cell model suitable for HTS. The results identified several compounds that had heretofore not been associated with inhibitory activity against Kv7.2 potassium channels that may hold therapeutic value for KCNQ2 patients with EE.
Poster Number: 2
First Author: Irene Vuu
Contributing Authors: Lisa Coles, Ilo Leppik, Michael Rogawski, Dorota Zolkowska, Jimmy Wu, Ned Patterson, James Cloyd

Author Affiliations: Center for Orphan Drug Research, College of Pharmacy, University of Minnesota, Minneapolis, MN, Experimental and Clinical Pharmacology Department, College of Pharmacy, University of Minnesota, Minneapolis, MN, College of Veterinary Medicine, University of Minnesota, Saint Paul, MN, Department of Neurology, University of California - Davis, Sacramento, CA

Subject Area: Neurological
Keywords: epilepsy status epilepticus dogs allopregnanolone

Abstract Title: Characterization of Allopregnanolone Pharmacokinetics in Dogs with Naturally-Occurring Epilepsy to Support Use in the Initial Treatment of Status Epilepticus

Abstract: Allopregnanolone is a naturally-occurring neurosteroid that is a positive allosteric modulator of synaptic and extrasynaptic GABAA receptors. We hypothesize that allopregnanolone possesses the requisite pharmacokinetic (PK) and pharmacodynamic properties to support its use as an initial IV bolus treatment for SE. One approach to testing this hypothesis is the use of dogs with naturally-occurring epilepsy, which is similar to human epilepsy in electroencephalographic presentation and response to therapy. Our study objectives were to assess the tolerability and develop a PK model of allopregnanolone following a 5 minute IV infusion, a clinically applicable regimen for the initial treatment of SE. Four dogs with naturally-occurring epilepsy were used, with two on phenobarbital. A 1 mg/kg allopregnanolone dose (1.5 mg/mL in 24% sulfobutyl ether β-cyclodextrin) was infused over 5 minutes. Blood samples were collected between 0 to 8 hours following dosing. Animals were observed for ataxia, vomiting, diarrhea, and lethargy prior to and for 60 minutes after the infusion, and thereafter at each subsequent sampling time. Plasma concentrations were measured using a UPLC-MS/MS system. PK modeling performed using Certara Phoenix. The only adverse effects observed were two dogs on phenobarbital who exhibited ataxia and somnolence 3 minutes after start of infusion, and recovered within 10 minutes. Plasma concentrations at 2 minutes post-infusion were ~5000 ng/mL, declining to 130-700 ng/mL at 30 minutes. Concentration-time profiles were best fit by a two-compartment model. Phenobarbital was associated with a greater clearance and shorter elimination half-life. A 1 mg/kg allopregnanolone dose infused over 5 minutes appears safe and results in attainment of plasma concentrations greater than those associated with seizure cessation in rodents. At this dose, transient sedation may occur in dogs receiving phenobarbital. Our results support further evaluation of allopregnanolone for efficacy in the initial SE treatment in dogs and humans.
Optimizing cystine depleting therapy in nephropathic cystinosis: Two case reports

Abstract:

Nephropathic cystinosis is a rare inherited autosomal recessive disorder that causes accumulation of cystine in tissues throughout the body due to a defect in the cystine transport protein of the lysosomes. The mainstay of cystinosis treatment outside of kidney transplant is oral cystine-depleting therapy using cysteamine and cysteamine eye drops. Strict adherence to the oral medication dosing schedule while using maximum tolerated dose is required to achieve optimal outcomes. Immediate-release cysteamine requires an every 6 hour dosing regimen. Significant adverse gastrointestinal side effects have been a challenge for patient compliance. A newly approved delayed-release cysteamine has the potential to improve patient compliance through its 12 hour dosing regimen, but adverse gastrointestinal side effects may still remain challenging for patients. This report presents two patient cases in which patient specific pharmacotherapy plans resulted in consistent compliance with delayed-release cysteamine dosing schedule and achievement of goal leukocyte cystine levels, while minimizing adverse side effects of cysteamine.
Poster Number: 4
First Author: Elizabeth L. Thompson¹,²
Contributing Authors: Jakub Tolar²,³, Eric A. Hendrickson¹
Author Affiliations: ¹Department of Biochemistry, Molecular Biology and Biophysics, ²Department of Pediatrics, Division of Blood and Marrow Transplantation, ³Stem Cell Institute, University of Minnesota, Minneapolis
Subject Area: Hematological, Cancer
Keywords: Fanconi anemia, Chromosomal instability, Genetic variants, DNA repair, Cancer
Abstract Title: FANCN patient mutations are hypomorphic and rescue catastrophic genomic instability

Abstract: Fanconi anemia (FA) is a rare chromosomal instability disorder. Patients with mutations in FANCN/PALB2 (partner and localizer of BRCA2) typically have more severe disease with earlier onset of cancer and bone marrow failure. FANCN is known to promote homologous recombination (HR) by complexing with both BRCA1 and BRCA2 and facilitating RAD51 strand invasion. To further investigate the function of FANCN, we generated a conditional null FANCN cell line. We found that removal of the conditional allele resulted in spontaneous chromosomal breaks and rearrangements that resulted in chromosomal catastrophe within 48 hours. Due to this crucial function of FANCN in maintaining genome stability, we hypothesized that FANCN patient mutations are hypomorphic and performing an essential function to preserve cellular viability. To test this, we complemented the FANCN null cell line with three FA-associated FANCN truncating mutations and with three FANCN single nucleotide polymorphisms (SNPs) identified in breast cancer patients.

Results: We determined that FANCN patient mutations are hypomorphic and can rescue the viability of the FANCN null cell line. Furthermore, we have confirmed that truncated FANCN mutants cannot bind BRCA2, while the SNP mutants can. Additionally, all three truncating mutations and two of the three SNPs are defective in HR, and sensitive to mitomycin-C, PARP inhibitors and replication stress.

Conclusions: This research has revealed mechanistic understanding of FANCN and how these FANCN patient mutations result in disease. For example, the FANCN N-terminus serves an essential function in viability and genomic stability, whereas the FANCN C-terminus servers an essential role for cell proliferation and mitomycin-C resistance. Importantly, we have identified two cancer-associated FANCN SNPs that are sensitive to interstrand crosslinks, defective in DNA repair and potentially pathogenic mutations. Finally, this research has created novel FANCN cell lines that can be used to screen additional FANCN variants and for developing new FA therapeutics.
Abstract:

Acute respiratory distress syndrome (ARDS) is a severe, life threatening form of respiratory failure characterized by proteinaceous pulmonary edema, inflammation, and hypoxemia with a mortality rate of forty percent and no proven molecular therapies. The edema in ARDS results from epithelial damage with increased epithelial permeability and reduced alveolar fluid clearance (AFC). The mechanism for AFC is active sodium transport by the alveolar epithelium, utilizing coordinated action of epithelial sodium channels and the sodium pump, Na+-K+ ATPase. The lung is a target tissue of thyroid hormone, and triiodothyronine (T3) has multiple effects on type-II alveolar epithelial cells. In preclinical studies, T3 increased alveolar epithelial cell Na+-K+ ATPase pump activity in vitro and in vivo. When administered in vivo via intra-tracheal instillation, it increased AFC in both normal and hypoxia-injured rat lungs. In ARDS patients, postmortem analyses show low lung tissue T3. We are planning to initiate a clinical study to assess the safety and efficacy of a replacement thyroid hormone T3 formulation for increasing AFC and reducing extra-vascular lung water (EVLW) in ARDS patients. The safety of intratracheal T3 was assessed in a rat GLP toxicology study to provide data to support the use of this novel route of administration for this drug in human subjects. Intratracheal T3 was administered on 5 consecutive days in intubated rats, at a dose approximately 30-fold over the highest projected clinical dose. No adverse clinical findings, and no drug related safety findings were observed based on clinical pathology and histopathologic endpoints. Toxicokinetic parameters for plasma levels of T3 following intratracheal instillation were also determined. This preclinical safety study should pave the way for the planned phase I/II study to determine the safety and tolerability of a T3 formulation delivered into the lungs of ARDS patients and to measure the effect on EVLW.
Epilepsy is a neurological disorder characterized by high frequency action potentials in the brain. Delayed and misdiagnosis can lead to developmental difficulties in children. Hence there is a critical need for early diagnostic biomarkers. MicroRNAs (miRNAs) are small noncoding RNA molecules that have a significant role in health and disease. Recently, circulating miRNAs have been identified in body fluids allowing them to be used as biomarkers. However, the diurnal variation in miRNA expression is not known. We hypothesize that miRNA expression levels vary with sampling time. Our objective is to understand variation in blood miRNA expression following seizures. Whole blood samples were collected twice (AM and PM) in PAXgene blood RNA tubes (Qiagen, CA) from five canines with naturally occurring epilepsy, maintained at the University of Minnesota Veterinary Medical Center. MiRNAs were extracted using PAXgene blood miRNA kit (Qiagen, CA) and quantified by small RNA sequencing on an Illumina HiSeq 2000 platform. Statistical analyses were performed to determine the differences between groups. We identified ~180 unique canine miRNAs in whole blood. Four out of the five dogs displayed upregulation of miRNAs in the AM compared to PM samples. Our preliminary analyses revealed differential expression of miRNAs between the two time points of sample collection and that sampling time should be considered while interpreting miRNA expression analysis. We will perform further studies to investigate the temporal regulation of miRNA expression following seizures in these dogs to identify optimal sample collection time points in a clinical setting.
Abstract: Background
Rational investigation of therapeutic interventions in amyotrophic lateral sclerosis (ALS) will be aided by validated objective biomarkers. The aim of this study is to evaluate the ability of magnetic resonance spectroscopy (MRS) to provide brain metabolite markers of disease progression in ALS.

Design/Methods
A subset of the participants of our prior cross-sectional 7 tesla MRS study (Cheong 2017) returned for scans at 6 months (14 ALS, 16 controls) and at 12 months (10 ALS, 12 controls). Neurochemical profiles of the upper limb motor cortex were obtained at each scan session. Clinical assessments included the ALSFRS-R, El Escorial diagnostic classification (EEC), and King's staging.

Results
Linear mixed-effects model analyses of time trends indicate a decline in tNAA/mIns in the motor cortex in PALS (N=14, p=0.02) and no change in controls over a 12 month period. Furthermore, tNAA/mIns declined in all participants with ALS who demonstrated upper limb functional decline and was stable in those who did not. Low tNAA/mIns at first scan predicted inability to complete the 12 month study due to ALS progression.

Conclusions
Upper limb motor cortex tNAA/mIns correlates with upper limb functional status in ALS and predicts study withdrawal due to ALS. MRS is readily obtainable in the clinical setting and is promising as a biomarker of disease progression and method of measuring upper motor neuron involvement in ALS.
Mutations in Valosin-Containing Protein (VCP) cause dominantly inherited multisystem degenerative diseases, including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD) and inclusion body myopathy (IBM). The disease mechanism through VCP mutation is not clear. To elucidate the mechanism of VCP mutation-induced disease, we developed a Drosophila model of VCP mutation-dependent degeneration and performed a genetic screening.Ubiquitination factor E4B (UBE4B) which has ubiquitin ligase activity (E3) and chain elongation activity (E4) was the strongest dominant modifier. Knockdown of dUBE4B by RNAi and overexpression of truncated dUBE4B lacking a C-terminal catalytic domain rescued the degenerated eye phenotype and motor neuronal degeneration caused by VCP mutation. We also found that UBE4B mutant flies also have the same movement and flight defects and abnormal wing posture, which observed in VCP mutant flies. Moreover, UBE4B directly interacted more strongly to mutant VCP than to wild-type VCP, suggesting that mutant VCP’s toxicity is mediated through increased interaction with UBE4B and its ligase activity. Currently, we are investigating which domain is important for mediating VCP’s toxicity and testing whether inhibition of the increased interaction between VCP and UBE4B can be a therapeutic strategy. We are also identifying downstream molecular pathways of the VCP-UBE4B complexes which could be a potential drug target.
Gaucher disease is an autosomal recessive disorder caused by mutations in the gene encoding the lysosomal enzyme, glucocerebrosidase (GBA) resulting in an accumulation of glucosylceramides. It was thought the most common type, Type 1 Gaucher disease (GD1), did not affect the brain, however GD1 patients have a higher risk of developing Parkinsonism. Current therapies significantly improve outcome, but patients continue to present anemia, fatigue and pain. We hypothesize that an adjunctive therapy with an antioxidant/anti-inflammatory agent has the potential to ameliorate ongoing symptoms. Our objective is to characterize oxidative stress and inflammation in the blood and brain of individuals with GD1, and determine whether these factors can be altered with orally administered N-acetylcysteine (NAC), an antioxidant with anti-inflammatory properties. Thirty adult patients with GD1 and 20 healthy individuals are being enrolled in an open-label study. Blood sample collection for biomarker analysis and brain imaging using Magnetic Resonance Spectroscopy (MRS) will be done before (baseline) and following 3 months of NAC. Ten healthy controls and 4 patients have completed the study. The redox ratio of reduced/oxidized glutathione, which is a measure of oxidative stress was measured in red blood cells using validated liquid chromatography/mass spectrometry method. Intracellular antioxidant enzyme activity and plasma markers for lipid and protein modifications were measured using colorimetric and immunoassays. Preliminary analyses of baseline data indicate alterations in brain bioenergetics and systemic antioxidant biomarkers in patients with GD1, the details of which will be discussed.
Patients with malignant brain tumors have limited options; survival rates of grade IV glioblastoma multiforme is 5% at 5 years after the initial diagnosis. The emergence of Zika virus (ZKV) to the status of public health emergency led to increased funding towards mechanisms of ZKV related microcephaly. Our data suggest that ZKV infection of fetal brain induced increased expression of autophagy and apoptosis-related genes. Given the similarities between neural stem cells and glioma stem cells, we sought to determine if ZKV is a viable therapeutic option for malignant gliomas. Characterization of in vitro cultured human and murine glioma cell lines demonstrate expression of putative receptors for ZKV entry. Analysis of the kinetics of infection and active viral replication in glioma cell lines through the plaque-forming assay reveal variable kinetics in ZKV infected cells; murine gliomas are relatively resistant to ZKV replication while highly passaged human gliomas are highly amenable to ZKV propagation and virus-induced lysis. To interrogate ZKV as an oncolytic agent in vivo, we induced brain tumors in immunocompetent C57BL/6J mice through transplantation of the murine GL261 glioma cell line within the striatum followed immediately by injection of ZKV at the same coordinates. At all three concentrations tested, ZKV did not significantly prolong the overall survival of tumor-bearing mice, relative to untreated tumor-bearing mice. We next sought to determine if ZKV can be used in conjunction with immunotherapies. Our lab previously developed a vaccine immunotherapy in which ex vivo cultured tumor cells are irradiated then infused into tumor-bearing mice which modestly extended overall survival. In the current study, we induced brain tumors in C57BL/6J and immunodeficient NOD-SCID mice. Mice in the treatment condition were immediately injected with ZKV at the same coordinates. At 3-, 7-, and 14-days following brain tumor induction, mice were subcutaneously infused with irradiated GL261 tumor cells previously infected with ZKV. We observed an increase in overall survival of immunocompetent treated mice with roughly half of all treated mice surviving long-term. These long-term survivors were re-challenged with tumor cells and the immune response was analyzed using flow cytometry. Relative to age-matched tumor-bearing controls, we observed increases in activated microglia and infiltrating T-lymphocytes in the brain of treated tumor-bearing mice. These results suggest that ZKV acts as an adjuvant, signaling an immune response to the infected tumor cells in the brain.
Abstract:
Introduction: Corticospinal tract degeneration is a defining feature of ALS, yet to date there have been very few longitudinal, controlled studies assessing diffusion MRI (dMRI) and other MRI metrics in the spinal cord in ALS. In this study, we performed a tract-specific analysis of cervical spine dMRI data, showing the potential of spinal cord dMRI to be a diagnostic biomarker of ALS.

Methods: We acquired high quality cross-sectional data from the cervical spine from C2 to C6 in 20 ALS and 20 control participants. One year longitudinal data were also acquired from 11 ALS and 13 control participants. We segmented the spine into gray and white matter, and into individual ascending and descending fiber tracts within the white matter, for performing tract-specific analysis using a diffusion tensor imaging model.

Results: The diffusion metrics became more sensitive when we segmented the white matter from the gray matter (Fig. 1A and 1B). The sensitivity of the metrics further increased when we segmented the descending fiber tracts from the ascending fiber tracts (Fig. 1C). Two of the descending tracts, the lateral CST and the rubrospinal tract, demonstrated highly significant reductions in fractional anisotropy (FA), relative to controls, throughout the cord length analyzed from C2 to C6 (Fig. 2A and 2B). We also noted significant differences in the spinal lemniscus (Fig. 2C).

Longitudinal changes in diffusion metrics were noted primarily in the lateral CST, rubrospinal tract, spinal lemniscus, and the posterior column (Fig. 3). We observed strong correlations between cervical cord FA and the ALSFRS-R at enrollment (Fig. 4). We also observed significant differences in cord cross-sectional area throughout the cord length, C2 to C6 (Fig. 5), with strong correlations with ALSFRS-R at the C2 level (Fig. 4).
Mucopolysaccharidosis type I (MPS I) was added to the Recommended Uniform Screening Panel in 2016 due to overwhelming evidence that early treatment results in improved outcomes. The severe form of MPS I, Hurler syndrome, is characterized by progressive neurological involvement, which follows a predictable trajectory of normal cognitive development in the first year of life, slowing in the second year, and rapid decline thereafter. Allogeneic hematopoietic cell transplantation (HCT) stabilizes this deterioration and dramatically extends survival. Younger age at HCT is one of the strongest predictors of favorable cognitive outcomes, although the literature is limited regarding outcomes of patients transplanted during this first year of normal development. Previous data from our institution across a wide age range suggest a mean loss of 3.64 IQ points per year may be expected in the 2 years following HCT. To understand if earlier HCT could improve cognitive outcomes, we examined the IQ scores of all Hurler patients who underwent HCT at the University of Minnesota prior to age 12 months and had longitudinal neuropsychological follow up at least two years post transplantation (N=8). We found IQ scores at 2 years following HCT were the same as baseline IQ scores (p = 0.63), with baseline mean of 91.6 and 2-year post-HCT mean of 93.4, providing evidence there was no loss in IQ points from the time period prior to HCT to 2 years afterward. These results quantify a likely cognitive benefit when transplant is conducted at younger than 12 months of life, an important finding at the dawn of newborn screening. Longer-term analyses of a larger cohort are needed to determine if these cognitive outcomes remain superior from those transplanted at older ages.
Abstract Title: ZFN-mediated liver-targeting gene therapy corrects systemic and neurological diseases of mucopolysaccharidosis type I

Abstract: Mucopolysaccharidosis type I (MPS I) is characterized by progressive neurodegeneration, and premature death. Caused by α-L-iduronidase (IDUA) deficiency and subsequent accumulation of glycosaminoglycans (GAG), current therapies include stem cell transplant (with significant risk of morbidity and mortality), and enzyme replacement therapy (requiring costly and frequent long therapeutic infusion sessions). In contrast, we are proposing a one-time, single infusion of three AAV vectors to accomplish in vivo gene editing. This method employs insertion of the normal IDUA cDNA by means of dual zinc finger nuclease (ZFN)-mediated cutting and insertion into intron 1 of the albumin locus. A small number of hepatocytes are thereby edited in vivo to create a stable long-term source of corrective IDUA enzyme. MPS I mice (n=8 per gender, 4-9 weeks old) were injected with a single dose of AAV2/8 encoding albumin-targeted ZFN and a donor encoding a partial IDUA cDNA. MiSeq analysis showed that treated mice displayed significant levels of indels (56%) at the target locus, demonstrating efficient cutting by ZFN. IDUA levels in these animals increased significantly in liver (up to 14 fold), heart, lung, muscle and spleen. Tissue GAG levels were significantly reduced in liver (by 91%), heart (85%), lung (86%), muscle (68%) and spleen (84%). Barnes maze tests at the end of the study showed that ZFN+IDUA donor treated MPS I mice achieved significant neurological benefits compared with untreated MPS I mice. This study serves as a proof-of-concept for this platform-based approach that should be broadly applicable to the treatment of a wide array of monogenic diseases.

Further, IND (BB-16821) based on these results was approved in 2016 by the US FDA for the initiation of a Phase I study of in vivo genome editing. This protocol was recently opened as an “actively recruiting” clinical trial (clinicaltrials.gov ID: NCT02702115).
Podocytes are terminally differentiated cells with limited regeneration capacity. In young Fabry patients, we showed that podocyte GL-3 accumulation occurs early, is progressive with age, and is associated with podocyte injury and proteinuria (Najafian et al. Kidney Int 2011). Kidney biopsies from 61 males, age 26 [4-65], median [range], years with classical Fabry genotype/phenotype were studied by electron microscopic stereology. While podocytes (PC) enlarge as a result of GL-3 accumulation in Fabry disease, the fraction of the glomerulus made up of PC [Vv(PC/glom)] decreased with age (r=-0.49, p=0.0001). The numerical density of PC/glomerulus [Nv(PC/glom)] correlated inversely with volume of GL3 inclusions/PC [V(Inc/PC)] (r=-0.73, p=0.00001), indicative of PC loss with GL-3 accumulation. 

V(Inc/PC) also correlated with urinary protein excretion (UPER) (r=0.42, p=0.02), a strong predictor of glomerular filtration rate (GFR) loss. Also, the fraction of PC cytoplasm occupied by GL3 inclusions [Vv(Inc/PC)] correlated with UPER (r=0.39, p=0.007) and PC foot process width (FPW) (r=0.50, p=0.008), consistent with direct relationship between PC GL-3 accumulation and podocyte injury. Using simple linear regression analysis, Vv(PC/glom) correlated with GFR (r=0.27, p=0.04). Using a general linear model with age, Vv(PC/glom), Vv(Inc/PC), V(Inc/PC), and mean volume of PC [VPC] as variables, Vv(PC/glom) was the only independent predictor of GFR (p=0.01). Using multiple regression analysis, Vv(PC/glom), Vv(Inc/PC), V(PC) and V(Inc/PC) explained 32% of GFR variance (p=0.008), independent of age. In conclusion, PC GL-3 accumulation in Fabry disease is associated with podocyte injury manifested by increased FPW and proteinuria. PC but not mesangial or endothelial structural parameters predict GFR loss in male Fabry patients independent of age. This is consistent with the known association of PC loss with glomerular scarring and argues for intervention before critical levels of PC loss have occurred, this likely leading to progressive renal failure regardless of therapy.
VEGFA-Flt1 axis modulates the muscular dystrophy phenotype in Duchenne muscular dystrophy model mice

Duchenne Muscular Dystrophy (DMD) is an X-linked recessive genetic disease in which the gene coding for the membrane stabilizing protein, dystrophin, is absent. Interestingly the vasculature has also shown to be perturbed in DMD and the DMD model mdx mice. We using data-mining patient transcriptomics data, we link this defect to vascular endothelial growth factor A(VEGFA). In this report, we explore VEGFA-Flt1 in the skeletal muscle of the mdx mice. We used whole body and vascular specific Flt1 conditional knockout to show that while Flt1 constitutive deletion of Flt1 is detrimental, vascular specific Flt1 deletion improves the DMD associated phenotype in the mdx mouse. We can recapitulate the phenotype by using small molecules and monoclonal antibodies to Flt1 in the mdx mouse model. We confirm that VEGFA is involved in the skeletal muscle regeneration in DMD and validate Flt1 as a therapeutic target for the treatment of DMD.
Poster Number: 18
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Subject Area: Neurological, Neuromuscular
Keywords: Baclofen withdrawal, spasticity, orphan drug development
Abstract Title: Characterizing Baclofen Withdrawal: A Survey of Clinician Experience

Abstract: Research Objectives: Determine provider’s experience regarding the significance, prevention and management of baclofen withdrawal.

Design: Survey distributed via e-link by the American Academy of Physical Medicine and Rehabilitation (PM&R).

Setting: PM&R Members

Participants: 30 PM&R Physicians

Interventions: A 24 question, electronic survey

Main Outcome Measure(s): The content of the survey included questions addressing the significance of, and interventions to prevent and manage baclofen withdrawal.

Results: Approximately two-thirds of providers manage over 50 and 16 patients on oral (PO) and intrathecal (IT) baclofen respectively. The most common indications for treatment were similar for PO and IT therapies and included SCI, TBI and CP. All providers were concerned about the potential for withdrawal following abrupt discontinuation of PO or IT baclofen. 80% of providers have seen at least 1 episode of withdrawal from PO throughout his or her career. 72% have seen withdrawal from IT therapy in the last 12 months. Nevertheless, 13.8 and 62.5% have an established protocol when PO and IT baclofen interruption is anticipated. Most frequent symptoms were increased spasticity and pruritus. Replacement of baclofen when feasible and benzodiazepines were the most frequently used medications to manage withdrawal. 79.1 and 95.9% of physicians felt that an intravenous formulation of baclofen would be useful in the prevention and treatment of PO and IT baclofen withdrawal, respectively.

Conclusions: Baclofen withdrawal from PO or IT therapy is recognized as a significant concern and has been observed by almost all of the respondents. The survey identified a lack of consensus in the prevention or treatment of withdrawal. The physicians noted that intravenous baclofen would likely to be useful in the prevention and management of baclofen withdrawal.
Recessive dystrophic epidermolysis bullosa (RDEB) is a skin blistering disease caused by mutations in the COL7A1 gene. RDEB patients experience severe blistering and fragility of the skin and mucous membranes, leading to pseudosyndactyly, susceptibility to infections, esophageal strictures, and aggressive cutaneous squamous cell carcinoma (SCC), which accounts for more than two thirds of RDEB patient deaths. RDEB patients can have multiple primary tumors and these tumors develop and metastasize much more quickly than in the general population. This study aims to characterize the transcriptomes of SCC samples and non-cancerous keratinocytes from RDEB patients using RNA-seq in order to identify conserved genes and pathways involved in RDEBSCC development. The analysis identified 1683 genes as differentially expressed (DE) (q<0.005) between RDEBSCC cells and RDEB keratinocytes (RDEBK), using a log2 fold change of ±2.0. Several interesting trends were identified in the list of DE genes: 1. Four core pluripotency genes and two major transcription factor families were upregulated in RDEBSCC cells relative to RDEBK; 2. Numerous genes encoding proteins involved in the transport of ions and biomolecules were DE, including 38 channel proteins, 34 solute carrier proteins, 28 cell junction proteins, 14 transmembrane proteins, and 6 ATP-binding cassette proteins; 3. A family of genes (5) involved in evasion of apoptosis were upregulated; 4. Genes involved in mediating epithelial-to-mesenchymal transition (EMT) were massively upregulated; and 5. Two potential cancer stem cell markers were expressed at high levels in RDEBSCC cells. These groups of genes provide insight into the aggressive nature of these cancer cells, represent potentially clinically actionable targets for developing targeted therapeutics that can slow or stop cancer progression, and/or help identify RDEBSCC at a much earlier stage.
Poster Number: 20
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Subject Area: Connective Tissue and Musculoskeletal
Keywords: recessive dystrophic epidermolysis bullosa, microRNA switches, mosaicism
Abstract Title: Purification of Revertant Mosaic Fibroblasts from a patient with Recessive Dystrophic Epidermolysis Bullosa using Synthetic MicroRNA Switches

Abstract: Recessive dystrophic epidermolysis bullosa (RDEB) is a severe, lethal skin disorder characterized by chronic skin blistering and aberrant wound healing. It is caused by loss-of-function mutations in the critical extracellular matrix protein type VII Collagen (C7). While rare (1:1,000,000 live births), the disorder is extremely painful and treatment is typically limited to palliative care. One treatment that addresses the cause of the condition is the use of C7 expressing stem cells or differentiated skin cells to replace C7 at the dermal-epidermal junction, restoring skin architecture and integrity. This requires the use of gene therapy or allogeneic cells, which can be costly and cause adverse reactions in the recipient. In rare cases, genetic reversion causes natural gene correction of the underlying mutation leading to a population or patch of cells that is phenotypically distinct or mosaic. While these cells may be useful clinically, isolating and purifying them has proven difficult. Here, we describe a method utilizing synthetic micro RNA (miR) switches, whereby differences in endogenous miR activity can be exploited to purify mosaic cells in culture. We first show, by targeted sequencing and immunocytochemistry, that C7 is expressed at higher levels in the mosaic compared to the blistered cells. Using miR-sequencing, we identified miRs differentially expressed between mosaic and blistered cells and made miR switches for these. Transfection of the mosaic cells with the miR switches should separate the corrected from the un-corrected cells. Future work includes flow sorting and Western blotting to confirm C7 expression in the mosaic sub-population. This technique may be useful for generating pure populations of naturally gene corrected mosaic cells that can then be used in future clinical applications such as expansion of the naturally-gene corrected cells for autologous hematopoietic cell transplant or skin grafts for areas of the body that fail to heal properly.
Abstract: Studies of eye tracking have increasingly been used to evaluate cognitive processes in rare disorders such as Rett syndrome (RTT). Currently, few studies systematically examine the quality of eye tracking data quality within and between groups. It has been shown that data quality can affect estimates of several key variables in eye-tracking research, which can lead to inaccurate interpretation of experimental or between-group differences. Individuals with RTT may introduce artefacts due to excessive movement, difficulty following verbal instructions, and ophthalmologic problems. Therefore, objective measures of data quality are needed to improve the quality and replicability of eye tracking research in this population. The current study included six individuals with Rett syndrome (aged 2:11 to 26:7 years) and five healthy female controls (aged = 5:4 to 21:10 years). All participants completed a task designed to evaluate the accuracy and precision of the eye tracking data collected. For each stimulus presentation, accuracy was computed as the Euclidean distance (in pixels) from the location of the stimulus and the gaze data. Precision of the gaze measures was calculated as the root mean square (RMS) of successive X and Y gaze locations. Between-group differences in each of these variables were examined. Distance measures from the RTT group were consistently and significantly higher than those from the control group. Precision measures for the X coordinates did not differ significantly between groups, whereas variability for the Y coordinates was significantly greater in the RTT group. These results support the notion that between-group differences in data quality may affect results of eye tracking research when comparing data collected from individuals with RTT and healthy comparison samples. Additional work is needed to determine the degree to which the observed differences in data quality may affect key eye tracking variables, such as fixation time and eye movement latency.
Poster Number: 22
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Subject Area: Neurological
Keywords: Rett syndrome; pain and discomfort scale; modified quantitative sensory test
Abstract Title: Testing Two Observational System Approaches to Measure Behavioral Reactivity during Modified Quantitative Sensory Testing in Rett Syndrome

Abstract: There are conflicting reports about pain in Rett syndrome (RTT); on the one hand, diagnostic criteria and case reports reference pain insensitivity (Hagberg, 2002), but there is evidence of behavior consistent with pain expression (Barney, Feyma, Beisang, & Symons, 2015). The current ‘gold standard’ for determining the presence of pain remains verbal self-report, yet most individuals with RTT have severe communication impairment. One potential approach to clinically evaluate the functional integrity of the somatosensory and nociceptive systems without relying on self-report can be based on exam. The Pain and Discomfort Scale (PADS) is an observation based coding system designed to detect pain/discomfort related behaviors during a standardized pain examination procedure (Bodfish, Harper, Deacon, & Symons, 2001). The purpose of this study was to modify the PADS to successfully capture pain- and discomfort-related reactivity in RTT during a modified quantitative sensory test (mQST) in 20 participants with clinically diagnosed RTT. Reactivity was scored (1) as 18 individual behaviors (e.g. brow furrow) or (2) as 5 behavior classes (e.g. facial expressions) for each stimulus. There was a high correlation between both methods for total reactivity scores ($r = 0.71$, $p = 0.0004$). Both scoring systems led to similar patterns of reactivity across behavior classes and stimuli and preserved variability in responding across participants. Coding by behavior class significantly reduced the time needed to train coders to a high reliability standard and the time needed to code each individual protocol. Overall, both methods captured pain- and discomfort-related behavior. Results indicate that scoring classes of behavior may be sufficient to fully describe pain-and discomfort in RTT, while saving both time and financial resources. This method may provide a way to investigate somatosensory and nociceptive function in RTT, as well as a sensitive, relevant clinical trial outcome measure.
Abstract: One of the most prevalent features in neurodegenerative diseases is mitochondrial abnormalities. Indeed, it has been reported that all patients with amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) have mitochondrial dysfunction. However, it is unclear how mitochondrial dysfunction is caused and whether it is a cause or a result of ALS and FTD. Recently, several mutations of CHCHD10 encoding a mitochondrial protein were reported as a genetic cause of ALS and FTD. Although mutations in over 20 genes have been identified as the cause of ALS and FTD, only CHCHD10 primarily localizes in mitochondria. This suggests that mitochondrial defects might be one of the primary causes of neurodegeneration in ALS and FTD patients and that understanding of the CHCHD10-mediated mitochondrial pathogenesis would be helpful to understand mitochondrial dysfunction that is shared in all ALS and FTD patients but not fully understood. In addition, it will also be helpful to develop therapeutic strategies that can be applied to all patients regardless of their genetic causes. To understand the disease-causing mechanism of CHCHD10, we generated a fruit fly (Drosophila) model. When a mutant form of CHCHD10 was expressed in Drosophila eyes, motor neurons and muscles respectively, CHCHD10-mutant caused corresponding degenerative phenotypes such as rough eyes, larval crawling defects, and abnormal wing postures. Furthermore, mutant CHCHD10 overexpression in mammalian cells caused mutant-dependent mitochondrial fragmentation. To further investigate mitochondrial pathways important for CHCHD10-mediated pathogenesis, we performed a forward genetic screening against mitochondrial proteins with the Drosophila model and transgenic RNAi flies. Surprisingly, knockdown of two Parkinson’s disease-causing genes (PINK1 and Parkin) significantly rescued the degenerative phenotypes. We are currently investigating how the PINK1/Parkin pathway contributes to the pathogenesis of mutant CHCHD10-induced ALS and FTD and whether it can be a therapeutic target.
Poster Number: 24

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Subject Area: Neurological

Keywords: acute repetitive seizures, status epilepticus, prodrug, intranasal, benzodiazepines

Abstract Title: Intranasal Delivery of Prodrug/Enzyme Combinations for the Treatment of Seizure Emergencies

Abstract: Seizure emergencies occur when an individual experiences a seizure that lasts for more than 5 minutes (status epilepticus) or two or more distinct seizures with incomplete recovery (acute repetitive seizures). Both conditions meet the FDA definition of a rare medical disorder i.e. fewer than 200,000 individuals per year with either disorder. Patients experiencing a seizure emergency must be treated as quickly as possible to avoid lasting neurological damage and other life-threatening complications. Benzodiazepines are the primary rescue medications used to treat seizure emergencies, the most commonly used being intravenous lorazepam or rectal diazepam. Despite the effectiveness of these drugs, the delivery routes are not ideal for first-line, outpatient treatments considering that a skilled caregiver must administer drugs intravenously and that the social stigma associated with rectal administration results in low compliance. Intranasal delivery is an attractive alternative because it requires little training, is easily performed by non-medical personnel, carries a low risk of injury to the patient, and has a rapid onset of therapeutic action. However, the low aqueous solubility of benzodiazepines presents a challenge in formulating these drugs in a volume small enough to be effectively delivered as a nasal spray. Our group has developed a novel intranasal drug delivery system: water-soluble prodrugs of benzodiazepines that are administered concomitantly with an enzyme. Conversion of the prodrug produces a supersaturated solution of the active drug, which further promotes rapid absorption across the nasal mucosal membrane. This prodrug/enzyme approach is a robust strategy to generate predictable levels of supersaturated drug for rapid intranasal delivery and represents a new paradigm in drug delivery. A drug product based on this system is expected to result in fewer emergency department visits and improved quality of life for patients who experience a seizure emergency.
Abstract: Background: Immune responses to gene therapy have been recognized as a threat to efficacy and safety since the early days of gene therapy research. As the number of gene therapies moving into clinical trials increases, immune responses that can occur towards the vector vehicle, the transgene, or both continue to be of primary concern. Several gene therapy clinical trials have employed immunosuppressive regimens to tolerize the patient to the gene therapy in order to maximize efficacy and minimize the toxicity of the gene therapy. But the immunosuppressive agents themselves also impose toxicities, including both short-term and long-term risks. The optimal immunosuppressive regimen should result in immune tolerance and a sustained and therapeutic level of gene expression, while imposing minimal number of adverse effects. Objectives: The objectives of this study are to provide a review of specific immunosuppressive regimens that have been used in gene therapy clinical trials, describe their efficacy and toxicity, as well as provide an overview of immunosuppressive agents. Mechanisms of action, specific immune system targets, and toxicities are described. Although targeted immunosuppressive approaches continue to be studied, successful immune tolerance to in-vivo gene therapy, to date, has been achieved using agents with multiple immune system targets. Immune responses to gene therapy create limitations on the number of gene therapy doses a patient may expect to receive, with the current understanding that the patient will likely be able to receive only one dose per lifetime. The efficacy of the single gene therapy dose is of paramount importance. A broader spectrum immune suppression may accommodate the present incomplete understanding of immune responses to gene therapy, and the consideration that patients will likely not be eligible for a second dose if the first dose is sub-therapeutic.
Abstract:

Introduction: We investigated the associations of hippocampus and amygdala volumes with cognitive and behavioral/psychological aspects in 6 Hurler-Scheie patients with the L238Q mutation and compared to 6 Hurler-Scheie patients without the L238Q mutation, matched in age range (14-25 years) from a cross-sectional study (NIH-U54NS065768). We hypothesized hippocampus volumes would be related to cognition, amygdala volumes to behavioral outcomes and there would be differences in hippocampus and amygdala volumes between the L238Q and control or non-L238Q group.

Methods: Among the 6 patients with an L238Q mutation, 4 were paired with nonsense, 1 with a deletion, and 1 with a splice site mutation. For the 6 in the non-L238Q group, 3 patients had a missense paired with nonsense, 2 with missense paired with missense, and 1 with nonsense and splice site mutations. T1-weighted images from 3T brain MRI were used for the volumetric analysis of amygdala and hippocampus by manual tracing using BRAINS2. Cognitive function was measured by WASI (Wechsler Abbreviated Scale of Intelligence) for IQ and BVMT (Brief Visual Spatial Memory Test) to assess nonverbal memory and learning. Behavioral outcomes were measured by the BASC-PRS and BASC-SRP (Behavior Assessment System for Children-Parent Rating Scale and Self-Report).

Results and conclusion: The total volume of hippocampus was positively related with BVMT (P=<0.001) but not statistically significantly associated with IQ in the L238Q group (P=0.150) when comparing the slopes between the 2 groups. The association between total amygdala volume and BASC scores were not significantly different between the two groups, though PRS anxiety and SRP depression had large estimated differences. We have found larger hippocampus volumes were associated with higher BVMT scores for the L238Q but the opposite for non-L238Q group. Future analysis of longitudinal data will provide more detailed information.
GM1 gangliosidosis (GM1) is a lysosomal disease caused by mutations in the GLB1 gene, which encodes the lysosomal hydrolase β-galactosidase (β-gal; EC 3.2.1.23). Insufficient β-gal catalytic activity results in the accumulation of the gangliosides GM1 and GA1 within the nervous system, resulting in progressive neurodegeneration and death. Treatments for this debilitating disease are not currently available, thus the development and testing of novel therapies in a model organism is of grave importance. To generate a mouse model of GM1, CRISPR/Cas9 genome editing was used to target exon 8 of the Glb1 gene. Of the 106 zygotes that were injected, one animal harbored a 20bp frameshift deletion that encompassed the predicted catalytic residue of β-gal. Subsequent analysis of enzyme activity in β-gal deficient animals (β-gal/-/-) showed that this mutation resulted in nearly a complete loss of β-gal enzyme activity (0-1% of wildtype levels, and 0-2% of heterozygous). Over the course of 6 months, animals were monitored for weight and behavioral changes, at which time they were tested with a battery of neurocognitive and motor function tests to elucidate the severity and phenotype of the disease. By 6 months of age, animals displayed features of a neurological disease, such as ataxia, bodily tremors, and abnormal gait. Neurocognitive testing using the Barnes maze and spontaneous alternation in the T-maze showed that β-gal(-/-) mice had significant spatial reference memory and spatial working memory impairments. To test motor function, animals were subjected to four tests: the balance beam, pole, inverted screen, and rotarod; which showed that β-gal mice have significant motor impairments. Overall, the results of this study show that the β-gal(-/-) mice harboring the 20bp deletion recapitulate many phenotypes of human GM1, making it a compelling model for potential future gene therapy studies.
Abstract: Introduction. The long-standing clinical observations and diagnostic features of ‘cold hands/feet’ associated with Rett syndrome have not received sustained scientific scrutiny. There are well known autonomic regulatory issues associated with Rett, but no work has directly investigated whether there is a peripheral basis underlying the observations. To begin to investigate, we have performed epidermal punch biopsies with girls affected by Rett.

Methods. Three adolescent girls with clinically confirmed Rett syndrome (ages 12, 13, 15) participated. Single punch epidermal biopsies were obtained from the postero-medial calf to compare directly with normative samples from approximate age-matched, body-site matched control children without developmental disability. Epidermal nerve fibers were traced, from confocal images, using Neurolucida software (MicroBrightField, Colchester, VT.) according to established counting criteria and reported as number of epidermal nerve fibers per mm in a 50 um thick section.

Results. We observed merkel cells that were quite large; abnormal/atypical vascular innervation, elongated mast cells, densely innervated hair follicles, and abnormal/atypical looking epidermal nerve fiber (ENF) branching. To begin to quantify, we derived ENF density value estimates were 12.2 ENFs/mm, 39.3 and 53.2 for the three girls, which cf. 8 female controls (age 12-17), the average ENFs/mm was 27.3 (SD = 9.7; range = 15.3 - 41.1) in an adolescent sample without developmental disability.

Discussion. All observations were preliminary and were not uniform across all three girls. The apparent differences in the degree of peripheral innervation in general and the atypical patterns specific to arterioles warrants further investigation and are consistent with a recent report regarding microvascular abnormalities in Rett.