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## Intraoral Spectroscopy for the Identification and Study of Molar Hypomineralization

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OBJECTIVES/GOALS: Molar hypomineralization (MH) is a highly prevalent dental disease that leads to rapid enamel decay even with preventative measures. While the harm is apparent, the etiology of the condition is not. Further, MH is difficult to study due to limited intact extracted teeth. Therefore, we need a method to study MH non-destructively and intraorally. METHODS/STUDY POPULATION: Recent work has shown excess proteins not commonly found in enamel are present in teeth with MH. This is theorized to be due to disruption and infiltration in the cells that form enamel leading to leftover protein and under mineralized enamel. We hypothesizethese proteins have specific spectroscopic signatures that can be detected using light. We further hypothesize that modern fiber optic probes can provide a method for non-destructive, pain-free, and rapid intraoral examination of MH. Initially, Micro Raman spectroscopy was used to detect specific vibrational bands associated with organics in teeth with MH followed by the collection of spectra of teeth with MH and heathy enamel using a spectrophotometer. These spectra were examined for any obvious differences. RESULTS/ANTICIPATED RESULTS: Currently 12 teeth were collected, and micro-computed tomography reconstructions confirmed location in 3D of MH lesions. Micro Raman of a MH-affected tooth revealed clear organic associated Amide I and III vibrational bands when compared to a synthetic hydroxyapatite (mineral in enamel) powder standard. We determined the wavelength of light that can be used to detect spectral differences between healthy enamel and teeth with MH. The next steps include optimization of the protocol of the intraoral spectrometer with the determined wavelength for implementation in clinic to allow for collection of spectra without the need for tooth extraction. DISCUSSION/SIGNIFICANCE: We hope that this work will lead to advancements in our understanding of the mechanism of MH as well as act as a proof of concept for a MH diagnostic tool. In the long term, the goal is to ideally lead to improvements in dental health care, decrease dental costs, and improve overall quality of life for the children with this condition.

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## Revealing Candidate Inherited Retinal Disease Candidates via Genome-wide Screening of Knockout Mice

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OBJECTIVES/GOALS: The goal of this study is to reveal strong candidate genes for inherited retinal diseases (IRDs) in humans to better understand the mechanisms behind IRD development and reveal potential therapeutic targets. We hope these findings will help improve our understanding of IRDs and, subsequently, diagnostic accuracy and prognosis for IRD patients. METHODS/STUDY POPULATION: The goal of the International Mouse Phenotyping Consortium is to identify the function of all protein-coding genes in the mouse genome via generation and phenotypic characterization of single gene knockout (KO) mice. Using this database, we identified all KO strains associated with abnormal retinal phenotypes, removed all RNA coding genes and pseudogenes, converted to human orthologues, and conducted a literature search for existing research regarding candidate IRD genes and retinal function/abnormalities. A similar process was used for RetNet genes. Subsequently, we performed bioinformatics analysis, including functional annotation (e.g. panther-db), pathway analysis (e.g. KEGG), and string-db to visualize known and predicted protein-protein interactions between the two data sets. RESULTS/ANTICIPATED RESULTS: Analysis of the IMPC database revealed, out of 8481 phenotyped genes, 572 unique protein coding genes were associated with 14 categories of retinal abnormalities such as abnormal retinal vasculature and abnormal retinal thickness, 377 of which have never been associated with retinal pathology in humans or mice. Pathway analysis of the IMPC database highlighted a general metabolism pathway as well as PI3K-Akt and MAPK pathways, not found in RetNet pathway results. Unique clusters from functional annotation clustering of the IMPC include DNA methylation and protein ubiquination. Visualization of protein-protein interactions in string-db between the IMPC (mouse) and RetNet (human) revealed 4 clusters of interest with gold standard RetNet IRD proteins interacting with candidate IMPC IRD proteins. DISCUSSION/SIGNIFICANCE: IMPC analysis revealed 572 candidate IRD genes, 377 of which are novel with no existing independent research related to the retina outside of the IMPC. Bioinformatic analysis reveals 4 strong clusters of interest through string-db where a gold standard RetNet gene interacts with candidate IRD genes as well as many functional pathways of interest.

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## Targeting Cdk8 to improve Ischemic Fracture Healing

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OBJECTIVES/GOALS: There are178 million bone fractures globally each year, and 46% of fractures that have accompanying vascular damage (ischemia) will not heal without surgical intervention. Using single cell RNAseq we identified Cdk8 as a gene upregulated under ischemic fracture. Our work seeks to inhibit Cdk8 to assess its potential as a therapeutic target. METHODS/STUDY POPULATION: Most bone injuries heal through a cartilage intermediate that requires mesenchymal progenitor cells (hMSCs) to become cartilage forming chondrocytes. hMSCs underwent pelleted 3D chondrogenic differentiation in the presence of Cdk8 inhibitor, Senexin B. Chondrogenic gene expression was assessed via gene analysis of Aggrecan, Collagen II, and Collagen X. Content of sulfated glycosaminoglycans (sGAGs) was quantified through DMMB analysis. With IACUC approval, C57Bl/6 WT mice underwent femoral artery isolation and resection to create an ischemic environment prior to a transverse tibia fracture. Mice underwent


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intraperitoneal injections of Senexin B from day -1 to 8 and were harvested at 10 days post fracture. Control mice received vehicle injection. Calluses were analyzed through μCT and histomorphometry. RESULTS/ANTICIPATED RESULTS: At 14 days, Senexin B increased chondrogenic gene expression and improved sGAG content in hMSCs. This persisted to day 21, suggesting that Cdk8 inhibition via Senexin B promotes chondrogenesis and matrix deposition. Histomorphometric analysis reveals thatin vivotreatment with Senexin B increases cartilage content and reduces mineralization of the fracture callus compared to the Control. μCT analysis corroborates this, with distinctly less peri-cortical mineral present in Senexin B-treated calluses, and a decrease in total bone volume. These results suggest an altered progression of cartilage formation and endochondral ossification with Cdk8 inhibition. DISCUSSION/SIGNIFICANCE: Our findings reveal that increased Cdk8 is associated with poor healing in ischemic fractures. Inhibition of Cdk8 appears to increase chondrogenesis of hMSCsin vitroand in the murine fracture callusin vivo. Targeting Cdk8 offers potential to improve callus formation in impaired healing scenarios.

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## Effects of SARS-CoV-2 Variants on CD8+ T cell Epitope Diversity: Estimating Clinical Severity in the United States*

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OBJECTIVES/GOALS: Our goal was to distinguish SARS-CoV-2 CD8+ T cell epitopes of spike, membrane, and nucleocapsid products in 27 of the most frequent HLA-Aand -B alleles.We hypothesize that differences mediated by variation in SARS-CoV-2 and host HLA genetics affect the differential clinical severity and presentation of acute infection and PASC. METHODS/STUDY POPULATION: Genomic sequences of SARS-CoV-2 variants were blasted against the original Wuhan strain using Ensembl's SARS-CoV-2 browser. We examined 16 COVID variants: 2 Alpha (B.1 and B.1.1.7), 5 Delta (AY.100, AY.25, AY.3, AY.3.1, and AY.44), and 9 Omicron (BA.1, BA.1.1, BA.2, BA.4, BA.5, BQ.1, BQ.1.1, XBB.1, and XBB.1.5), sequenced from the Louisiana patient population. cDNA sequences were translated using the Expasy tool. To predict MHC-I epitope binding, we used the Immune Epitope Database and Analysis Resource, via TepiTool utilizing the IEDB recommended default prediction and the 27 most frequent HLA-A and -B alleles. In silico peptide docking was conducted on FoldX, utilizing HLA-B*15:01 structures (n= 7) from the Protein Data Bank. RESULTS/ANTICIPATED RESULTS: CD8+ epitope conservation was estimated at 87.6-96.5% in S, 92.5-99.6% in M, and 94.6-99% for N. As the virus mutated, an increasing proportion of S epitopes experienced reduced predicted binding affinity: 70% of Omicron BQ.1- XBB.1.5 S epitopes experienced decreased predicted binding, as compared to ~ 3% and ~15% in Delta AY.100-AY.44 and Omicron BA.1-BA.5 respectively. Additionally, we identified several novel candidate haplotypes that may be susceptible to severe disease, notably HLA-A*32:01, -A*26:01, -B*58:01, and -B*53:01, and relatively protected from disease, such as -A*01:01, -A*02:01,-A*31:01, -B*15:01, -B*40:01, -B*44:03, and -B*57:01. In silico analysis of COVID peptides and HLA-B*15:01, a common allotype in the United States, largely matched predicted binding patterns. DISCUSSION/SIGNIFICANCE: To elicit long term COVID-19 immunity and prevent PASC, it is important to understand the relationship between T-cells, viral variants, and HLA genetics. This project is one of the first to explore the interaction between CD8+ epitope diversity and viral genetics for the majority of the United States population.

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## Digital Physical Activity Phenotype before Cerebrovascular Disease: A Retrospective Study of Accelerometer-Measured Behavior in UK Biobank Observational Cohort*

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OBJECTIVES/GOALS: To investigate digital behavior patterns before cerebrovascular disease (CeVD), we compared accelerometer-measured physical activity (PA) phenotypes of future CeVD patients versus controls in UK Biobank. METHODS/STUDY POPULATION: Accelerometer data from 76,525 eligible participants monitored for 7-days (Jan. 2013-Dec. 2015) was classified into four categories: sedentary, light PA (LPA), moderate-to-vigorous PA (MVPA), and sleep. Covariables and diagnoses were defined using baseline data and patient records. Daily PA patterns associated with incident CeVD were compared to controls using negative binomial regression models. RESULTS/ANTICIPATED RESULTS: Adult participants with future CeVD (n = 2,163) spent 4.4% less time in MVPA (Incident Rate-Ratio (IRR) 0.956; 95% CI = 0.923-0.992; p = 0.016) compared to controls. During 0:00-5:59h (midnight to 5:59AM), future CeVD patients were less likely to sleep (IRR = 0.985; 95% CI = 0.977-0.992; p <0.001) but more likely to be sedentary (IRR = 1.189; 95% CI = 1.098-1.290; p <0.001) or in LPA (IRR = 1.108; 95% CI = 1.015-1.211; p <0.001). In subgroup analyses, decreased MVPA was observed in current/former smokers (IRR = 0.887; 95% CI = 0.819-0.963), males (IRR = 0.931; 95% CI = 0.870-0.997), and the unemployed/retired (IRR = 0.923; 95% CI = 0.856-0.998), an effect more pronounced in depressed patients (p for interaction = 0.044) and prolonged (> 2 hr/day) screen users (p for interaction = 0.018). DISCUSSION/SIGNIFICANCE: The digital phenotype of PA prior to CeVD is characterized by less sleep during 0:00-5:59h and less daily MVPA, demonstrating the utility of accelerometer data in identifying candidates for preventative intervention.

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## Democratizing access to clinical data for research: Implementation and evaluation strategies in an academic medical center and lessons learned

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OBJECTIVES/GOALS: To facilitate data exploration at an academic medical center, we piloted self-service data science tools to provide easy access to research data and provide analytical workspace. The


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