Southern California Rare Disease Genetics Meeting – March 8,2019

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UCI University of California, Irvine

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NOVEL MOLECULAR GENOTYPE IN THE *POLR3B* GENE, CONSISTENT WITH POLR3 RELATED LEUKODYSTROPHY – A CASE REPORT

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Here we report a case of a 13-year-old boy with global delays, tremors, progressive ataxia and brain MRI findings of leukodystrophy. Whole exome sequencing noted him to have a likely pathogenic variant (c.2303G>A p.Arg786) and a variant of uncertain significance (c.1135A>G p.Lys379Glu) in the *POLR3B* gene. The latter variant of uncertain significance has not been reported in the literature in association with the disease phenotype. The phase of these genetic variants is being determined. In this abstract we describe our patient's phenotype in detail and compare it to patients who have been diagnosed and reported in the literature with having POLR-3 related leukodystrophy. We argue that our patient's clinical presentation is consistent with a diagnosis of autosomal recessive POLR-3 related leukodystrophy and the variant of uncertain significance (c.1135A>G p.Lys379Glu) is likely a causative /pathogenic variant based on clinical interpretation. Additional case reports and functional studies of the variant of uncertain significance will help classify its pathogenicity.

The Rare and Precious: How Rare Diseases Are Revolutionizing Medicine

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"There are varying statistical definitions of rare diseases that may result in a sense of low relevance to everyday's medicine. While this erroneous perception may have been excused in the past, our view of these diseases has been fundamentally changed thanks to technological advances in genomics. These diseases are so common in aggregate that around 5% of the general population has molecular evidence of being affected by dominant forms, and the nearly ubiquitous carrier state for the recessive forms is the basis for the growing number of expanded carrier screening programs. The contribution of these diseases to our basic understanding of the structure and function of the human body at the molecular level cannot be overstated. In fact, these diseases are informing the rapidly evolving field of "druggable genome", and the entry of several new drugs to the market for the treatment of common diseases as a result of this approach only signals a beginning of an exciting era.

Pompe Disease

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Pompe Disease is a rare autosomal recessive disorder that occurs with the deficiency of enzyme α-glucosidase (GAA), located on the chromosome 17q25.2-q25.3, categorized under a lysosomal storage disorder. The disease results in the accumulation of glycogen, a polysaccharide of glucose, in the smooth, cardiac, and skeletal muscles . Late Onset Pompe is further characterized by skeletal muscle dysfunction, proximal muscle weakness, and early

respiratory insufficiency. Based on this, many cases of patients have exhibited decreased bone density as symptoms of Pompe Disease and in asymptomatic vertebral fractures. Our cohort included 15 Pompe patients ranging from 21- 74 years on routine ERT (enzyme replacement therapy) treatment, which administered Lumizyme or algucosidase alfa to supplement or replace α-glucosidase (GAA), the enzyme absent or reduced in Pompe Disease. A previous study on 8 patients indicated that reduced bone mineral density or osteopenia was linked to metabolic imbalance and bone homeostasis for Pompe Disease. We tested the progressive impact of ERT treatment on a varied cohort of Pompe patients with DXA bone density studies to assess osteopenia or osteoporosis. Bone mineral density tests such as DXA scans measure the amount of calcium, minerals, & porosity in the bone examined through z and t-score analysis. Our data results demonstrated that one year increase from ERT treatment lead to 0.152 unit score significant increase in z-scores with p-value=0.0128 in the lumbar z-score. In addition to lumbar, femur z-score showed 0.079 unit score increase from one year of treatment with p-value=0.1002. For females specifically, there was 2.172 units lower average z-score compared to males in lumbar z-score and 0.563 units lower average z-score compared to males in femur z-score. Increase in age also changed the dynamics for Pompe disease as one-year increase in age led to 0.086 unit score significant increase in lumbar z-scores with p-value=0.0133 and 0.042 unit score significant increase in femur z-scores with p-value=0.0429. The results, overall, denoted that ERT treatment shows a positive/effective correlation with increasing z-scores, hence patients are at a lower risk of osteoporosis/osteopenia. ERT treatment increases the overall lumbar z-score value therefore the patient is at a lower risk of osteoporotic development with time. The majority of females are, although, at a higher prompted risk of developing osteoporosis compared to males. This study can further be useful for genotype-phenotype correlation analysis and to study the importance of early management to prevent osteopenia and bone fractures.

CYLD Variants in Brooke-Spiegler Syndrome, a Tumor Predisposition Condition

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Purpose:

Brooke-Spiegler Syndrome (BSS) or *CYLD* cutaneous syndrome is characterized by skin appendage tumors (cylindromas, trichoepitheliomas, and spiradenomas) that form typically in the head/neck region. Tumors can be disfiguring, with some individuals requiring complete surgical removal of the scalp. There is a risk for malignant transformation in 5-10% of patients. There are rare reports of internal tumors as well. This condition is not included in the ACMG referral indications for cancer predisposition assessment guidelines. Here we describe a case of BSS with a novel mutation and a review of all reported BSS cases. We analyzed all pathogenic variants reported in the literature to date in *CYLD*, and suspected impact of variants on protein function.

Methods:

We performed a review of all published *CYLD* mutations and molecular results. We mapped reported variants in relation to known functional domains, and compared against locations of benign variants found in gnomAD. For our new case we performed capture-based NGS on 510 cancer-related genes, total sequence footprint of 2.8 Mb from blood/excised tumor.

Results:

We examined the type and distribution of 115 different pathogenic variants in the literature. These suggest LoF as a mechanism as 50% are frameshift, 21% are nonsense, 14% are splice site variants, and almost 3% are large

deletions. Missense variants, which account for 13% of the total, are limited to the ubiquitin c-terminal hydrolase (UCH) functional domain. We identified a novel frameshift variant in a Hispanic woman, *CYLD* c.1759dupA p.M587fs (in 49% of 874 reads). Tumor cells showed 95% mutation, implicating tumor LOH. Interestingly, of the 500 additional cancer genes tested, no significant variants were detected in tumor.

Conclusions:

We found, out of molecularly defined cases with ethnicity noted, only about 1% were Hispanic. We report a novel *CYLD* variant that may not have previously been described due to under-ascertainment of Hispanic individuals. The restriction of pathogenic missense variants to the UCH domain highlights its importance in tumor suppression pathways. The UCH domain is not devoid of missense variation in the general population however the enrichment of pathogenic variants in the UCH domain is highly significant. Indeed, the CYLD gene product has been characterized as a deubiquitinating enzyme and loss of the deubiquitinating activity correlates with tumorigenesis. Future studies could elucidate the effect of missense variants on protein folding and function. While LOH is an important mechanism of tumorigenesis in BSS (in 48%), it remains unclear what are the other drivers of tumor formation, in particular in malignant transformation. A recent clinical trial with a topical kinase inhibitor for BSS showed muted promise. Clinical management should include monitoring for transformation.

B-cell lymphoma, non-hodgkin's lymphoma, and myelodysplastic syndrome in gaucher disease

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Gaucher Disease (GD) Type I is the most common lysosomal disorder and affects nearly 1 in 40,000 live births. It is the most frequent genetic disorder within the Ashkenazi Jewish population and individuals display a wide range of phenotypes from childhood-onset to asymptomatic adults. Several studies have reported that Gaucher patients are at a higher risk for multiple myeloma and hematological malignancies relative to the general population. Despite the reported risk increase in these cancers, the causation is unknown. Several interesting theories have implicated sphingolipids, gene modifiers, and chronically stimulated B-cells.

Here, we present two patients with GD type I and lymphoma from our cohort of 4 patients. We describe a 66 year-old Ashkenazi Jewish male with GD Type 1 who started Cerezyme at age 57 years and developed large B-cell lymphoma three times; once at 58 years and twice at 62 years. He also developed myelodysplastic syndrome at 62 years and both diseases were subsequently treated with bone marrow transplant (BMT). His Gaucher disease improved after the BMT, however he succumbed to graft versus host reaction. We also report a 38 year-old Ashkenazi Jewish male with GD Type I who was diagnosed with non-Hodgkin lymphoma (NHL) at age 22 years which was treated with chemotherapy and radiation therapy. His Cerezyme was initiated at 25 years and the patient was switched to Cerdelga at 35 years. He reports a sharp increase in energy since this change. Additionally, we performed a meta-analysis of the reports in the literature of the association of B-cell lymphoma and NHL with GD Type 1.

We conclude that the high incidence of lymphoma in our Gaucher patients (50%) and the meta-analysis of current literature highlights the need for more research focused on defining the relationship of Gaucher disease with B-cell lymphoma and NHL.

Single Cell RNA Sequencing of Gastrulating Mouse Embryos to Understand Developmental Origins of Heart Defects

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Congenital heart defects (CHD) occur commonly in the general population and at high frequency (~30%) in individuals with the most common form of Cornelia de Lange Syndrome (CdLS), haploinsufficiency for *NIPBL*. Our recent findings suggest that CHDs may arise from reduced numbers of cardiac progenitor cells of the second heart field (SHF) in *Nipbl+/-* mice (Santos et al., 2016). To investigate this, we performed single-cell RNA sequencing (10X Genomics Chromium; Illumina HiSeq) on *Nipbl+/-* and wildtype littermate embryos at embryonic day 7.5: the stage when cardiogenic progenitors are emerging from the primitive streak. We identified 18 presumptive cell types using Seurat, including progenitors of first heart field (FHF; *Mesp1-high)* and SHF (*Mesp1-low, Mef2c*, *Isl1*), as well as more differentiated cardiac progenitors (*cTNT*, *Nkx2.5*). Observed differences in the proportions of progenitors suggested that *Nipbl +/-* embryos are either delayed or deficient in the generation of SHF, as compared with FHF, progenitors. To test this idea, we used SoptSC (a recent single-cell analysis tool) to identify a trajectory of wildtype cells from late epiblast to definitive cardiac progenitor. We are currently studying how the early cell-state transitions in the wildtype trajectory are disrupted by *Nipbl* haploinsufficiency, in order to define its effects on cardiac lineage specification of cells emerging from the primitive streak.

A Mouse Model of X-linked Intellectual Disability Protein PHF6 Associated with Impaired Neuronal Maturation

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Mutations in PHF6 are implicated Börjeson-Forssman-Lehmann syndrome (BFLS), an X-linked intellectual disability (XLID) characterized by mental impairment, developmental delay and seizures. However, the mechanisms by which mutations of PHF6 contribute to the pathogenesis of BFLS remain poorly understood. Here, we report a novel patient-specific PHF6 mutation mouse of BFLS. We generated a patient specific mutation C99F knock-in mice using CRISPR/Cas9 system. We have found that PHF6 C99F knock-in mice display deficits in cognitive, adaptive behaviors, and reduced threshold to seizures. Electrophysiological studies reveal that the intrinsic excitability of entorhinal cortical stellate neurons is increased, providing a mechanistic basis for susceptibility of BFLS mice to seizures. Transcriptomic analysis of the cerebral cortex in C99F knock-in mice and PHF6 knockout mice show that PHF6 promotes the expression of neurogenesis genes and comcomitantly suppresses the expression of synaptic genes, suggesting PHF6 maintains neurons in an immature state. Remarkably, PHF6-regulated genes overlap with autism spectrum genes in developmental modules related to synaptic function. The integrative analysis in our mouse model provides molecular mechanism of BFLS pathogenesis and offers new links between BFLS and autism spectrum disorders.

Regulatory T cells suppress novel skeletal muscle macrophages identified by single-cell RNA sequencing

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Duchenne muscular dystrophy (DMD) is a lethal X-linked disorder that results from mutations in the dystrophin gene causing necrosis and muscle inflammation. In mouse models of DMD, muscle macrophages are major effector immune cells playing a dual role by promoting injury through chronic activation of M1-like macrophages and facilitating muscle regeneration by inducing M2-like macrophages. Despite the recognition that the M1:M2 macrophage balance regulates the severity and progression of muscular dystrophy, muscle macrophages heterogeneity has been poorly characterized. To rigorously assess the phenotypic and functional characteristics of dystrophic muscle macrophages, we have applied single-cell RNA sequencing (scRNAseq) as an unbiased profiling strategy to interrogate and classify muscle macrophage heterogeneity at the single-cell level in DMD. Herein, we identified previously unrecognized macrophage populations and their gene expression signature, suggesting specialized functions and particularly a novel population of $CD52^{+}$ muscle macrophages that express high levels of the pro-inflammatory IL-1. Furthermore, work from our laboratory and others recently revealed that Regulatory T cells (Tregs), which were elevated in muscle of DMD patients and mdx mice, modulate macrophage activation and suppress the severity of muscular dystrophy. Given the immunosuppressive nature of Tregs and their ability to suppress pro-inflammatory responses, depletion of Tregs exacerbated inflammation in mdx mice, exhibiting large expansion of the CD52+ population together with an increase of its transcriptional signature. Understanding how Tregs suppress muscle inflammation will reveal pathways that may be targeted for novel and safer anti-inflammatory therapy for muscular dystrophies.

Intralesional injection of collagenase Clostridium histolyticum for the management of Peyronie's disease with indentation or hourglass deformity

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Introduction: Intralesional injection (ILI) of collagenase Clostridium histolyticum (CCH) is the only Food and Drug Administration approved medical treatment for the management of stable phase Peyronie's disease (PD). The prevalence of diagnosed PD in the United States has been estimated to be 0.5% in adult males. PD, which is rare condition with genetic factors, have no published reports specifically examining outcomes of treatment in patients with PD and significant indentation or hourglass deformity. We sought to assess outcomes in patients receiving ILI CCH for these specific deformities.

Methods: We conducted a retrospective review of 13 patients with PD and significant indentation or hourglass deformity who began ILI CCH between November 2016 and June 2018. A penile duplex Doppler ultrasound (PDDU) was performed prior to initiation of therapy in all patients. Degree of curvature was measured at the time of PDDU, after the second cycle, and 4-6 weeks after completion of the fourth cycle. Patients were called after completing treatment and asked whether or not they were satisfied with their injections and if they would pursue this treatment again. Patients answered the symptom bother domain of the Peyronie's Disease Questionnaire (PDQ) and were scored on a scale of 0-16 before, during and after treatment.

Results: Mean age was 52.9 ± 12.6 years. Nine patients (69.2%) had significant indentation and four patients (30.8%) had an hourglass deformity. Mean pre-operative curvature was 48.1^{o} ± 15.9. Mean number of injections

was 7.2 ± 2.9, median 6 (range 2-12). Overall, 7.7% completed 2 injections, 7.7% 4 injections, 7.7% 5 injections, 30.8% 6 injections, 23.1% 8 injections, 7.7% 10 injections, and 15.4% 12 injections. Mean change in curvature was -11.2° ± 9.4 (20.4% ± 14.2) and 53.8% of patients had significant ≥20% improvement in curvature. Mean change in PDQ Bother score was -6.80 ± 2.86. Overall, 55.5% reported subjective resolution of their indentation and 75.0% reported significant improvement in hourglass deformity. Responders were satisfied with treatment (100%) and said they would pursue this treatment again (80%). One patient successfully completed 8 injections and reported resolution of indentation, but ultimately sustained a penile fracture which required surgical repair. No other complications were reported in the remaining patients.

Conclusions: Patients with PD and significant indentation or hourglass deformity demonstrate favorable improvements in penile curvature and bother. Resolution or improvement of indentation and/or hourglass deformity can be attained in certain patients. ILI CCH indications could be expanded to include patients with these deformities.

Treatment Related Outcomes for Patients with Atypical Peyronie's Disease

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Introduction and Objective: Peyronie's disease (PD) is an acquired condition with genetic factors typically characterized by plaque formation and dorsal/lateral penile curvature. Atypical PD is a term used to describe less common PD presentations such as ventral curvature, multiplanar curvature, penile indentation and hourglass deformity. The prevalence of diagnosed PD in the United States has been estimated to be 0.5% in adult males. We sought to assess the outcomes of patients with atypical PD presenting to a men's health clinic.

Methods: We conducted a retrospective review of charts of men who presented to the clinic with atypical PD between October 2016 and October 2018. Patients were included if they were considered to be in the stable phase of the disease and had completed a penile duplex Doppler ultrasound (PDDU) prior to any intervention. Gathered data included patient demographics and treatment details, outcomes and complications. Offered treatments included intralesional injection of Collagenase Clostridium Histolyticum (CCH), tunical plication (TP), partial excision and grafting (PEG) and insertion of inflatable penile prosthesis (IPP).

Results: During this period, 74 men with stable PD underwent PDDU, of which 42 (56.8%) were found to have atypical PD. Of these, 32 (76.2%) had penile indentation, 14 (33.3%) had a multiplanar curvature, 10 (23.8%) had a ventral curvature, and 10 (23.8%) had an hourglass deformity. Overall, 31/42 (73.8%) patients opted for treatment: 22 (70.9%) CCH injections, 4 (12.9%) TP, 4 (12.9%) IPP, and 1 (3.3%) PEG. The remaining 11 patients did not pursue treatment, mostly due to insurance coverage issues. Patients with ventral curvature underwent CCH (62.5%), PEG, TP and IPP (12.5% each); multiplanar curvature underwent CCH (77.8%) and TP (22.2%); penile indentation CCH (72.0%), TP, IPP (12.0% each) and PEG (4%); hourglass deformity CCH (77.8%) and PEG (22.2%). Patients who underwent surgery had a mean improvement in curvature of 45.0 ± 10.8 degrees (85.5 ± 17.5%); 100% with significant (≥ 20%) change in curvature. Patients who underwent CCH injections had a mean improvement in curvature of 13.5 ± 5.7 degrees (33.1 ± 10.4%); 90% with ≥ 20% change in curvature. The mean number of CCH injections used was 6.4 ± 3. There was 1 penile fracture in the CCH group which required surgical repair. There were no complications in the surgery groups.

Conclusions: Patients with atypical PD represent a significant proportion of men with PD. Surgery represents an excellent option in this group. Outcomes with CCH in patients with atypical PD are comparable to those reported in the IMPRESS trials.

Variable Clinical Features of Fabry Disease in Patients with Novel Mutations

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Fabry disease (FD) is an X-linked disorder of glycosphingolipid metabolism, caused by a deficiency or absent lysosomal enzyme activity alpha-galactosidase A due to mutations in the α-Galactosidase A (GLA) gene. Affected hemizygous males with little or absent GLA activity may present the classic form of FD with progressive renal disease, cardiomyopathy, neuropathy, gastrointestinal complaints, acroparesthesia, angiokeratomas, hypohidrosis, corneal verticillata, tinnitus, and hearing loss in early childhood. Heterozygous females typically present with milder symptoms and later onset and rarely have classic FD. We report the clinical and molecular findings in 27 patients with FD from our multidisciplinary clinic study at University of California, Irvine. Our cohort includes 25 adults and two children (13 M and 14 F) from 16 families, ranging in age from 6-66 years, with ages of diagnosis ranging from 3-59 years.

We describe 2 novel mutations not previously reported in the literature in patients with classic FD. The first novel mutation c.1041_1042insG was found in a 34 y old male with angiokeratomas, corneal whorling and significant neuropathy but no major organ involvement. The novel mutation c.1226_1231delCCACAG, p.Pro409_Gly411delinsArg was seen in a 62 y. old male with involvement of his kidneys, lungs and heart and a history of two strokes.
We report clinical presentation and progression in our patients including renal involvement in 37% of patients, the majority presented with varying levels of proteinuria 15% of whom had end-stage renal disease, one required a kidney transplant. 74% experienced acroparasthesia, 52% have angiokeratomas, 63% exhibit corneal whorls, 19% percent of patients had left ventricular hypertrophy/ cardiomyopathy. Additionally, 50% of patients experienced gastrointestinal problems, 59% had hypohidrosis, and 26% had lymphedema. Among the adults, 48% had tinnitus and 33% of adults experienced hearing loss. One male had several strokes and MRI changes of multifocal encephalomalacia and gliosis of the left cerebral frontal and parietal lobes.

Amongst the 14 females, 71% had corneal whorls, often the first index of Fabry in the family, 42% had angiokeratomas, and 50% had GI involvement. One female patient (7%) has severe cardiac and neurologic involvement.

Enzyme replacement therapy with recombinant human α-galactosidase A available since 2001 was administered at a dose of 1.0 mg/kg every other week. The participants in this cohort completed 6 monthly evaluations monitoring the effects of ERT. 89% (8/9) males and 33.3% (3/10) of the females receive enzyme replacement therapy (ERT). One male has discontinued ERT because of severe Fabrazyme reactions including meningitis and anaphylaxis. He is currently participating in the lucerastat trial. Only two families in our cohort have a mutation that is amenable to migalastat. Two children received ERT treatment before age of 5 y. with improvement in symptoms and GL3 levels. Each individual demonstrated different response rates of symptom improvement and slowing of organ deterioration with ERT.

We present the phenotypic diversity in patients with Fabry disease, the long-term effects of ERT, and highlight patients with unusual phenotypes and novel mutations not previously reported.

Wanted! Pathogens that lead to life-threatening hyperammonemia

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Background: Hyperammonemia is a potentially life-threatening emergency that can be due to liver disease, portosystemic shunt, inborn errors of metabolism, drug toxicity and other causes. In addition, the term "idiopathic hyperammonemia" refers to hyperammonemia of unknown cause in the setting of malignancy or immunosuppression. Infection is another, lesser recognized cause of hyperammonemia. We present two cases of infection that caused or contributed to severe hyperammonemia.

Case 1: A 16-year-old, immunocompromised female with acute myelogenous leukemia developed acute encephalopathy and persistent hyperammonemia up to 705 μmol/L (normal 0-47 μmol/L) refractory to multiple therapies including hemodialysis. After learning about a similar, unpublished case due to *Ureaplasma* infection, empiric therapy was started with azithromycin, followed by doxycycline. The patient showed immediate and sustained neurologic improvement and normalization of ammonia levels. Blood PCR was positive for *Ureaplasma parvum*. Metabolic workup including plasma amino acids, plasma acylcarnitine profile, urine orotic acid, urine organic acids and hyperammonemia gene panel did not suggest an underlying metabolic disorder.

Case 2: A 12-year-old male with ornithine transcarbamylase deficiency and poor metabolic control was admitted for hyperammonemia up to 431 μmol/L without a clear trigger. In addition, he had unexplained weight loss, alternating diarrhea and constipation, and previous history of small intestinal bacterial overgrowth (SIBO). Ammonia normalized on high-dextrose IV fluids, intralipid, IV ammonia scavengers and arginine drip and remained normal with IV fluid wean and advancement of enteral feeds. Upon transition to home ammonia scavengers of alternating enteral sodium benzoate and glycerol phenylbutyrate, which has a triglyceride molecular structure, hyperammonemia recurred and correlated with vomiting and altered mental status. SIBO was suspected and suggested by increased fecal fat and plasma folate. Treatment with rifaximin resulted in rapid weight gain, normalization of stool studies and improved metabolic control. Sodium phenylbutyrate was substituted for glycerol phenylbutyrate until fat malabsorption resolved.

Conclusions: Disseminated infection with *Ureaplasma* species or other urease-producing pathogens should be considered as a potential cause of idiopathic hyperammonemia, an unexplained, often fatal condition in immunocompromised patients. In patients with urea cycle disorders, small intestinal bacterial overgrowth is a likely underrecognized example of complex gene-environment interactions that can contribute to poor metabolic control. These two cases illustrate the importance of including infection in the differential diagnosis for acute hyperammonemia. Treatment potentially could be life-saving.

Characterization of copy number variants (CNVs) identified by genetic testing of inherited retinal disorders

Lucia Guidugli
Blueprint Genetics

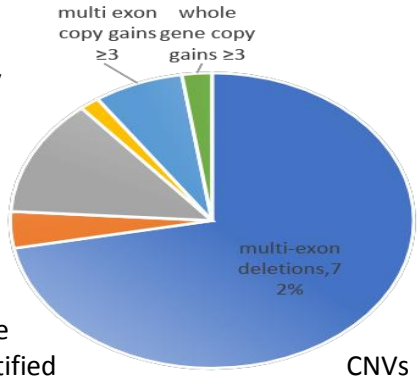
Purpose: Retinal dystrophies include a heterogeneous group of disorders that damage the photoreceptors in the retina and cause visual impairment. Prompt and comprehensive genetic diagnosis of these disorders is essential for risk assessment measures, management of symptoms and selection of the appropriate targeted treatment. In

order to provide a comprehensive diagnosis, the genetic testing strategy needs to take into account sequence alterations as well as copy number variants. The purpose of this study is to evaluate the rates and characteristics of copy number variants (CNVs) in a cohort of 2649 patients tested on a retinal dystrophy panel including 266 genes.

Methods: The testing approach is based on whole exome sequencing using the IDT xGEN Exome Research Panel with added custom clinical content and the Illumina NovaSeq platform.

Results: CNVs reported as primary finding matching the patient phenotype were identified in a total of 125 cases (4.6%): 95 (76%) were partial gene deletions including five cases with one exon deletion and 16 (13%) were whole gene deletion. In addition, we identified two (2%) partial gene duplication, nine (7%) partial gene copy number gains (≥3), and three (2%) whole gene copy number gains (≥3) (Figure 1).

Figure 1: percentage of different types of CNVs detected on the RD panel.



The majority of identified CNVs (113, 90.4%) were either likely pathogenic or pathogenic while 12 (9.6%) were variants of uncertain significance. Of the likely pathogenic and pathogenic CNVs 92 (73.6%) were diagnostic: 66 (71.7%) in autosomal recessive diseases, 15 (16.3%) in autosomal dominant disorders and 11 (12%) in X-linked disorders and. The *USH2A* gene was enriched in CNVs compared to other genes and all of the identified *USH2A* CNVs were classified as either likely pathogenic or pathogenic.

Conclusions: Overall, these results highlight the importance of a comprehensive genetic testing approach for the diagnosis of retinal dystrophies. We have identified CNVs ranging from one exon deletion to whole gene deletions and copy number variants in multiple genes and confirmed the relevance of *USH2A* in these disorders. In addition, we have detected a relative high percentage (9%) of high copy number gains (≥3) that warrant further investigation.

Semi-quantitative MR muscle analysis of VCP inclusion bodymyopathy

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Introduction
Inclusion body myopathy associated with Paget and dementia (IBMPFD) is a rare disease associated with mutations in the VCP gene[1]. The disease is characterized by adult onset proximal myopathy and muscle biopsy findings of vacuoles and inclusions that stain positive for TDP43, ubiquitin and autophagy markers. MRI is the pivotal imaging tool to support clinical diagnosis and monitoring progression of several inherited and acquired neuromuscular disorders, revealing anatomic details and changes in signal intensity within the muscles, however, very limited MRI data is available in VCP disease. T1-weighted images (T1WI) disclose chronic alterations including fatty infiltration and muscle volume decrease. Semi-quantitative scoring systems exist for the evaluation of fatty infiltration, MRI quantification methods have been recently applied to evaluate fatty infiltration in clinical research. However, a relationship between semi-quantitative method and MRI quantification has not been fully investigated in muscle involvement of neuromuscular diseases. Therefore, the

purpose of this study was to correlate semi-quantification of fatty infiltration with MRI quantification based on fat fraction analysis and to characterize specific patterns of chronic myopathy in VCP IBM with semi-quantitative methods.

Methods

The study protocol was approved by the Institutional Review Board, and all subjects gave written informed consent. We prospectively evaluated 12 patients with varying degrees of myopathy (7 women and 5 men; mean age, 50.3 years; range, 28-64) and 8 healthy volunteers (5 women and 8 men; mean age, 43.1 years; range, 27-65). All MR studies were performed on a 3T scanner (Achieva, Philips Healthcare, Netherlands) using a body coil. Axial images of the thighs were acquired including T1WI. The acquisition parameters were as follows: 2D turbo spin-echo; repetition time (TR)/echo time (TE) = 886/15ms, number of excitation (NEX) = 1, and total acquisition time = 5 minutes, field of view (FOV) = 380mm, slice thickness/gap = 5/5mm, image matrix = 512×512, and number of slices = 20-25. The thigh muscles were assessed for fatty infiltration in the middle section, where following muscles were all visible: the rectus femoris, quadriceps except for rectus femoris, sartorius, gracilis, semimembranosus, semitendinosus, biceps femoris, adductor longus, and adductor magnus.
For the semi-quantitative assessment, the degree of fatty infiltration was graded by a radiologist according to 5-point scale[6](Table 1). The presence or absence of muscle volume decrease was graded on a/b sub-scale: a, with presence of muscle volume decrease; and b, with absence of muscle volume decrease. For the MRI quantification, T1W images were first corrected for shading or intensity inhomogeneity across the FOV based on N3 algorithm and then segmented for different tissue types based on a 3-class fuzzy c-means (FCM) algorithm using MIPAV software (http://mipav.cit.nih.gov). Also, manual muscle segmentation of the bilateral thigh muscles was performed by a radiologist using MIPAV software. Then, the fat fraction value in each muscle was calculated. The Pearson correlation coefficient was used to correlate between fat fraction value and semi-quantitative scale. Kruskal-Wallis rank sum test and the Steel-Dwass post-hoc test were used to compare the relationship between fat fraction value and the semi-quantitative scale.

Results

The semi-quantification provided equivalent accuracy as quantification. There was an association between fat fraction value and 5-point semi-quantitative scale. The fat fraction value showed a stepwise increase in higher point semi-quantitative scales ($p < 0.001$). Fat fraction values showed a stepwise increase in higher point semi-quantitative scales ($p < 0,1$). The sartorius and adductor magnus were most affected by fatty infiltration, while the adductor longus and rectus femoris were relatively well-preserved. The semitendinosus was most subject to decrease in muscle volume, that was frequently seen in the hamstring muscles (semimembranosus, semitendinosus, biceps femoris) and the adductor muscles (adductor longus, adductor magnus).

Conclusion

This study demonstrated the usefulness of semi-quantitative MR muscle analysis of VCP associated inclusion body myopathy associated with Paget and dementia (IBMPFD) which provided equivalent accuracy as quantification. The sartorius and adductor magnus were most affected by fatty infiltration, while the adductor longus and rectus femoris were well-preserved. Muscle volume decrease was more frequently seen in the hamstring and adductor muscles. This semi-quantitative method can be widely available in clinical settings and assist noninvasive initial/follow-up IBMPFD diagnosis.

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Normative QCT Data for Evaluating Bone Health in Patients Less Than 5 Years Old

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Evaluating bone health in pediatric patients less than 5 years old (<5yo) would allow early diagnosis and treatment of bone disorders. In adults, bone health is evaluated by comparing a patient's quantitative CT scan (QCT) bone mineral density (BMD, mg/cm^3) with normative QCT BMD data. However, studies of bone health in pediatric subjects <5yo are limited to 2-D dual-energy x-ray absorptiometry, which suffers from the confounding effect of bone thickness [1,2]. The goal of this study was to develop a normative QCT database for subjects <5yo. CT scans of UCIMC Emergency Department patients <5yo were retrospectively obtained with IRB approval and waiver of informed consent. All scans included a calibration phantom with concentrations of 0, 50, 100, 200 mg/cc calcium hydroxyapatite (INTable^{TM}, Image Analysis Inc., Columbia, KY, USA) and were analyzed with in-house software. BMD, bone mineral content (BMC, mg) and volume (Vol, cm^3) of the L1 vertebral body were evaluated in a region of interest (ROI) consisting of the portion of the vertebral body that falls anterior to a plane tangent to the anterior of the spinal canal. No distinction was made between cortical and trabecular bone. This ROI ensured that the same ROI was evaluated regardless of anatomical changes during vertebral maturation. Medical records were reviewed to rule out L1 fracture and to obtain sex, age (y), race/ethnicity, weight (kg), height/length (cm) and BMI (kg/cm^2). Backwards stepwise regression analysis was used to identify QCT predictors. We obtained 55 QCT scans with contrast of 42 male and 13 female subjects (Table 1; 29 Caucasian, 16 Hispanic, 2 Asian/Pacific, 1 African American, 6 Other, and 1 Unavailable). BMD was not associated with sex ($p=0.93$) or with age, weight, height or BMI ($p\geq0.68$). Age and weight were independent predictors of BMC (age, $p=0.014$; weight, $p=0.002$, $R^2=0.59$) and Vol (age, $p=0.004$; weight, $p<0.001$, $R^2=0.68$). Sex, height and BMI were not associated with BMC ($p>0.25$) or Vol ($p>0.14$). In this small study we have begun the process of developing a normative QCT database. Our data do not represent a random sample of the population and are disproportionately male, so our results should be interpreted cautiously. In this cross-sectional study of subjects <5yo, BMD was not correlated with age and was generally between that of the elderly (mean trabecular BMD=90 mg/cm^3) [3] and young adults (mean=300 mg/cm^3) [4]. CT scans of patients with known bone disorders will be needed to determine if BMD in patients <5yo with bone disorders fall outside the normative range. In contrast to BMD, age and weight are predictors of BMC and Vol, so linear regression can be used to adjust for age and weight when evaluating BMC and Vol. Additional work is needed to create a large normative database comprised of a broad demographic sample. Once created, the database will provide an objective tool to help distinguish patients <5yo who have healthy bone from those with bone disorders that predispose them to fracture.

Table 1. Subject descriptors. Only height was normally distributed.

Variable	N	Mean	SD	Min	Median	Max
Age (y)	55	3.15	1.17	0.61	3.07	4.87
Weight (kg)	55	16.2	4.5	9.7	15.2	33.2
Height/Length (cm)	54	97.6	12.5	68	98	122
BMI (kg/cm^2)	54	16.9	2.7	10.1	16.8	26.6
BMD (mg/cm^3)	55	243	28	190	248	329
BMC (mg)	55	1310	506	553	1210	2830
Volume (cm^3)	55	5.39	1.94	2.59	4.93	10.8

REFERENCES: [1] Gilsanz et al, J Clin Endocrinol Metab 1997; [2] Kalkwarf et al, J Clin Densitom 2014; [3] Lang et al, Bone 2002; [4] Gilsanz et al, Radiology 2009.

Epithelium mediated mechanisms for soft tissue syndactyly in Van der Woude syndrome

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Syndactyly, the fusion of digits or toes, is the most common congenital limb malformation. It can be inherited as an isolated abnormality or as part of complex syndromes, including Van der Woude syndrome where it is associated with cleft lip and/or palate. While Van der Woude syndrome is most commonly caused by mutations in the transcription factor IRF6, a subset of cases is caused by dominant negative mutations in the epithelial transcription factor Grainyhead like-3 (GRHL3). Interestingly, mice deleted for *Grhl3* exhibit soft tissue syndactyly, similar to that in Van der Woude patients. Previous studies in multiple different genetic mutants have found that syndactyly is caused by impaired interdigital cell death of mesenchymal cells between the forming digits. Surprisingly, mice deleted for *Grhl3* had normal mesenchymal interdigital cell death, suggesting alternative mechanisms for abnormal digit separation in Van der Woude syndrome. Using 3D reconstructions of multiple tissue sections from embryonic mouse forelimbs, we found that during digit separation, the overlying epidermis forms an interdigital epithelial tongue (IET) at the leading edge as the epithelium moves in between the separating digits. The anti-adhesive property of the surface periderm cells allows the IET to bifurcate as the digits separate. In *Grhl3-/-* mice the IET moves normally between the digits but fails to bifurcated because of hyperadhesion of the periderm. Morphological analysis indicates that Grhl3 is required for normal polarity of periderm cells such that adhesion factors are limited to the basolateral surfaces. Our data identify a novel epidermal developmental process that is required for digit separation and explain mechanistically why patients with Van der Woude syndrome develop syndactyly.

Dysmorphology Features in Prader-Willi Syndrome is influenced by Molecular Class and Growth Hormone.

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Objective: Prader-Willi syndrome (PWS) is a complex genetic disorder associated with paternal deletions, uniparental maternal disomy (UPD) or imprinting defects (ID) of chromosome15q11-q13. We sought to compare distinctive features of PWS including neonatal hypotonia, feeding difficulties, facial dysmorphisms such as narrow bitemporal diameter, upslanting palpebral fissures, cryptorchidism, small hands and feet in these genetic subtypes in a large cohort of patients from the NIH PWS Rare Diseases Clinical Research Network (RDCRN). We also compared the effects of growth hormone (GH) on these differences.

Methods: Clinical and dysmorphic features from 355 patients (61% deletion, 36% UPD and 3% IC defect) recruited in NIH PWS RDCRN study was analyzed. Continuous variables of the clinical data were adjusted to standardized centiles from growth charts. We compared the dysmorphic features in the deletion versus UPD subtypes and compared these differences in the groups who received GH versus did not receive GH.

Results: When comparing deletion with UPD subtypes, patients with deletions were found to be heavier with a mean weight centile of 75 ± 30 versus 62 ± 35 ($p=0.002$); a smaller head circumference (HC) with median HC centile of 51 (range 0-117) versus 61 (range 2-110) ($p= 0.009$) and flat occiput (27 vs 14 %; $p=0.005$) respectively. The patients in the deletion group were also more fair-haired (40% vs 15%; $p<0.001$) than in their family members due to loss of a single *OCA2* albinism gene allele in the 15q11-q13 region ($p=0.018$), They also had a

higher incidence of abnormal dentition (42 % vs 25%; p=0.009); low anterior hairline (26% vs 15%; p=0.04); abdominal striae (29 % vs 16 %; p=0.045), nail abnormalities 26% vs 13 % (p=0.050) and interestingly a lower incidence of strabismus 39% vs 53% (p=0.03), and downward slanting of the fissures as 6% vs 13% the (p=0.015) than found in the UPD group.

Patients in both deletion and UPD groups who received GH had a heavier body weight 71 ± 31 vs 62 ±30 (p=0.012), larger head circumferences (58 ± 32 vs 52 ±29 centiles, p=0.005), and larger hand length (p=0.049). The GH treated cohort was taller 44 ± 34 vs 41 ± 32 centiles although this difference was not significant (p=0.813). Other differences also were not significant.
Overall, upslanting palpebral fissures were seen in 23%, small hands and feet in 63%, and 70% respectively; craniosynostosis in 0.8%, strabismus in 42%, abnormal dentition in 32%; hypopigmented hair in 30%, striae in 32 % and skin picking in 26%. Cryptorchidism was present in 29% and hypoplastic scrotum in 84% of males, hypoplastic clitoris and labia minora in 3% and 10% females respectively.

Conclusions: The prevalence of dysmorphic features was delineated in a large cohort of patients with PWS. There were statistically significant correlations between phenotype and molecular subtypes –patients with deletions having a higher incidence of being overweight, have smaller head circumferences and several other striking features of PWS. Interestingly the GH treated group were heavier, had larger heads and were taller, although height and other dysmorphic features were not significantly different.

Neural regeneration strategies to treat degenerative diseases of the retina

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Our group focuses on the application of neural regeneration strategies to degenerative diseases of the retina. We first isolated retinal progenitor cells (RPCs) from immature human tissue and propagated these cells in culture using serum-free conditions. With colleagues, we repeatedly showed that RPCs could be isolated from various mammalian species and used to ameliorate visual dysfunction following transplantation. Surprisingly, RPCs are tolerated as allografts within the eye, despite variability in MHC expression. That insight, along with the equally surprising discovery that unmodified RPCs possess endogenous neurotrophic activity led to initiation of a preclinical development program and the founding of a startup company (jCyte). An open label phase 1/2a trial in 28 patients with RP was completed in 2017. Results showed a high degree of tolerability for the cells, along with indications of improved visual acuity in treated versus untreated eyes and an apparent dose response. Based on these findings, a masked phase 2b trial was initiated and a total of 84 patients enrolled, with results expected in H2 of 2019. Preparations for a final clinical study are underway.

The "transcriptomopathy" concept: Cornelia de Lange Syndrome and its relationship to other rare—and not so rare—disorders

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Cornelia de Lange Syndrome (CdLS) is characterized by structural and functional abnormalities in multiple organ systems, including, with variable penetrance, limb truncations, heart defects, growth retardation, urogenital abnormalities, craniofacial dysmorphia, and cognitive, behavioral and neurological impairment. Known causal mutations for CdLS implicate a set of nuclear proteins involved in the regulation of gene expression, especially the cohesin complex and its associated factor Nipped-B-like (NIPBL). The most severe (and most common) forms of CdLS are caused by simple haploinsufficiency for *NIPBL*, which is remarkable given that compensatory autoregulation appears to maintain *NIPBL* transcripts at levels only 30-40% below normal in affected individuals. Even more remarkable is that the sole direct consequence of *NIPBL* haploinsufficiency—in both human cells and animal models—seems to be a subtle derangement of the transcriptome. Specifically, gene expression levels change for up to a few thousand genes (with different genes affected in different cell types), but the changes are rarely as much as two-fold, and usually much lower. These observations have led to a view of CdLS as being caused by collective, potentially synergistic effects of many small gene expression differences, each of which, on its own, would likely be benign. We have used animal models—including zebrafish and mouse models—to investigate this idea, and to identify some of the transcriptional perturbations that underlie heart and limb defects. At the same time, we've begun to question whether similar etiologies underlie other disorders. A review of the literature suggests that over 100 OMIM-indexed genetic disorders should be classified with CdLS as "transcriptomopathies", as they are caused by mutations in genes that, like *NIPBL*, play roles in "general" (i.e. not tissue- or function-specific) transcriptional regulation. That many such genes belong to families with additional members suggests that full range of transcriptomopathies may be even broader. Interestingly, phenotypic similarities shared among transcriptomopathies suggest that certain developmental events are uniquely sensitive to small changes in gene expression. Understanding such sensitivity could shed light not only on these (mostly) rare disorders, but also on how genetic heterogeneity in the general population (which is now thought to manifest primarily in gene expression level differences) contributes to common, non-syndromic developmental abnormalities.

Moving Treatment Forward for children with rare neuromuscular disease

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Neuromuscular diseases have had a long history of treatment failure. Genetic diseases like Duchenne muscular dystrophy (DMD) and Spinal muscular atrophy (SMA) have been notably refractory to intervention with a long-history of failure. For DMD we have had only a single treatment since the late 80's in the form of glucocorticoids. This class of drugs can clearly change the natural history promoting prolonged ambulation but the course is inevitably downward leading to dependency on wheelchairs, and respiratory and cardiac compromise. We have tried to move the field forward with replacement gene delivery initially reaching very favorable results in pre-clinical studies. Currently we are doing a micro-dystrophin gene delivery trial using adeno-associated virus (AAV) with promising results after 1 year in an open label trial that has now moved to the next level using a double-blind, placebo controlled protocol.

We also have promising data from gene delivery replacing the mutated survival motor neuron (SMN) gene in SMA. In this study we have tested two doses identifying a therapeutic dose that rescues the lives of infants that usually die by age 2. We have 15 patients now followed for 4-5 years (depending on time of enrollment), all of whom continue to improve. We have children, receiving gene therapy in infancy who would never learn to sit, walk, talk or feed independently. These limited milestone achievements are now reversed and will be reviewed in this presentation.

A Case of First Trimester Prenatal Diagnosis of Bardet-Biedl Syndrome Associated with Increased Nuchal Translucency

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We report a case of prenatal diagnosis of Bardet-Biedl syndrome by chorionic villus sampling following abnormal carrier screening and increased nuchal translucency. Bardet-Biedl syndrome (BBS), first described in 1866 and further defined in the 1920's, occurs in about 1 in 100,000-160,000 people of non-consanguineous unions. It is associated with rod-cone dystrophy, postaxial polydactyly, truncal obesity, learning disabilities, and genitourinary abnormalities, and is primarily diagnosed during childhood or later. Several reports document prenatal diagnosis of BBS during the second trimester due to abnormal ultrasound findings. We present a case of first trimester diagnosis of BBS, *BBS1*-related. This pregnancy was the first of a healthy nonconsanguineous 26-year-old female of Italian and German descent and 28-year-old male of Irish and Italian descent. Expanded carrier screening after confirmation of pregnancy identified that both parents carry the same mutation in the *BBS1* gene (c.1169T>G, M390R). Prior to diagnostic testing with chorionic villus sampling, nuchal translucency ultrasound was performed and noted an increased nuchal translucency of 3.0mm. Genetic testing identified that the fetus was homozygous for the *BBS1* mutation and therefore predicted to be affected with BBS. This case exemplifies a first trimester ultrasound anomaly in association with a diagnosis of BBS.

A de novo CTNNB1 gene defect in a patient with autism spectrum disorder: Case report and review of the literature

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We report a 9 years old female presented with Autism spectrum disorder, developmental delays, hypotonia, and intellectual disability. She attends at 4th grade special education classes. However, she is at the 5-7 years of age level. She exhibited aggressive behavior at school including throwing chairs, biting and melt downs. Physical examination revealed her overall size was less than 5th percentile. She had close set eyes, thin upper lip, soft philtrum, space between her front to teeth. She had brown hair color with normal texture and amount. She has esotropia. She did not have spasticity, but had frequent falls most likely associated with ataxia. She was able to make full sentences that can be understandable to some extent. A brain MRI was normal. Her routine chromosome analysis and microarray, Prader Willi syndrome testing were all normal. Her Intellectual disability panel at Fulgent genetic labs showed a heterozygous pathogenic variant at CTNNB1 gene: c. 197G>A (p.Trp66*)

which is a non-sense variant introduces an early stop codon at position 66 and results in truncated protein encoded by this gene. This change has not been reported in Broad ExAC dataset or previous cases with this gene defect. According to ACMG guidelines , laboratory considered this variant to be pathogenic .

There have been less than 10 patients reported with CTNNB1 gene defect. CTNNB1 gene encodes for beta 1 catenin which is a component of the cadherin adhesion complex. It functions for cell –cell adhesion and part of wnt signaling pathway. This pathway is important to control cell growth and differentiation both in normal and tumor tissues. Loss of function mutations in CTNNB1 gene have been reported in individuals with intellectual disability (OMIM #615075), spasticity, microcephaly and central hypotonia suggesting a recognizable phenotype associated with haploinsufficiency for this gene. Some patients also demonstrated fair hair, and skin and distinctive facial features.

Our patient's phenotype is comparable with other reported cases. All of the patients had autistic behavior with aggressive outbursts and temper tantrums. They all presented with intellectual disability and truncal hypertonia. Our case confirms findings in previously reported 10 patients associated with CTNNB1 gene defects.

Soluble glycosylated protein released from glycogen by rhGAA

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In studies on the degradation of glycogen by rhGAA a glycosylated protein core material glycogen was found which consists of about 5-6% of the total. However, there was a question of why the glucose released by rhGAA still does not equal the amount of starting glycogen. Approximately 25% of the glycogen was unaccounted for based on glucose released. After incubation of glycogen with rhGAA until no more glucose is released no carbohydrate was detected from the incubation medium that elutes after glucose by HPAEC-PAD. If the incubation medium is boiled in 0.1N HCl first, several oligosaccharides are then detectable. Incubation with trypsin or chymotrypsin also exposes oligosaccharides for detection. This appears to be a case of protein masking carbohydrate. The masked material in the medium binds to Dowex 50W which is evidence of a charge such as a protein but it is not bound by Concanavalin A. Glycogen does bind Concanavalin A. Given its characteristics the presence of this material in serum was suspected. It is present in serum either in an HCl extract or in a trypsin or chymotrypsin digest. The characteristics of the serum material were the same as the material from the incubation medium. One oligosaccharide, which can not be degraded further by rhGAA, from the incubation medium and serum co-elute. The oligosaccharides contain *m*-inositol, *e*-inositol, sorbitol, mannose, galactose and glucose with three of them also containing xylose. The presence of this glycosylated protein in serum is taken as an indication of a fraction of glycogen being degraded outside the lysosome and outside of the cell. This material appears to be a terminal degradation product of glycogen by GAA. This now raises the question of where this material may be degraded and if it is related to the residual glycogen in the Pompe mouse tissue following ERT.

De novo 3p21.31 Loss of *DHX30*, a new CNV that Expands the Phenotypic Spectrum of Neurodevelopmental Disorder with Severe Motor Impairment and Absent Language (NEDMIAL)

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The *Death Box Polypeptide 30* (*DHX30*) gene, member of the RNA helicase family that includes DHX and DDX proteins, is involved in DNA transcription, splicing, and translation, and is highly expressed during brain neurogenesis. Heterozygous, *de novo* single nucleotide variants (SNVs) in *DHX30* have been detected in 16 patients with Neurodevelopmental Disorder with Severe Motor Impairment and Absent Language (NEDMIAL) [Lessel et al. 2017 and Eldomery et al. 2017] (MIM:617804). However, full gene deletion of *DHX30* has not been previously described.

We report on a 3-year old male with hypotonia, developmental delay, and speech delay referred to the UCLA Neuromuscular Clinic at 2.9 years of age for motor impairment who also had Chiari malformation and hypomyelination. Chromosomal microarray analysis detected a *de novo* heterozygous, 1.1 Mb copy number (CNV) loss of 3p21.31 including 12 RefSeq genes of which five are predicted to be dosage-sensitive. The true frequency of a loss of this size in the general population is uncertain. At present, only *DHX30* and *SETD2* have phenotype associations relevant to some of the patient's features. Because this patient lacks the facial dysmorphism, macrocephaly, skeletal anomalies, and endocrine abnormalities typically observed with Luscan-Lumisch Syndrome (MIM: 616831), the *SETD2* variant was excluded.

Presently, the impact of loss of *DHX30* in humans is unknown. Individuals with a similar loss have not been reported. Mice homozygous null for *Dhx30* exhibit embryonic lethality; however, heterozygous mice, while apparently healthy, have not undergone in-depth neurobehavioral phenotyping, precluding comparison. Notably, other members of this gene family are recognized as candidates for intellectual disability and developmental delay (*DDX3X*) and brain malformations (*DHX37*), suggesting a role for this and other gene family members that function as RNA helicases in central nervous system development. Our patient has a complete null *DHX30* allele that apparently confers a much milder phenotype than the severity associated with missense SNVs in patients with NEDMIAL- who are non-verbal, have no independent walking, and present with delayed myelination with cerebellar or cerebral atrophy, and dilated ventricles. Given the overall evidence we interpreted this CNV loss as likely to be pathogenic.

This report highlights the complexity of interpreting novel whole gene losses in cases where missense SNVs, are more frequently associated with a severe disease phenotype, and is an example of the challenges involved in interpreting and managing patients in which apparently more severe genetic perturbations result in a milder manifestation. It also adds to the limited phenotypic spectrum of patients with whole gene deletions of *DHX30 and* demonstrates the necessity of collecting genotypes and better phenotypes on other patients with *DHX30* deletion or truncating SNVs, to effectively guide novel CNV interpretation and inform the clinical team on a treatment plan and prognosis for a young child with this CNV loss.

Transcript studies support evidence for altered calcium signaling and altered mitochondrial function in autism

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Increasing evidence suggests that a disruption in intracellular calcium homeostasis is involved in the pathophysiology of autism spectrum disorder (ASD). In prior studies, Schmunk et al. used a high throughput Fluorometric Imaging Plate Reader (FLIPR) assay to monitor inositol triphosphate-mediated calcium release from the endoplasmic reticulum. These studies revealed altered calcium release in fibroblasts of autism cases, where in most autism cases (>75%), calcium signaling levels were below the extreme limit seen in controls[1]. However, in one case, levels of calcium release were markedly high, far exceeding the upper limit of controls and nearing levels obtained with the ionophore ionomycin, a calcium releasing agent.

To evaluate the molecular bases for altered calcium signaling, we carried out a transcription analysis that yielded data on expression levels of 28,085 transcripts, using fibroblast RNA from two control cases and three autism cases - one case with the extremely low-signaling levels seen in most sporadic ASD cases, one case with intermediate-low signaling levels, seen in a minority of ASD cases, and the unique case with the markedly elevated high calcium signaling.

Based on reviews of the literature, 78 genes were identified that encode proteins involves in calcium signaling, calcium storage and transfer of calcium between organelles. Literature reports revealed that 46 genes involved in mitochondrial functions were altered in autism. In our studies, we compared levels of transcription in 3 cases and 2 controls to identify genes where transcript level, either in a case or in a control, differed by 5 standard deviations or more compared with the other 4. Of the 78 calcium signaling related genes, 28 showed altered expression in affected cases and 2 showed altered expression in controls. Of particular interest, relevant to the FLIPR assay of calcium signaling, the case with the abnormally high signaling showed significantly increased expression in 13 genes that included purinergic receptors genes and ATP2A3 (SERCA3), an endoplasmic reticulum calcium sequestering pump. This case also showed significantly low expression of VDAC2, a protein involved in mitochondrial calcium uptake.

Transcript level analyses of the 46 selected genes involved in mitochondrial function revealed that the case with intermediate-low calcium signaling had altered levels in 20 genes, one gene was altered in the high calcium signaling case and one gene was altered in controls. Mitochondrial related genes with the most significantly altered levels in the intermediate low calcium signaling autism case included decreased levels of MCU (mitochondrial calcium uniporter) and increased expression of UCP2, an uncoupling protein that reduces the mitochondrial membrane potential in mammalian cells. This limited transcription analysis provides additional evidence for roles of altered calcium homeostasis and for altered mitochondrial function in autism.

The *Iliad* follows the *Odyssey*? Challenges in treating and caring for novel rare diseases

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Creating translational resources for clinicians and families has lagged behind our improved diagnostic capability, creating challenges in prognosis, management, and treatment. A 6 month-old twin boy, Kavish, with early suggestion of infantile spasms had a quick diagnostic odyssey in 2014. His parents had noted he was different from his fraternal twin at 2 months of age and began treatment for reflux. Not tracking and with poor response to voice, Ophthalmology (normal fundus) and Audiology (OAE and ABR) follow-up to NICU concerns were normal. At 3 months, he developed gentle subtle rhythmic jerking of feet > hands, lasting a few seconds, without Sandifer posturing. At 4 months a brain MRI was normal. The family traveled abroad for a month, and on return raised concern he only seemed alert and "with it" about 15-20% of the time. At 6 months, EEG suggested evolving hypsarrhythmia, with slow and disorganized background along with frequent multifocal spikes. After normal metabolic evaluations, cytogenetic microarray, and refractory treatment response ,whole exome sequencing revealed a *de novo* missense mutation p.G937D in the *DNM1* gene. Kavish was the 5th individual in the world found to carry a *DNM1* mutation, prior to publications to publications establishing the role of *DNM1* in developmental encephalopathy. *DNM1* encodes a mechanochemical protein which allows for clathrin mediated endocytosis of vesicles: “pinching” synaptic vesicles from the pre-synaptic axonal membrane. This would ultimately be revealed to be a novel mechanism for seizures and encephalopathy (*Neurology* 2017 89 (4):385-394 PMCID: PMC5574673), but at the outset parent and provider trod the path alone.

The Jackson Labs had described the “fitful mouse” *ftfl* (Rebecca Boumil, 2001) as a model for epilepsy, and in 2010 Dr. Boumil mapped the *ftfl* mutation to *Dnm1* (PMCID: PMC2916854). The missense mutation in the mouse model was the direct analog to the mutation found in Kavish: a precise mouse model for this patient! A Google search revealed that a chemical agent “dynasore” inhibits dynamin function. But in 2015, no rapid funding mechanism existed to quickly translate treatment from mouse to man. In the ensuing years, the family became experts in their son’s rare condition, networking with other families (14 as of the 2017 publication, but how to find “unpublished” families?). Ominously, the *fitful* mouse had a broader developmental encephalopathy, and milder phenotypes were not ascertained in humans. Optimistically, human patients caused resurgent interest (and funding opportunities) in the mouse model. Selective serotonin reuptake inhibitors (SSRI) seemed to inhibit dynamin, providing a rationale for (unsuccessful) empiric treatment.

Beginning in 2016, public resources through NIH National Center for Advancing Translational Sciences (NCATS) were devoted to establishing patient registries, drive patient-focused pre-clinical R&D, and streamline NIH and FDA pipelines. Traditional rare disease advocacy organizations and novel groups (People-Centered Research Foundation (PCRF)) are entering the fray. The experience of this family illustrates the challenges in moving beyond the Diagnostic Odyssey.

Lesch-Nyhan Disease

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Lesch-Nyhan disease is a heritable disorder of metabolism in which the mutated gene is on the X-chromosome. Patients are virtually all male, but a few symptomatic females have been reported. The abnormal enzyme hypoxanthine guanine phosphoribosyltransferase normally converts to be purine base hypoxanthine and guanine to their respective nucleotides. This leads to an enormous over production of uric acid, leading to all of the clinical manifestations of gout. Cerebral manifestations include mental retardation. Behavioral manifestation includes self-injurious behavior.

Homozygous B4GALNT1 Mutation and Clinical Glutaric Acidemia Type II: A Case Report

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Introduction: The beta-1,4-N-acetylgalactosaminyl transferase-1 (B4GALNT1) gene encodes the GM2/GD2 synthase protein, which is responsible for the synthesis of complex gangliosides necessary for central nervous system development. Mutations in the B4GALNT1 gene lead to a loss of function of the synthase protein, resulting in the loss of GM3 synthetase activity and thus affecting important glycosphingolipid biosynthesis and signaling pathways of the central nervous system.[1] Hereditary spastic paraplegia subtype 26 (SPG26) has been linked to B4GALNT1 mutations and is characterized by weakness and spasticity of the lower extremities as well as intellectual disability and dysarthria due to neurological involvement. No cases in the literature have yet reported associated Glutaric Acidemia Type II (GAII) or other metabolic disorders.

Method: We report a 36 year old female who initially presented for workup of cognitive deficits, seizures and clinical spastic paraplegia. She was diagnosed with clinical glutaric acidemia type II after extensive metabolic studies. NGS and whole exome sequencing (WES) revealed no abnormalities in the GAII electron transfer genes implicated in glutaric acidemia type II, however she was found to have novel homozygous *B4GALNT1* mutations, which explained her spastic paraplegia phenotype.
Conclusion: To our knowledge, this is the first case of SPG26 associated with a clinical diagnosis of GAII, suggesting a novel mechanism for GAII. We expect this report will lead to additional cases of this potential new role of B4GALNT1 in mitochondrial function.

Developing 3D facial Data and Tools to Transform Clinical Genetics

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Our NIDCR funded FaceBase project aims to develop 3D cranial Morphometry data and tools to transform dysmorphology. Specific aims are to: 1) build a broad and deep 3D morphometric facial scan “library” of syndromes, 2) develop 3D analytical tools to systematically analyze and distinguish dysmorphic syndromes, and 3) develop an automated, prototype clinical diagnostic aid. Using automated landmarking, we combine geometric

morphometrics and dense representation analysis; the former uses facial landmarks and statistical shape analysis to assess variation and the latter uses the full surface contour map of the face. Our current database includes 3D facial images from 4,000 individuals with >400 different genetic syndromes. Here, we review our current progress on this project.

Safety and effectiveness of resistance exercise training in a pilot study of patients with late onset Pompe disease

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Pompe Disease is a rare inherited disorder associated with muscle weakness and respiratory insufficiency which can affect all ages, ethnicities, and gender. It is caused by the accumulation of glycogen primarily in cardiac and skeletal muscle. There are two forms of the disease, classic infantile form and juvenile/adult form. The purpose of this study is to evaluate the effectiveness of the supervised resistance training program on muscle strength, functional capacity and body composition in patients with late onset Pompe disease. The study includes 10 patients over a 32 week study of increased resistance exercise 3 times a week in a gym with a personal trainer. The patients also undertook respiratory muscle exercise training daily with increasing resistance every 2 months. Each patient served as their own control (Weeks 1 - 8 baseline period) and subsequently physical strength, stamina and respiratory function was tested with the Biodex and hand held dynamometers, 6 minute walk test (6MWT), maximum/minimum inspiratory pressure (MIP/MEP), SF-36 questionnaire, muscle volume and texture change using MRI, and blood and urine collections of tetrasaccharide.

Throughout the study, we found that there was an overall improvement in the patient's muscle strength by Biodex dynamometry, MRC scores and 6MWT. For example, knee and elbow extension torque improved by a mean of 21.6% and a 41.6% respectively. The 6MWT showed an average improvement of 6.58%, and total MRC scores showed an improvement of 2.1% from initial to final visit. There was a marked improvement in the respiratory parameters in the majority of patients; the MIP showed an increase of 25% while MEP increased by 10%. Overall, patients also had an improved sense of well being with very few adverse side effects.

TK2-related mitochondrial depletion syndrome in a pair of siblings – a diagnostic odyssey leading to possible treatment

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Diagnosing mitochondrial disease is quite challenging because there can be a wide and varied degree of organ system involvement, even among affected individuals within the same family. Two siblings are presented who were ultimately determined to have TK2-related mitochondrial depletion syndrome.

The proband was 11 years old when she first presented to the Genetics Clinic for poor ability to gain weight and gastroparesis. Her cognitive abilities were normal, but there were mild gross motor concerns (poorer performance in sports, climbs stairs slowly). A basic inborn error of metabolism work-up was normal (including

lactate), SNP chromosome microarray revealed a likely benign CNV but also remote consanguinity, and CPK was elevated. Muscle biopsy showed non-specific findings on pathology as well as respiratory chain enzymatic analyses.

The proband's brother is 2.5 years younger in age, and has a more prominent myopathic phenotype. However, in contrast to his sister, he is without GI or weight gain concerns. Although he walked on time (14 months old), his hypotonia became more apparent through childhood. As a preteen, he continues with PT, still falls on occasion, and needs to climb stairs while holding on. His initial work-up was similarly negative, except for his CPK being mildly elevated.

Clinical whole exome sequencing uncovered homozygous likely pathogenic variants in TK2 for both of the siblings. TK2 is an initial step in the generation of intramitochondrial nucleotides used in mitochondrial DNA replication. Pathogenic variants in a number of genes within these pathways result in mitochondrial DNA depletion syndromes, although in some instances (such as with TK2 mutations), quantification of mtDNA content can be normal. Studies in mice have been published showing clinical improvement with oral therapy aimed at bypassing the blocked synthesis step with exogenously administered nucleosides. Anecdotal indications are of a similar response in humans, and these siblings have been enrolled in pre-clinical trials with preliminary evidence of a positive clinical benefit.

These two siblings illustrate the variability of clinical phenotypes in mitochondrial disease, but also highlight a less common (but arguably more gratifying) benefit of arriving at a genetic diagnosis: a treatment may be available to more durably treat the underlying condition.

Antisense oligonucleotide treatment targeting glycogen synthase (GYS1) in a mouse model of Pompe disease

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Pompe disease is a progressive myopathy resulting from the deficiency of acid □□glucosidase (GAA). The standard of care is enzyme replacement therapy (ERT) with recombinant human (rh) GAA. ERT works well in alleviating the cardiomyopathy; however, many patients continue to have progressive muscle weakness from muscle glycogen accumulation produced by muscle glycogen synthase (GYS1). Previous studies have shown that inhibition of GYS1 reduced lysosomal glycogen. Knockdown of GYS1 mRNA by phosphorodiamidate morpholino oligonucleotide conjugated with a cell penetrating peptide however was nephrotoxic. Antisense Oligonucleotides (ASO) technology has emerged as a powerful therapeutic alternative for the treatment of genetic disorders by targeting RNA. In order to impart specificity for the muscle variant of GYS1, we propose the use of ASO-mediated gene silencing through the RNaseH1 dependent degradation mechanism. Most recently nusinersen has been developed as the only therapy for spinal muscular atrophy using this technology, and our hope is that ASO technology will be successful in Pompe disease.

Over 150 ASOs were designed and screened in vitro to identify the most efficacious ASO to be tested in mice. The lead from the screen were validated in a dose response study and the top 10 ASOs were screened in vivo. Three ASOs (GYS1 ASO#1, GYS1 ASO#2, GYS1 ASO#3) showed the best tolerability and efficacy profile leading to the knock-down of GYS1 mRNA by approximately 50%. We have performed a pilot study of the efficacy of three GYS1 ASOs in Pompe mice as monotherapy and have reduced muscle GYS1 mRNA levels compared to PBS and a mismatch ASO. We studied the effects of the GYS1 ASOs on skeletal muscle glycogen, histology, GYS1 RNA, and

muscle function in Pompe mice. Based on Western blot data, there is successful knock-down of GYS1 protein in ASO#1, ASO#2, and ASO#3. Autophagy markers, such as LC3, p62, and mTOR, in mice treated with ASO#3 were comparable to wildtype mice.

These preliminary studies provide proof of principle for a promising adjunct treatment for Pompe disease.

Expanding the Phenotype of Type XII Collagen Myopathy: Congenital Cataracts, Microcephaly and Global Developmental Delay?

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Hereditary congenital myopathies may be caused by mutations in genes encoding several classes of proteins, including extracellular matrix proteins, contractile proteins, and Golgi-resident modifying enzymes. The archetypal extracellular matrix proteins involved in congenital myopathies are the type VI collagens, which are defective in Bethlem and Ullrich myopathies. Recently, type XII collagen has been recognized as involved in a phenotypically similar congenital myopathy, and both autosomal dominant and recessive forms have been described. We report two siblings with a congenital myopathy who are compound heterozygotes for missense mutations in COL12A1, the gene encoding type XII collagen. As neonates they presented with hypotonia, which evolved to spasticity with hyperreflexia, finger flexion contractures, and inability to ambulate. Bilateral congenital cataracts were evident in both sibs, and one underwent unilateral phakectomy. Head circumferences at birth were normal but by age 3 years were below the 3rd percentile; brain MRI at age one year in one sib was unremarkable. Both sibs, currently ages 7 and 12 years, are nonverbal. Whole exome sequencing revealed missense mutations in COL12A1, predicted to result in the amino acid substitutions Pro591Arg (maternal) and Ser2042Leu (paternal). Neither parent had a history of motor or cognitive delay or cataracts. Cultured skin fibroblasts from one sib exhibited decreased intracellular immunoreactivity and absent extracellular immunoreactivity against type XII collagen. In the absence of other exomic data to explain cataract formation, brain atrophy, and global developmental delay, we propose that compound heterozygosity for missense mutations in COL12A1 may play a role in the development of these additional clinical findings.

A Recurrent De Novo Heterozygous COG4 Substitution Leads to Saul-Wilson Syndrome

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Saul-Wilson syndrome is a rare sporadic rare form of primordial dwarfism with characteristic facial and radiographic features. Our patient is a three-year-old Korean girl who presented with intrauterine growth retardation and a concern for a skeletal dysplasia. After birth, she exhibited mild dysmorphic features, ptosis, growth retardation, conductive hearing loss, neutropenia, platyspondyly, metaphyseal flaring, and brachydactyly. Whole exome sequencing revealed heterozygosity for a missense variant in COG4, a gene encoding one of the eight components of the conserved oligomeric Golgi (COG) complex. An additional 13 individuals with Saul-Wilson syndrome were identified as part of an international collaboration. All affected subjects harbored heterozygous de novo variants in COG4, giving rise to the same recurrent amino acid substitution (p.Gly516Arg). Affected individuals' fibroblasts, whose COG4 mRNA and protein were not decreased, exhibited delayed anterograde vesicular trafficking from the ER to the Golgi and accelerated retrograde vesicular recycling from the Golgi to the ER. This altered steady-state equilibrium led to a decrease in Golgi volume, as well as ultrastructural abnormalities with collapse of the Golgi stacks. Protein glycosylation in sera and fibroblasts from affected subjects was not notably altered, but decorin, a secreted proteoglycan, showed altered Golgi-dependent

glycosylation. In summary, we define a specific heterozygous gain-of-function COG4 substitution as the molecular basis of Saul-Wilson syndrome, a rare skeletal dysplasia distinct from the allelic recessive disorder COG4-related congenital disorder of glycosylation (type IIj).

EXPLORE: A Prospective, Multinational Natural History Study of Acute Hepatic Porphyria Patients with Recurrent Attacks

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Background: Acute hepatic porphyrias are rare, often misdiagnosed genetic diseases caused by a mutation in one of the enzymes of heme biosynthesis. This results in the accumulation of neurotoxic heme intermediates, aminolevulinic acid (ALA) and porphobilinogen (PBG), that can cause severe neurovisceral pain, life-threatening attacks and chronic debilitating symptoms (pain, nausea, and fatigue). We will be presenting updated ≥12-month data.

Methods: EXPLORE is a prospective, international, observational natural history study and clinical management of patients with hepatic porphyrias with recurrent attacks (≥3 attacks/year) or who receive prophylactic treatment to prevent attacks. Patient medical history, physical examination and porphyrin biomarkers, and questionnaires on porphyria activity were collected.

Results: 112 patients enrolled from 13 countries and were followed for 12 months. Mean patient age: 39 years, 89% female, 93% with acute intermittent porphyria, 4% variegate porphyria, and 3% hereditary coproporphyria. Patients reported a mean of 9.3 attacks in the 12 months prior to the study, pain being the most common symptom, occurring in 99% of attacks. Mean attack duration was 7 days. Chronic symptoms were reported by 65% of patients, pain being the most frequent symptom. On-study attack rate was 3.7 attacks/person/year, of which 69% required treatment with hemin or a healthcare visit. For those patients on hemin prophylactically, mean attack rate was 3.5 attacks/person/year. Mean ALA and PBG levels at screening (during non-attack) were markedly increased to 9 and 23 times the upper limit of normal, respectively.

Conclusion: EXPLORE, the first international natural history study in patients with hepatic porphyria and recurrent attacks, demonstrates that patients suffer from attacks and chronic symptoms. Given morbidity and mortality, there remains an unmet need for novel therapies to prevent attacks and treat chronic symptoms.

HSPB8 Mutations is Associated with a Novel Rimmed Vacuolar Myopathy

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Background: Heat shock protein beta 8 (*HSPB8*), also known as heat shock protein 22, is involved in chaperone assisted selective autophagy (CASA) in cells. CASA is a process that maintains muscle cytoskeleton by facilitating the degradation of damaged components. Disruption of genes in the chaperone-assisted degradation pathway is implicated in several myopathies.

Objective: We clinically and molecularly characterize a new family with autosomal dominant rimmed vacuolar myopathy (RVM) caused by mutations in the *HSPB8* gene. HSPB8 chaperone defect has previously been associated with Charcot Marie Tooth disease and only recently with RVM.
Approach: We performed whole exome and whole genome sequencing (WGS) in the family. Western blot and immunocytochemistry were used to analyze three patient fibroblasts and compared with their age, sex match controls.
Results: Affected patients have distal and proximal myopathy with muscle biopsy showing rimmed vacuoles, muscle fiber atrophy and endomysial fibrosis typical of RVM. Muscle MRI showed severe relatively symmetric multifocal fatty degenerative changes of the lower extremities. We identified a duplication of C at position 515 of the HSPB8 gene (c.515dupC) on WGS. This mutation caused a frameshift with a predicted alternate stop codon p.P173SFS*43 in all affected individuals, resulting in an elongated protein product. Western blot and Immunocytochemistry studies revealed reduced expression of HSPB8 in patient fibroblasts, in addition to disrupted autophagy pathology compared with control fibroblasts. The pathology is most likely due to a dominant negative effect.

Conclusion: We report a novel family with autosomal dominant rimmed vacuolar myopathy caused by a c.515dupC in the HSPB8 gene which causes a translational frameshift resulting in an elongated protein. As next generation sequencing becomes more available, additional myopathy families will be identified with HSPB8 mutations. Understanding the mechanism for the RVM pathology caused by mutated chaperone will permit novel targeted strategies to alter the natural history in HSPB8 and related diseases.

VCP inhibitor: Potential treatment for VCP myopathy and ALS

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Background: Valosin-containing protein (VCP) disease mutants cause inclusion body myopathy with early-onset Paget disease and frontotemporal dementia (IBMPFD) through a gain of function activity of the VCP gene. Studies in drosophila models as well as in patient fibroblasts indicate that VCP inhibitors improve the disease pathology. CB-5083 is a reversible and competitive specific inhibitor of AAA ATPase p97/VCP and has a documented safety, and is well tolerated in human studies for cancer treatment. Thus, we hypothesize that normalizing the gain of VCP function in patient myoblasts and VCP mutant mice will correct the disease phenotype.
Objectives: Correction of muscle pathology in vivo in the homozygous VCPR155H/R155H disease mouse model with VCP inhibitor CB-5083.

Approach: VCP $^{R155H/+}$knock in mouse model developed by Kimonis lab displays pathological features of human VCP-associated disease however is a slow model of the disease. The homozygous VCP $^{R155H/R155H}$ mouse (2-month old, both sexes) were divided into 2 randomly assigned groups of 12 mice each for daily gavage treatment with selected doses of CB-5083 (½ of the maximum tolerated dose) and vehicle group, for 2-4 months. Findings were compared with wild type mice.

Results: Homozygous VCP mice tolerated the CB-5083 treatment well with normal behavior and body weights. They also showed improvement of muscle strength when compared with vehicle group and wild type littermates.
Conclusions: VCP inclusion body myopathy with early-onset Paget disease and/or frontotemporal dementia (IBMPFD) is caused by a gain of function mutation of the VCP gene. Preliminary studies show that Homozygous VCP mice tolerated the CB-5083 treatment well with improvement of muscle strength when compared with littermates. If successful in patients with VCP disease, ALS and other sporadic IBM could be treated using this strategy.

Patient-specific iPSC disease modeling reveals tissue-specific molecular mechanisms of *LMNA*-associated cardiac disease

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Lamin A/C are intermediate filament proteins that construct the nuclear lamina of a cell encoded by the *LMNA* gene. Lamin A/C has been implicated in processes such as chromatin localization and cell differentiation. Consequently, mutations in *LMNA* gene have detrimental effect in cell functions which results in diseases collectively termed as laminopathies which include *LMNA*-related dilated cardiomyopathy. In a large affected family, we recently identified a heterozygous novel *LMNA* splice-site mutation (*LMNA* c.357-2A>G) predicted to result in exon skipping and premature truncation. Our group aims to elucidate the molecular mechanisms contributing to the pathology of *LMNA*-related cardiac disease by developing an *in-vitro*, patient-specific disease model. Dermal fibroblasts, obtained from affected and unaffected family members, were reprogrammed to induced pluripotent stem cells (iPSCs). One control and one patient-derived iPSCs were differentiated into cardiomyocytes (iPSC-CMs) for molecular and protein studies. Sanger sequencing of *LMNA* transcripts revealed evidence of the predicted exon skipping and frame shift exclusively in patient iPSC-CMs, indicating the expression of the mutant allele albeit at a low level compared to the normal allele. Western Blot studies showed that patient iPSC-CMs had a reduced level of Lamin A/C protein compared to control iPSC-CMs but no evidence of the mutant truncated protein. Interestingly, when the same studies were performed on patient and control fibroblast, mutant allele expression or reduction in Lamin A/C protein level was not observed. Taken together, our results provide insight to the molecular mechanism of *LMNA*-associated cardiomyopathy and reveal the tissue specificity of this mutation.

The cohesin loader NIPBL interacts with TCOF1 and RNA to regulate pre-ribosomal RNA synthesis

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NIPBL is an essential protein, mutations in which cause Cornelia de Lange Syndrome (CdLS). The canonical function of NIPBL is to load cohesin onto chromatin to mediate sister chromatid cohesion and chromatin loop organization. How NIPBL's chromatin binding specificity is determined is not fully understood. We found that a subpopulation of NIPBL tightly binds to Treacle, the protein encoded by the *TCOF1* gene mutated in Treacher Collins syndrome (TCS), and localizes to the fibrillar center of the nucleolus in interphase and to the nucleolar organizing center in mitosis in a treacle-dependent manner. NIPBL localization to the nucleolus is further promoted by its direct interaction with pre-ribosomal RNA (rRNA) transcript. We identified two RNA binding domains in NIPBL in vitro, both of which are required for efficient rRNA binding in vivo. NIPBL binds to ribosomal DNA (rDNA) in an RNA-dependent manner, in turn recruits PAF1, and further stimulates pre-rRNA transcription. Stress that inhibits rRNA synthesis displaces NIPBL from rDNA and clusters it to the nucleolar cap in a treacle-dependent manner, indicating that they are together part of the nucleolar stress response. The results reveal that a subpopulation of NIPBL exist as a stable complex with treacle in the cell, and a novel treacle- and transcript-dependent recruitment of NIPBL to the rDNA region is important for pre-rRNA transcriptional regulation and nucleolar stress response. Our results indicate the novel physical link between CdLS and TCS.

iPSC disease modeling of bone dysostosis in a family with *LMNA*-related cardiomyopathy and brachydactyly

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Lamin A/C (*LMNA*) are filamentous proteins of the nuclear lamina with roles in nuclear structure and cell differentiation. Despite constitutive expression in somatic cells, *LMNA* mutations primarily affect mesodermal/mesenchymal-derived lineages. We report our preliminary findings for a family with *LMNA*-related Heart-Hand Syndrome IV: cardiomyopathy and brachydactyly (Zaragoza *et al*., 2017). We test our hypothesis: *pathogenesis involves impaired osteogenic differentiation of mutant MSCs*. Fibroblasts from three affected and three unaffected family members were reprogrammed into iPSCs. One mutant and one control MSC lines were created and compared for quality and nuclear structure. Although both displayed normal quality, nuclei of mutant MSCs had more nuclear defects, differences not observed in fibroblasts. After MSC differentiation, mutant osteoblasts had decreased calcium deposits consistent with impaired osteogenic differentiation. Mutant adipocytes and chondrocytes had no differences in lipids and glycosaminoglycans, respectively. Our preliminary observations support *LMNA* tissue-specific pathology and provide an *in vitro* model for human bone development and dysostosis.