Examining Marketing Strategies to Increase School Meal Participation to Reduce Food Insecurity among Latino Children in California’s San Joaquin Valley

Context/Purpose: California’s San Joaquin Valley (SJV) has a large community of Latino, agricultural-worker families with low-income who disproportionately experience food insecurity and diet-related diseases. School meals, which meet federal nutrition standards, provide a readily accessible program, free of charge to most students, that promotes food security and health. Our community-academic-policy team’s prior research suggests that school meal participation is suboptimal, yet few studies examine strategies to promote participation.

Methods: SJV elementary schools were cluster-randomized to a marketing intervention (n=2) or control (n=1). Thirty intervention classrooms were randomized to one of four marketing messages communicated through school district texts and emails, fliers in classrooms, and sent home via students. Messages were informed by existing literature, community partner input, and cognitive interviews with 17 parents. Parents of students (3rd-5th grade; n=1012) in study schools were eligible to complete surveys that captured child/family demographics, school meal perceptions and participation, and child/household food insecurity. Meal participation data was obtained from the district. Teachers completed surveys to examine intervention implementation.

Results: Study schools served primarily low-income (95% free reduced-price meal eligible), Latino (96%) and English-learner (65%) students. Of the 283 parents of students participating, 234 completed both baseline and follow-up surveys. The majority (61.5% or 91/148) of intervention parents reported seeing marketing messages. Parents who didn’t see messages reported challenges navigating technology, that children did not share fliers, or that privacy concerns prevented them from clicking links. Teacher surveys showed high intervention fidelity.

Interpretation: Implementation of a school meal marketing campaign was feasible, but many parents did not see the materials. Suggestions for improvement include short educational videos or fliers mailed to homes.

Conclusions: Marketing to improve uptake in meals is feasible to implement but should include multiple methods for distribution. We are examining how marketing affects meal perceptions, meal participation, and food insecurity.
Understanding unmet needs of long-term childhood cancer survivors in the Salinas Valley: a community-academic partnership to develop programs for families after cancer treatment

Context/Purpose: Childhood cancer survivors with socioeconomic disadvantages are vulnerable to receiving suboptimal survivorship care and may have unmet needs that contribute to health disparities. We partnered with Jacob’s Heart Children’s Cancer Support Services, a nonprofit serving families of children with cancer in the Salinas Valley, to identify post-treatment needs, concerns, and barriers to care for adolescent/young adult (AYA) cancer survivors.

Methods: In this qualitative study, we interviewed Jacob’s Heart staff, AYA cancer survivors, and parents of childhood cancer survivors (≥5 years post diagnosis, English or Spanish speaking, received services from Jacob’s Heart). Jacob’s Heart led recruitment using purposive sampling to ensure representation of Latinx participants. We performed team-based reflexive thematic analysis supplemented by discussions with community partners.

Results: Participants included 12 AYA cancer survivors (11 Latinx, 11 speak English/Spanish), 11 parents (8 Latinx, 7 limited English proficiency), and 7 Jacob’s Heart staff (5 Latinx, 5 speak English/Spanish). Interviews revealed gaps in AYA cancer survivors’ preparedness to manage their healthcare, including lacking knowledge about their treatment, feeling overwhelmed, and coping through avoidance. Contributing factors included parent/child language discordance, parents’ health literacy, hesitance to discuss cancer history, and healthcare system barriers around care transitions. There was a clear need for mental health services for cancer survivors, parents, and siblings. Exacerbating factors included being alone (family in other countries, deportation), lack of access to and cost of mental health care, Hispanic cultural stigma around mental health, and cancer misconceptions in schools and community.

Interpretation: AYA cancer survivors and parents in the Salinas Valley have unmet needs that are magnified by language, cultural, and structural factors and may contribute to health disparities.

Conclusion: This study highlights the strength of a community-academic partnership to engage research participants and identify opportunities to improve care. Findings will inform future collaborative projects in the community and clinic.
FOXP3-deficient patients have expanded autoreactive T cells originating from both regulatory and effector T cells

Immune dysregulation polyendocrinopathy enteropathy X linked (IPEX) syndrome is a life-threatening disease caused by loss of function mutations in the FOXP3 gene. FOXP3 is a critical transcription factor for the suppressive function of regulatory T cells (Treg). The physiological role of Treg is to prevent undesirable immune responses. Yet, the presence of autoreactive T-cell and their origin has never been characterized in IPEX and thus the role of Treg in controlling autoreactive T-cell expansion in humans remains ill-defined. Using epigenetic analyses as a lineage marker for Treg origin and TCR receptor sequencing, we showed that IPEX patients have expanded autoreactive T cells originating from two sources. The first originates from autoreactive effector T cells (Teff), most likely due to loss of Treg suppressive function, since T-cell autoreactivity is normal in FOXP3 carrier mothers in which the majority of Treg express the wild-type form of FOXP3 and a half of Teff expresses the mutated form of the gene. The second originates from Treg which are expanded but lost their phenotypic markers including CD25 and FOXP3. We call these cells loss-of-identity Treg and showed that they are not present in patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy nor in FOXP3 mutation carrier mothers. Moreover, we show that loss-of-identity Treg are prevented by the presence of healthy donor Treg in an IPEX patient with low donor chimerism post stem cell transplantation. Importantly, we show that FOXP3 knockout in Treg in vitro results in an increased expansion in response to TCR stimulation and confers the ability to produce proinflammatory cytokines, shown to be associated with IPEX syndrome. Thus, loss-of-identity Treg could directly contribute to the disease pathology. Collectively, we provide the first analyses of autoreactive T cells in IPEX which could be used to monitor disease progression or treatments’ efficacy.
Defining mechanisms for failure of pancreatic beta cell development in a novel mouse model of neonatal diabetes

Neonatal diabetes mellitus (NDM) is a genetic form of diabetes presenting in the first 6 months of life. Identifying the genetic etiology influences treatment and prognosis and has informed our understanding of pancreas development in humans. In around 20% of cases the genetic cause remains unknown, partly due to limitations in identifying and interpreting non-coding genetic variants related to disease risk.

We report a novel spontaneous mouse model of NDM characterized by reduced pancreatic islet mass leading to insulin deficiency and hyperglycemia. We used traditional linkage approaches to identify an 8Mb region on chromosome 8 which co-segregates with neonatal diabetes. Initial analysis by whole exome sequencing failed to identify any causal coding variants, we therefore hypothesized that the causal mutation was in a non-coding region with a regulatory function. Whole genome sequencing revealed a novel homozygous intronic single nucleotide variant in the Gins4 gene, which co-segregated with diabetes. We demonstrate that this variant alters GINS4 splicing, resulting in a longer isoform.

We investigated the role of this region in human diabetes risk. Non-coding variation at the human GINS4 region is associated with altered Type 2 diabetes (T2D) risk, supporting a role for the locus in multiple types of diabetes. GINS4 is expressed during endocrine cell development and GINS4 loss in a human beta-cell model results in reduced beta-cell number. We identified T2D-associated variants in islet open chromatin, supporting a regulatory effect. Luciferase reporter assays demonstrate repressor activity of this region.

In summary, we have identified evidence in both mice and humans for a role of dysregulation of GINS4 causing defects in beta-cell development and function which contributes to elevated diabetes risk. On-going work will determine timing and relevance of Gins4 during beta-cell development, its role in adult beta-cell function, and how non-coding regions of Gins4 regulate nearby gene expression.
Access to Pediatric ECMO Centers: A Geospatial Analysis of the Influence of Minority Status and Poverty

INTRODUCTION: As ECMO becomes a standard of care for resuscitation efforts, gaps in the delivery of this life saving technology must be evaluated. The relationship between rural-urban, metro-nonmetro, race/ethnicity, income, insurance status and other sociodemographic factors may impact access. We evaluated minority status and poverty rate in populations that have access to pediatric ECMO and those that do not.

METHODS: Addresses of ECMO programs were obtained from the Extracorporeal Life Support Organization Registry. We defined access to pediatric ECMO as a 200-mile driving distance (LPCH’s Pediatric ECMO catchment area) and created service areas. We obtained census tract level information on minority status and poverty rates using the “CDC Social Vulnerability Index 2018 – USA” layer in ArcGIS. We used ArcGIS Pro for geospatial analysis and data representation and used ArcGIS Pro and R Studio for statistical comparisons.

RESULTS: Forty-three percent of the total US land area and 4.1% of the US population does not have access to Pediatric ECMO. When compared to US averages, in areas that lack access to pediatric ECMO, 28.8% (vs 38.9% US, p <0.001) of the population are considered minority and 14.4% (vs 13.7% US, p <0.001) are considered living below the poverty level. In areas with access to pediatric ECMO, 39.4% (p <0.001) of the population are considered minorities and 13.69% (p <0.001) live below the poverty line.

CONCLUSION: A significant portion of the United States lacks access to pediatric ECMO, with 1 in 25 people living in a region without access. The Rocky Mountain, Great Plains and Midwest regions have the greatest dearth of pediatric ECMO programs, as does northeast New England, and a portion of the Pacific Coast. Extending ECMO access to children would involve a necessary investment into health equity given the higher level of poverty.
Novel humanized loss-of-function NF1 mouse model of juvenile myelomonocytic leukemia

Juvenile myelomonocytic leukemia (JMML) is a deadly pediatric cancer characterized by splenomegaly, monocytosis, and myelodysplastic features with RAS pathway mutations being major drivers. The loss-of-function of Neurofibromin1 gene (NF1-LOF) occurs in ~20% of patients with dismal outcomes. NF1 gene negatively regulates RAS/MAPK/PI3K pathways to control proliferation/differentiation of immature myeloid cells. In JMML patients, NF1 mutations result in loss of either protein translation or function which upregulates the RAS pathway. Hematopoietic stem cell transplantation is the only curative option, but relapse occurs in ~50% of patients. There is an urgent need for novel therapeutic strategies but clarifying disease development mechanism has been challenging due to low patients sample availability and lack of reliable humanized animal models which are the gold standards for studying rare diseases. We used CRISPR/Cas9 to create NF1-LOF in healthy juvenile hematopoietic stem and progenitor cells (HSPCs) from healthy donor umbilical cord blood (UCBs) to mimic the loss of protein function found in JMML patient cells. We consistently obtained high KO scores of ~85% in modified UCB HSPCs, loss of full-length NF1 protein by western blot, and significant hypersensitivity of NF1-KO cells to GM-CSF in colony-forming unit assays in vitro, a unique characteristic feature of JMML patient cells. Thereafter, we assessed the ability of NF1-KO HSPCs to initiate leukemia in humanized immunodeficient NSG-SGM3 neonates. We observed signs of leukemia (including weight loss, weakness, monocytosis in blood, anemia, enlarged spleen and liver) in all NF1-KO recipients with 5 weeks median survival, abrogation of lymphocytes and accumulation of myeloid cells. Extensive leukemia infiltration was also observed in spleen, liver and lungs, similar to JMML patients. In conclusion, we have successfully developed a novel humanized mouse model of JMML carrying NF1-LOF mutation using CRISPR/Cas9 genome editing technology, with features that have been reported as unique characteristics of this disease.
A novel in vitro stem cell model to study maternal endothelial function in preeclampsia

Context/Purpose: Maternal endothelium dysfunction is a central component to preeclampsia pathogenesis. Studies to dissect the maternal endothelial dysfunction, particularly on a patient-specific basis, is hampered by access to systemic primary arterial endothelial cells (ECs). The aim of this study is to establish a replenishable patient-specific in vitro maternal ECs model to allow robust mechanistic studies to dissect preeclampsia endothelial dysfunction.

Methods: Induced pluripotent stem cells (iPSCs) derived from three women were differentiated into ECs using our recently developed protocol. The differentiated ECs were exposed to pooled banked sera from normotensive pregnancies, preeclamptic pregnancies, normotensive postpartum for non-pregnant comparison and controls (basal media and FBS). ECs functional analyses (nitric oxide release, migration, and tube formation) were evaluated in 3 lines. Multiple group comparisons were performed by one-way ANOVAs followed by Tukey tests.

Results: Patient-specific iPSCs were established and subsequently differentiated into ECs. Levels of nitric oxide release were significantly lower after incubation with preeclamptic sera compared to the FBS control, and normotensive and non-pregnant (postpartum) sera treatments were also lower than FBS but higher than PE treatments. Tube formation and migration also demonstrated significantly impaired functions by preeclamptic sera compared to FBS controls. Cell viabilities were not affected by sera treatment. Similar results were obtained for all three patient-specific lines.

Interpretation: Establishment of patient-derived iPSCs-ECs treated with pregnancy sera serves as a novel model to study maternal endothelial dysfunction. Our model demonstrated the impaired endothelial function in preeclampsia, indicating the circulating factors in the preeclamptic sera exert negative effects on endothelial cells. The reduced endothelial function is not attributed by cell death.

Conclusion: This system makes it possible to define the interplay between individual maternal endothelial health and the circulating factors that lead to preeclampsia endothelial dysfunction.
Building the Evidence to Address Disparities in Type 1 Diabetes (BEAD-T1D): Recruitment Practices to Engage Underrepresented Youth and Their Families

Underrepresented youth have less access to diabetes technology research and usage. The BEAD-T1D study aims to build an evidence-based intervention to reduce disparities in diabetes technology uptake in youth aged &lt;12 years, with type 1 diabetes (T1D), and public insurance. We present our strategies to optimize recruitment of underrepresented groups.

A bilingual/bicultural Latino research assistant (RA) was hired to facilitate culturally competent recruitment. The RA screened youth in advance of their clinic visits and coordinated with clinic teams to contact potential participants. The RA introduced the study to families using their preferred language, time of contact, and answered personal concerns around research. Families were given the option to consent in-clinic, in person, or virtually (video or phone call) at a pace set by the parent/guardian to ensure understanding.

56 families (64% Hispanic, 25% Non-Hispanic White [NHW], 2% Non-Hispanic Black [NHB], and 9% Other) were eligible. 14 eligible families were not approached: 6 no showed, 2 provider preference, and 6 clinic dismissals before RA contact. Of 42 approached, 23 consented (age 39+/−9 years; 78% female; 65% Hispanic, 26% NHW, and 9% Other). 19 did not consent: 2 declined (1 NHW and 1 NHB), 13 did not answer calls, and 4 plan to consent in upcoming months. In-clinic approach was important to successful consent: 78% consented in-clinic. Barriers to in-clinic approach for RA included late/no response from providers, care team ending clinic visit, and bandwidth/connectivity issues.

Culturally and linguistically congruent staff, flexible recruitment practices, and prioritizing families’ availability contributed to a study population that is 65% Hispanic, significantly higher than typical T1D clinical trials. Cultural interpersonal relationships formed with our families, addressed barriers to research participation underrepresented families face within and outside of the medical system. Researchers should modify their hiring practices and recruitment protocols to support inclusion of historically excluded families.
Violence, mental health, and HIV: Elucidating key risk factors for poor antiretroviral therapy adherence in adolescent girls and young women in western Kenya

Context/Purpose
Intimate partner violence, poor mental health, and HIV are intertwined threats to the health of adolescent girls and young women aged 15-24 worldwide. Among women who have experienced violence, 50-90% develop mental health conditions, and young women who have experienced more than one episode of violence are 50% more likely to become HIV-infected than those with no violent experiences. To reach global HIV prevention and treatment goals, a better understanding of the complex structural and social barriers to care is urgently needed.

Methods:
Cross-sectional data was collected at urban and rural health facilities in Kisumu, Kenya from January–June 2022 from adolescent girls and young women (AGYW) aged 15 – 24 with a confirmed HIV diagnosis. Surveys included questions on intimate partner violence (emotional, physical, and sexual violence) in the last year, the 9-item Patient Health Questionnaire to assess for depression, and the Center for Adherence Support’s adherence index to assess for antiretroviral therapy (ART) adherence. Adherence was categorized as poor if AGYW received poor scores in the adherence index or if a participant’s latest viral load was &gt;200 copies/mL per CDC guidelines. The association of poor ART adherence with types of violence (physical, emotional, and sexual) and depression were analyzed using logistic regression.

Results/Interpretation:
309 AGYW were recruited to participate. After adjusting for confounding, AGYW with experiences of emotional IPV showed significantly greater odds of poor adherence when compared to AGYW without reported experiences of emotional violence (Odds Ratio [OR]=2.13, 95% Confidence Interval [CI]=1.09-4.18). Those with moderate or severe depression also showed significantly greater odds of poor adherence than those with no depression (OR=2.32, 95% CI=1.06-5.07). Physical and sexual violence were not significantly associated with poor adherence in this sample.

Conclusion:
Understanding what experiences and risk factors are significantly associated with poor adherence allows us to identify potential targets for future interventions.
A hybrid gene correction strategy for Cystic Fibrosis

Cystic fibrosis (CF) is a monogenic disease that affects more than 30000 Americans. While many organs are affected, respiratory complications are the primary cause of death. This disease is caused by mutations in the CF transmembrane conductance regulator gene. While some medications have improved the survival of CF patients, there is still no cure. A potential therapeutic strategy is to fix the problem at the DNA level and repair the defective gene. Our group has previously shown that it is possible to correct the most common mutation by electroporation of editing reagents into airway cells, however autologous airway stem cell transplantation remains a challenge. Therefore, we propose to take this editing approach one step further and edit whole lungs or lobes of lungs ex vivo while in normothermic perfusion and later transplant them into patients. Devices such as the Toronto Ex Vivo Lung Perfusion System are currently used to preserve and assess lungs prior to transplant but can potentially be used for ex vivo gene therapy. First, we need to identify and validate viral and non-viral vectors that can be used to efficiently deliver the editing reagents to lung tissue and confirm that it is possible to genetically edit the lower airway stem cells to ensure durable correction of the defect. We are using precision-cut lung slices (PCLS) prepared from normal lung tissue for the initial validation of reagents and we have already identified lipid nanoparticles that work efficiently in PCLS. Moreover, we are performing selection of AAV vectors from libraries to find serotypes that perform better than AAV6 in PCLS. These reagents should allow editing directly in the lungs to enable clinical translation in a hybrid approach where cells are engineered in situ but avoiding the toxicity observed after systemic administration of vectors designed for in vivo gene therapy.

Background: The rate of cesarean birth was 31.7% in 2019 in the US. Medically indicated cesarean section can be a life-saving procedure, whereas medically unnecessary cesarean can lead to avoidable adverse outcomes. Racial/ethnic disparities in cesarean rates persist; the contribution of inequitable hospital quality of care to this disparity is uncertain.

Objective: Using a population-based dataset of hospital births in California from 2007-2018, we examined the contribution of birth hospital to racial/ethnic disparities in low-risk cesarean births.

Study Design: We used live birth and fetal death certificates linked with maternal birth hospitalization data from California, 2007-18. We examined nulliparous, term, singleton, vertex (NTSV) births. Modified Poisson regression models with a mixed effect for hospital and bootstrapped errors were used to compare racial/ethnic differences in cesarean rate, adjusted for socio-demographic and hospital characteristics. We used G-computation to assess how the rates of NTSV cesarean section by racial/ethnic group would change if all births occurred at the same distribution of hospitals as births to non-Hispanic White individuals.

Results: Among 1,747,312 NTSV births at 214 hospitals, 27% were cesarean (range: 25.3% among US-born Hispanic to 31.6% among Non-Hispanic Black individuals). The adjusted risk ratios of cesarean section ranged from 1.00 (95% CI 0.98-1.04) among US-born Hispanic to 1.27 (95% CI 1.20-1.32) among Non-Hispanic Black individuals, relative to the Non-Hispanic White population. When assuming the same distribution of births across hospitals as for Non-Hispanic White population, rates of NTSV cesarean section ranged from 74 excess events in the non-Hispanic Black population to 6,606 avoided events among US-born Hispanics.

Conclusions: Racial/ethnic disparities in NTSV cesarean section rates in California are not explained by individual-level sociodemographic characteristics or hospital characteristics. Future efforts to reduce low-risk cesarean birth should address potential inequities in quality of care by race/ethnicity and the reduction of inter-hospital variability.
Assessing Treatment of Children with Attention-Deficit/Hyperactivity Disorder: A Novel Application of Machine Learning Methods

Background
Current quality measures for attention-deficit/hyperactivity disorder (ADHD) rely on limited claims-based data that are disconnected from practice guidelines, driving many healthcare organizations to perform costly, labor-intensive chart reviews.

Objective
To develop and test a natural language processing classifier of electronic health records that captures pediatrician adherence to ADHD guidelines, which recommend parent training in behavior management as first-line for young children with ADHD.

Methods
We extracted clinical notes of all office visits of children aged 4-6 years, seen ≥2 times in 2015-2019 in a community-based primary care network, who had ≥1 visit with an ADHD diagnosis (cohort n=423). Two pediatricians annotated notes of the first ADHD visit for each patient. Inter-annotator agreement was assessed for recommendation of behavioral treatment; disagreements (13%) were reconciled. We used a random subset of first-visit notes (n=296, 70%) to train and validate a classification algorithm based on Clinical Bidirectional Encoder Representations from Transformers (Clinical-BERT). The untrained test set of first-visit notes (n=127, 30%) was used to assess model performance compared to manual chart review. We then chose model thresholds to maximize sensitivity (recall) and minimize false negative rate. We completed external validation deploying the model on all other notes of ADHD or well-care visits in the same cohort (other notes=1,020); we annotated all notes the model classified positive (n=50) and 5% of notes classified negative (n=50).

Results
The model achieved acceptable performance (F1=0.75) classifying first ADHD visits (Table 1), capturing low rates of behavioral treatment recommendations (40% of visits). Following threshold selection, external validation yielded improved model performance with recall=0.92 (Table 2) and revealed that pediatricians recommended behavioral treatment in only 5% of non-first-ADHD visits.

Conclusion
Deploying a natural language processing algorithm on a large and variable set of clinical notes could enable scalable and continuous quality measurement of clinical care for ADHD and other chronic conditions.
A cost-effectiveness analysis of gestational carriers for infertile patients of very advanced maternal age

Background: According to SART 2016 national data, ~36% of women begin pregnancy after 40 years of age (very advanced maternal age, vAMA). Both infertility and vAMA have been identified as independent risk factors for adverse pregnancy outcomes. In particular, older women face an increased risk of severe maternal morbidity (SMM), defined by the CDC as an indicator of a life-threatening condition at the time of delivery. Gestational carriers (GCs), who are typically younger and at lower risk for SMM, can carry a pregnancy on behalf of vAMA infertile women in some cases. While GCs involve high up-front costs for patients, SMM is associated with expensive pregnancy-related, long-term costs related to poor maternal and neonatal health outcomes. Objective data to guide decision-making regarding the cost-effectiveness of GC for vAMA infertile patients are lacking.

Objective: To analyze the cost-effectiveness of embryo transfer to a vAMA infertile patient versus to a GC in order to calculate the cost per quality-adjusted life year (QALY).

Materials and Methods: A decision analysis tree was constructed to compare a single embryo transfer (donor oocyte, without preimplantation genetic testing) to vAMA infertile patients versus to GCs. Model inputs including live birth and SMM rates, costs (US$, healthcare perspective), and QALYs (maternal perspective incorporating neonatal prematurity) were derived from the literature and national data registers. Three age cohorts (40-44, 45-49, and 50-54), each with 1,000 theoretical patients, were studied over their lifetimes to account for possible long-term health impacts of SMM. The incremental cost-effectiveness ratio (ICER=US$/QALY) for each age cohort was the main study outcome. One-way sensitivity analyses were also performed to vary the cost of using a GC and the QALY impact due to SMM given the heterogeneity of clinical indicators.

Results: Embryo transfer to a GC was cost-effective across all three age cohorts given all ICERs fell below the willingness-to-pay threshold of $100,000/QALY, suggesting economic utility. Additionally, using a GC was increasingly cost-effective with advancing age, as evidenced by ICERs that progressively decreased from age 40-44 ($44,898/QALY) to age 45-49 ($34,082/QALY) to age 50-54 ($28,863/QALY). One-way sensitivity analyses confirmed that embryo transfer to a GC remained cost-effective over a range of GC costs from $85,000 to $190,000 and a range of QALY impacts due to SMM from more severe to less severe clinical indicators (0.60-0.99).
Conclusions: vAMA infertile patients face higher risk of SMM, and in some cases, a GC may be considered to optimize maternal outcomes. Our study shows that a GC is a cost-effective option to consider for vAMA infertile patients across a range of age groups, GC costs, and SMM clinical indicators.
EXAMINING SEX-LINKED TRENDS IN THE PLACENTAL TRANSCRIPTOME DURING THE FIRST WINDOW OF FETAL ANDROGEN PRODUCTION

Objectives
Sex differences in human placenta exist from early pregnancy to term, however it is unclear what proportion of prenatal sex differences can be ascribed to sex chromosome complement versus male fetal androgens. Here we survey the first trimester chorionic villus (CV) transcriptome for sex-linked signatures that vary in males from 11 to 16 gestational weeks, corresponding to the window of escalating androgen production in male fetuses.

Methods
Illumina HiSeq RNA sequencing was performed on Lexogen Quantseq 3' libraries derived from human CV biopsies. Differential expression (DE) was determined using a Limma-voom pipeline ($\alpha<0.05$), followed by post-hoc linear regressions to identify transcripts that trend over time ($R^2 \geq 0.30, m \geq +/-0.04$).

Results
Overall we observe 322 transcripts DE between male (n=11) and female (n=11) placentas in the time window from 11 to 16 weeks. Contrary to our predictions, the difference between male and female expression in the DE set was more pronounced at the earlier gestational ages (11-13.5 weeks) vs. late (13.5-16 weeks), $p=0.026$ (for $\geq$2x DE transcripts $p=0.0006$). We also identify 23 transcripts that show linear changes in expression across this window in males only (n=8), in both sexes in the same direction (n=11), and both sexes in the opposite direction (n=4). No female-specific trends were found. The majority of transcripts that varied in males showed decreased expression from week 11 to 16 (n=9).

Conclusion
Sex differences in the placental transcriptome are pronounced in the late first trimester, and contrary to our prediction, these differences begin to converge moving into the second trimester. Only a subset of DE genes show distinct trends in expression in males over this window suggesting that fetal androgens may not be the main drivers of sex differences in placentation, and that sex chromosome complement is likely to play a primary role.
Factors Associated with Opioid Use Disorder following Spinal Fusion for Adolescent Idiopathic Scoliosis

Context/Purpose: Post-operative pain management following spinal fusion (SF) for Adolescent Idiopathic Scoliosis (AIS) typically involves opioids. Postoperative analgesia is often a child or adolescent’s first opioid exposure, we sought to describe associations between OUD and analgesic prescribing practices following SF for AIS.

Methods: We identified patients ages 10-18 undergoing primary SF for AIS between 2007-2015 in the IBM® MarketScan® Truven Data Mart. Demographic variables and prescription patterns (in morphine milligram equivalents [MMEs]) were analyzed to determine any potential association with OUD in the 1 year following SF.

Results: We identified 5,366 operative AIS cases (75.4% Female, 24.6% Male), of which 120 patients (2.2%) developed an OUD. AIS patients were prescribed on average 597.5 (+/− 601.9) total MME and 62 (+/− 47.1) MME/day post-operatively. Our multivariable model demonstrated no association of total MME, MMEs per day, or number of refills prescribed with the subsequent development of OUD within one year of SF. Presence and number of complex chronic conditions (CCC) diagnosed were significantly associated with development of an OUD (OR=1.76, p=0.021). Geographic Region was also associated with rates of development of OUD (p<0.001); operative AIS patients in the Northeast were more likely to be diagnosed with an OUD compared to North Central (p=0.005), South (p=0.002) and West (p=0.005) regions. No association was found between OUD and sex, age, reoperation rates, post-operative complications, or number of levels fused during surgery.

Interpretation: Although prescriber variability is especially present after posterior spinal fusion for AIS, the quantity of opioids and refills prescribed after the index surgery were not significantly associated with the development of opiate use disorder. Patients with a prescription for a tranquilizer or antipsychotic had a significantly increased risk of development of an OUD.

Conclusion: We used a multivariable model to demonstrate that variability in opioid prescribing is not associated with subsequent risk of development of OUD.
Development of hKIT Chimeric Antigen Receptor T-Cells as Dual Hematopoietic Stem Cell Transplantation Conditioning and Immunotherapeutic Agents for Cure of Pediatric Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is a frequent subtype of cancer that affects thousands of children and adults worldwide. Current treatments generally consist of harsh, conventional chemotherapy and/or hematopoietic stem cell transplantation (HSCT); therapies that cause significant morbidity and mortality, but often do not cure the disease. Unfortunately, AML patients experience an overall 5-yr survival rate of only 50%. Leukemic stem cells (LSCs) have been shown to perpetuate and maintain AML, and standard chemotherapies targeting the bulk of AML blasts tend to minimally effect LSCs. In this project we aim to leverage longstanding work on non-myeloablative conditioning strategies to develop a novel array of chimeric antigen receptor (CAR) T cells that target the hKIT receptor expressed by human HSCs, LSCs, and leukemic blasts in order to improve AML treatments and facilitate long lasting remissions. We developed hKIT CAR-T that cells exhibited a range of anti-HSPC and anti-leukemic cytotoxicity in vitro with complementary IL2 and IFNg production. While heavy and light chain orientation did not significantly impact CAR expression, we did observe significant differences in expansion kinetics, T-cell phenotype distribution, and exhaustion marker profile. Our results indicate that hKIT CAR-T cell therapy may be a tractable strategy for elimination of HSPCs and AML cells. Future studies will expand this approach in vitro and in vivo with AML blasts and leukemic stem cells to determine the role of anti-hKIT immune pressure in leukemia initiation. We will also directly compare the impact of CD28 vs. 41BB intracellular signaling domains on in vitro and in vivo anti-leukemia activity to adequately guide the translational development of this targeted dual function non-genotoxic conditioning regimen with anti-leukemic effects.
Dynamic dual control mechanism in children with ADHD: deficits in learning from performance feedback and trial history

Cognitive control deficits are a hallmark of childhood Attention Deficit Hyperactivity Disorder (ADHD) but the underlying component mechanisms still remain unknown. Theoretical models posit that cognitive control involves two distinct reactive and proactive control modes but their distinct roles in ADHD are not known, and the contributions of proactive control remain vastly understudied. Here we decompose behavioral performance on two cognitively challenging tasks into multi-componential measures. Specifically, we investigated three different conditions where typical developing (TD) children are capable of adapting response strategies based on their knowledge of task contexts and learning from performance feedback and trial history.

Eighty children (9-12 years old), including 50 children with ADHD (16F/34M) and 30 TD children (14F/16M) completed a stop-signal task (SST) and a conditional SST. Proactive control driven by context, feedback and anticipation from trial history was examined by rule modulation in conditional SST, post-error slowing in the SST, and Dynamic Belief Model in the SST, respectively. Reactive control was measured using stop-signal reaction time (SSRT) in the SST.

We found that children with ADHD can apply context-driven proactive control just like TD children, but they have significant deficits in implementing proactive control based on their learning from performance and trial history compared to TD children (p<0.05). Children with ADHD also have longer SSRTs than TD children (p<0.01). Moreover, while TD children exhibit interrelatedness between proactive and reactive control functions, such coordinated dual control mechanisms were not present in children with ADHD. Last, not only dual control functions are associated with behavioral problems in ADHD, multi-dimensional features derived from dynamic dual control framework can predict clinical symptoms, such as inattention and hyperactivity/impulsivity, in the unseen data.

Together, our findings provide novel insights in the dual control mechanism in childhood ADHD and the multi-componential cognitive measures can serve robust predictor for clinical symptoms.
Improving youth helmet safety with liquid shock absorbers

Context/Purpose: Repeated head impacts and concussions are commonly sustained by youth athletes. Recent advances in shock absorber technology have yielded soft, collapsible, hydraulic shock absorbers, with simulation models suggesting promising improvements in reducing brain injury risk if implemented into American football helmets.

Methods: Drop test impacts at two velocities (2.5, 3.2 m/s) and two masses (2.51, 3.81 kg) were performed on a prototype hydraulic shock absorber and seven technologies currently being used for impact attenuation in American football helmets. Impact tests were conducted at low (-18ºC), ambient (19ºC), and high temperatures (50ºC). High-speed video and force were recorded for each impact. Comfort pads in a youth football helmet were replaced with the prototype hydraulic shock absorbers while the rest of the helmet was left as designed by the manufacturer. The prototype helmet and an unmodified control helmet were fit to an anthropomorphic headform while a pneumatic linear impactor delivered impacts to the helmeted headform at two velocities (3.5, 5.0 m/s) and four locations (Side, Side Upper, Oblique Front, Oblique Rear). Linear and angular head kinematics were recorded for each impact.

Results: The hydraulic shock absorber exhibited superior performance in dissipating impact energy across all impact conditions (average 98%) and maintained consistent performance across low, ambient, and high temperatures (variations smaller than 3%). Upon implementation to the youth football helmet, Head Acceleration Response Metric, a brain injury risk function that factors both linear and angular head kinematics, was reduced by 21% overall when compared to the standard helmet.

Interpretation: Hydraulic shock absorbers offer considerable advantages over existing shock absorbing technologies in their ability to dissipate impact energy and attenuate the severity of head impacts representative of youth American football concussions.

Conclusion: Soft, collapsible, hydraulic shock absorbers exhibit promise as a new technology for improving the safety of youth American football helmets.
Care practices for birthing parents with COVID-19 and their infants at a tertiary-level teaching hospital in California, USA

Background: The safety of breastfeeding and birthing parent-infant contact were of grave concern as the COVID-19 pandemic exploded. A tertiary children’s hospital in California, USA, implemented a shared decision-making approach for rooming-in, skin-to-skin contact, and breastfeeding for the population positive for COVID-19.

Aims: To assess the safety of the care practices for the infant, breastfeeding outcomes, adequacy of inpatient lactation support, and infection prevention teaching for the obstetric population positive for COVID-19.

Method: This was a retrospective study using medical record review and follow-up telephone surveys. The study evaluated the cases of infant infection, and compared the rates of breastfeeding, skin-to-skin contact, lactation consultations, and breast stimulation between the COVID-19, positive and negative, obstetric populations from April to October, 2020.

Results: Forty-four dyads participated in this study, 95.5% (42/44) identified as Hispanic and 90.9% (40/44) roomed-in. Only 2% (1/44) of the infants tested positive for COVID-19 after 24 hours of life. Both rates of exclusive breastfeeding (40.9%, 18/44) and skin-to-skin contact (54.5%, 24/44) in the study population were significantly lower than those of the negative obstetric populations. In the post-discharge surveys, 100% (38/38) of the birthing parents reported they received adequate teaching and 86.8% (33/38) confirmed they were able to follow the instructions.

Conclusion: Birthing parent-infant contact and breastfeeding with infection prevention precautions were associated with infants 98% (43/44) free of COVID-19 infection. COVID-19 impacted breastfeeding establishments. Hands-on hospital lactation support needed. Discharge teaching that focuses on addressing barriers to infection prevention could benefit this population during their isolation periods.
The Antimicrobial Peptide LL-37 Promotes Penetration of Antibiotics through Infected Sputum

Cystic Fibrosis (CF) is an inherited autosomal recessive genetic disease that results in progressive multisystem disease and mortality. The buildup of thick mucus in the lung airways impairs bacterial clearance and allows for colonization by bacterial pathogens. Inhaled antibiotics are critical for managing chronic pulmonary infections in CF; however, little is known about how environments in CF lung airways impacts antibiotic diffusion. Pseudomonas aeruginosa (Pa) is one of the most common pathogens cause infections in CF airways. We previously identified a role for Pf bacteriophage as a structural element in Pa biofilms. In particular, Pf phage organizes host and bacterial polymers present in sputum that encases P. aeruginosa and promotes antibiotic tolerance. We also reported that Pf phage are abundant and highly prevalent in the lungs of individuals with CF. This is associated with increased bacterial colonization and worsening respiratory function. Through a Fluorescent Recovery after Photobleaching assay (FRAP), diffusion of various inhaled antibiotics were determined to understand how Pf phages and polymers affect antibiotic diffusion. Then isothermal calorimetry assay (ITC) was used to determine the interaction between phage, polymers, and antibiotics. Finally, FRAP and ITC were used to understand how to reverse phage and polymers’ interactions with antibiotics. We reveal that Pf phage organize sputum in ways that prevent the diffusion of antibiotics as well as reduce their antimicrobial efficacy. Moreover, we demonstrate that while Pf phage can attenuate the antimicrobial effects of LL-37, an antimicrobial peptide abundant in sputum, LL-37 disrupts Pf phage-mediated organization of sputum biofilms, enhances antibiotic penetration into biofilms, and improves antibiotic killing efficiency. Our study suggests that LL-37 and Pf phage have opposing effects on antibiotic diffusion and efficacy in sputum where LL-37 promotes antibiotic diffusion in biofilm and Pf phage disrupts antibiotic diffusion in biofilm.
Provider Experiences and Perspectives of Family Presence During Procedures for Children with Cardiac Disease

INTRODUCTION: The mother of a critically ill patient who had already watched her son undergo several resuscitations and invasive procedures asked if she could watch an endoscopy her son was to undergo to look for the source of a GI bleed. When she was told that she could not, she asked, “why not?” With growing national dialogue about transparency in healthcare, there is an increasing number of families requesting transparency of, and access to, what happens in the OR.

To better understand the potential impact of FP for pediatric cardiac procedures we conducted a qualitative study to examine how clinicians caring for children with cardiac disease perceive and experience FP.

METHODS: We conducted semi-structured, in-person, video-conference interviews of clinicians involved in procedural care of children with congenital or acquired heart disease at a quaternary care pediatric heart center. Three of the researchers conducted the data analysis to enhance credibility and dependability using content analysis.

RESULTS: Twenty clinicians were interviewed between August 2021 and June 2022. The inter-rater reliability for coding was 0.95. Four primary themes emerged: (i) the perceived benefit to patients of FP, (ii) the perceived burdens or challenges associated with FP, (iii) the limitations associated with achieving FP, (iv) and the policies, procedures, and processes associated with the implementation of FP. These themes and several subthemes are discussed.

CONCLUSIONS: Both benefits and barriers were identified. The benefits of FP may have variable impact on different patient sub-populations, underlying family emotional states, and severity of the clinical context. Medical-legal concerns, already heightened around peri-operative care, will need to be addressed so clinicians feel comfortable being observed. The perspectives and experiences of clinicians highlight the need for clear policies, procedures and staff support around FP if it is to be successful within the context of a heart center.
Utilization, Safety, and Efficacy of Telemedicine in Patients Presenting with Pediatric Lower Urinary Tract Symptoms (pLUTS) Before and During the COVID-19 Pandemic: A Single Tertiary Care Center’s Experience

Context/Purpose: Telemedicine for pediatric lower urinary tract symptoms (pLUTS) is a relatively new mode of delivering bladder health education with scant evidence supporting current practice. We reviewed our institution’s pLUTS-related care patterns specific to visit type (telemedicine versus in-person) during the initial COVID-19 pandemic to inform development of virtual bladder health education programs in our community.

Methods: We conducted a retrospective cohort study of new pLUTS patients at our institution’s pediatric urology clinics. Demographic variables and wait times were collected and compared before and after March 2020. In-depth chart review was performed if a patient underwent an initial telemedicine visit followed by an in-person visit to identify missed radiology, lab or physical exam findings.

Results: 612 patients total were seen for a pLUTS referral diagnosis from September 2018 to August 2021. 305 patients were seen in the pre-COVID period (before March 2020), and 307 patients were seen in the post-COVID period (after March 2020). Most were 5-10 years old (62.3%), female (56.2%), English-speaking (86.5%), White (39.4%) and had private insurance (67.2%). Average wait times were shorter for telemedicine (43.0 days) versus in-person (61.6 days). 11 patients (mean age = 10.4 years) underwent an in-depth chart review. 81.8% (9) had a comorbid condition and/or family history of LUTS. In-person visits revealed no missed clinical findings that changed management.

Interpretation: pLUTS care can be delivered via telemedicine without a significant change in our total volume or patient population. Overall, wait times remain high. Future qualitative work is needed to understand the needs of our non-English speaking patients and patients with comorbid conditions. The in-person exam can be omitted safely, supporting programs that address the volume and delays in care in our community.

Conclusion: Our findings support the continued use of telehealth visits for PLUTS-related care in our clinic and the development of virtual bladder health education programs.
No Increased Risk of Cognitive Delay at 3 in Children with Prenatal Zika Virus Exposure

Although ZIKV targets neural stem cells in the developing brain, neurological manifestations are not apparent at birth in the majority of ZIKV-exposed children. Were they protected from ZIKV neurotropism or do cognitive delays manifest as they age? We investigate this question in a large cohort of ZIKV-exposed and unexposed children in Grenada, West Indies. 384 mother–child pairs were enrolled during a period of active ZIKV transmission (April 2016–March 2017) and prospectively followed up at 12 (n=66), 36 (n=58), and 48 months (n=59). Child exposure status was based on laboratory assessment of maternal serum. Main outcome measures included the Oxford Neurodevelopment Assessment (OX-NDA), NEPSY® Second Edition (NEPSY®-II), Cardiff Vision Tests, and anthropometrics. Across ZIKV-exposed and unexposed infants, there were no differences in rates of neurodevelopmental delay at 12, 36 and 48 months. Visual acuity and contrast sensitivity did not differ between ZIKV-exposed and unexposed children at 36 and 48 months. Both groups had similarly low rates of microcephaly at birth (3% v 2%, F=0.96, p=0.33), as well as similarly low rates of stunting (0-11%) and wasting (2-10%). Delivery complications, male sex, younger maternal age, and lower levels of partner education were found to be significantly associated with neurodevelopmental delay, independent of ZIKV-exposure status. In our cohort, the majority of ZIKV-exposed children appeared to be protected from the effects of ZIKV neurotropism with normal neurodevelopmental trajectories from 12 months up to at least 4 years of age. This is reassuring as the absence of delays beyond this age lowers the risk for serious neurocognitive and mental health morbidity. Moreover, the association between perinatal and socioenvironmental indicators and neurodevelopmental delay was independent of prenatal ZIKV exposure, emphasizing the importance of the nurturing care model in offering the greatest impact on children’s developmental potential.
Building Enhancer-Gene Regulatory Maps of the Noncoding Genome for the Developing Human Heart

Genetic variation plays a key role in the etiology of single ventricle heart disease (SVD). Most candidate genetic variants are in non-protein coding regions of the genome, where they may affect the functions of enhancers that control gene expression in specific cell types during development. Human genetic studies have now identified thousands of candidate genetic variants, each of which could reveal molecular insights into heart development and the causes of SVD. However, several important questions remain unanswered, including: 1) which cell types and genes are critical for human cardiac development, 2) which of the known genetic variants are truly causative for SVD and, ultimately, 3) which cellular pathways are impaired by these genetic variants, as these may represent novel therapeutic targets. To address these questions and link variants to functions, a comprehensive regulatory map is required of all genes and enhancers active in human heart development at deep temporal and cell type/state-specific resolution.

We have applied single-cell RNA-sequencing and ATAC-sequencing to generate an initial atlas of >70,000 cells in 12 structurally normal human fetal hearts ranging from 7-21 weeks post conception. We have identified at least 27 cell types and states. Using these data, we have built cell-type specific enhancer-gene maps using the Activity-by-Contact (ABC) model. We used these maps to predict the regulatory effects of distal enhancers and single-nucleotide variants on gene expression by intersecting these enhancer maps with disease variants associated with SVD or quantitative measurements of heart morphology. These variant-to-function maps highlight numerous cases where noncoding risk variants appear to disrupt the functions of cell-type specific regulatory elements. Overall, this work will advance our fundamental knowledge of heart development by cataloguing cardiovascular cell states throughout development and providing a data-driven, unbiased approach to identify key cell types, genes and genetic variants that influence risk for SVD.
Stanford Medicine Participant Engagement Platform (PEP): supporting Maternal/Child Health research participant recruitment

The Research Participation Program (RPP) provides resources and tools to help study teams meet their participant recruitment goals, with a focus on engaging patients as partners in research. Services include Direct Email, Children’s Epic MyChart, Postal Mail, and Stanford Research Registry outreach. RPP partners with study teams to develop and send study invitations to potential participants identified using the Stanford Research Repository (STARR). Thanks to a close partnership with MCHRI and the Clinical Research Support Office (CRSO), RPP launched Children’s Epic honest broker MyChart services, Bulk MyChart messages, and Best Practice Alerts (BPAs) in 2020.

Figure 1. Almost half (54/114; 47.4%) of our consults are for maternal/child health studies, Stanford Children’s Epic MyChart is our most utilized honest broker service (24/114; 21.1%). From March 2020 to the present, we are thrilled to report a 3.1% interest rate for our Children’s Epic MyChart outreach, meaning 984 patients have responded “I’m interested” out of a total of 31,510 invitations. If we combine Direct Email and Postal Mail invitations with MyChart, we have sent a total of 81,087 invitations for maternal/child health studies, with 1,441 responding with interest for a rate of 1.8%. Direct Email applies to studies that include healthy controls, which does not imply the recipient has a certain condition, in compliance with HIPAA. In addition, our Stanford Research Registry supports maternal/child health studies. Out of 9,430 registry participants, 3,610 (38%) are under 18 years old, many of which were enrolled due to interest in pediatric COVID vaccine trials. Finally, our expanded partnership with StudyPages to include up to 100 non-COVID studies also provides participant recruitment and retention support for maternal/child health studies.

Please reach out to us at EngageParticipants@stanford.edu for a one-hour free consultation, during which time PEP honest broker services can be explored and questions answered.
Biomechanical Comparison of Single Semitendinosus 4 Strand Graft Versus Combined 4 Strand Semitendinosus + Gracilis Graft for ACL Reconstruction

Background
Tissue selection is an important aspect of ACLR. Due to lower rates of autograft failure in young, active patients, autograft may be preferred over allograft. Studies analyzing HT graft configurations for ACLR show comparable biomechanical properties to BPTB and QT autografts. Both ST-only and ST+GR autografts have much higher load to failure than the native ACL, suggesting that either of these graft options may be reasonable for ACL reconstruction. The purpose of this study is to compare the biomechanics of 4-strand ST-only grafts with 4-strand combination ST+GR grafts.

Methods
Twenty-four fresh-frozen HT allografts were used, with 16 ST and 8 GR tendons. In one group, the STs were halved with each half folded to create eight 4-strand ST-only constructs (ST-only). In the second group, the ST was single-folded and combined with a single-folded GR to make eight 4-stranded combination construct (ST+GR). Testing was performed for load to failure (10 mm/sec), stiffness, elongation at failure, and cyclical elongation, which were analyzed for statistical significance using unpaired two-tailed t-tests (P = .05).

Results
Load to failure was not significantly different (P=0.64) between the ST-only (1689.84 N; SD=129.74) and ST+GR (1626.59 N; SD=347.35) constructs. Elongation at failure was significantly different (P=0.01) between ST-only (5.67 mm; SD=1.34), and ST+GR (7.42 mm; SD=0.92) constructs. Stiffness (modulus of elasticity) was also significantly different (P=0.007) between ST-only (458.99 N/mm; SD=58.27) and ST+GR (364.67 N/mm; SD=61.12) constructs. Cyclic elongation was not significantly different (P=0.89) between the ST-only (0.259 mm; SD=0.509) and ST+GR (0.256; SD=0.506) constructs.

Conclusion
The ST-only autograft may be favored over a ST+GR option, as it has superior stiffness and elongation characteristics, harvests less tissue, and preserves one of the postero-medial thigh muscles. For surgeons and patients who prefer HT ACL graft options, this information may optimize outcomes.
Revealing the impact of lifestyle stressors on the risk of adverse pregnancy outcomes with multi-task machine learning

Introduction: Psychosocial and stress-related factors (PSFs) are potentially modifiable and accessible targets for intervention that are associated with adverse pregnancy outcomes (APOs). Although APOs are relatively infrequent and therefore challenging to model, multi-task machine learning (MML) is an ideal tool for exploring the interconnectedness of APOs and building on joint combinatorial outcomes to increase predictive power. By integrating single cell immunological profiling of underlying biological processes, the effects of stress-based therapeutics may be measurable, facilitating the development of precision medicine approaches.

Methods: In a prospective cohort study, PSFs were assessed during the first trimester with an extensive questionnaire for 200 women. We used MML to simultaneously model and predict APOs (severe preeclampsia, superimposed preeclampsia, gestational diabetes, early gestational age) and risk factors (BMI, diabetes, hypertension) for these patients based on PSFs. Strongly interrelated stressors were categorized to identify potential therapeutic targets. Furthermore, for a subset of 14 women, we modeled the connection of PSFs to the maternal immune system to APOs by building corresponding ML models based on an extensive single cell immune dataset generated with mass cytometry time of flight (CyTOF).

Results: Jointly modeling APOs in a MML setting significantly increased modeling capabilities and yielded a highly predictive integrated model of APOs underscoring their interconnectedness. Most APOs were associated with mental health, life stress, and perceived health risks. Biologically, stressors were associated with CD4/CD8 T cells and signaling pathways including JAK/STAT (STAT1, STAT3, STAT5), MyD88 (ERK), and CREB. Immune characteristics predicted based on stress were in turn found to be associated with APOs.

Discussion: Elucidating connections among stress, APOs, and immune characteristics has the potential to facilitate the implementation of ML-based, individualized, integrative models of
pregnancy in clinical decision making. The modifiable nature of stressors may enable the development of accessible interventions, with success measured through immune characteristics.
Longitudinal Characterization of Neonatal Health and Morbidity using Electronic Health Records and Machine Learning

Introduction: While prematurity is the largest cause of death in children under 5 years, the current definition of prematurity, based on gestational age, lacks the precision needed for guiding care decisions. Here we propose a longitudinal risk assessment for adverse neonatal outcomes in newborns based on a machine learning algorithm that uses electronic health records (EHRs) to predict a wide range of outcomes over a period starting shortly after the time of conception and ending months after birth.

Methods: By linking the EHRs of the Lucile Packard Children's Hospital and the Stanford Healthcare Adult Hospital, we developed a cohort of 32,354 mother-newborn dyads delivered between 2014 and 2020. From the EHR system, we extracted conditions, medications, laboratory measurements, procedures and clinical notes for each mother and newborn in the cohort. Based on these data, we trained, tested, and validated a multi-input multi-task deep learning model, featuring a long short-term memory neural network, to predict 24 different neonatal outcomes (assessed using conditions extracted from the EHRs). Predictive ability was assessed using the area under the ROC curve (AUC).

Results: The 24 outcomes varied in prevalence from 46.4% (jaundice) to 0.07% (periventricular leukomalacia). AUCs of the machine learning model to predict neonatal outcomes at delivery exceeded 0.9 for 10/24 outcomes considered and were between 0.8 and 0.9 for seven additional outcomes. One week before delivery the AUC was higher than 0.9 for death and ROP, and between 0.8 and 0.9 for 12/24 additional outcomes.

Discussion: To date, this is the largest study utilizing linked EHRs from mother-newborn dyads providing an important resource for the investigation of associations between maternal features and neonatal outcomes. We developed the first longitudinal clinical risk prediction tool for various neonatal outcomes.
Anatomical and functional maturation of the mid-gestation human intestine

Gastrointestinal (GI) maturation is a key determinant of survival for extremely preterm infants. The enteric nervous system (ENS) controls GI motility, and immature GI motility limits enteral feeding and causes severe health complications. Due to the significant challenges in obtaining and studying human fetal tissue, little is known about when the human ENS becomes mature enough to carry out vital functions. Here we define the progressive anatomical maturation of the human fetal ENS and analyze GI motility in the second trimester of in utero development. We identify substantial structural changes in the ENS including the emergence of striped neuronal cytoarchitecture and a shift in the representation of excitatory and inhibitory neurons. We further analyze and pharmacologically manipulate GI motility in freshly collected human fetal intestines, which, to our knowledge, is a first functional analysis of intact human fetal organs ex vivo. We find that the ENS influences GI motility beginning at 21 postconceptional weeks (PCW), the earliest reported evidence of neurogenic GI motility. Our study provides unprecedented insight into human fetal ENS development, foundational knowledge which facilitates comparisons with common animal models to advance translational disease investigations and testing of pharmacological agents to enhance GI motility in prematurity.
Cross-tissue assessment of local and peripheral immune determinants of preeclampsia

Preeclampsia (PE) is a severe pregnancy complication that affects 3-5% of all pregnancies worldwide and significantly impacts maternal and child mortality and morbidity. To date, no effective diagnostic test can predict PE before the onset of clinical symptoms. Dysregulation of pregnancy-induced immune adaptions are increasingly implicated in PE pathogenesis and provide a promising strategy to identify predictive factors of PE. In this study, 225 blood samples were collected in a cohort of 21 pregnant women for longitudinal analysis of immune trajectories (suspension mass cytometry) to differentiate normotensive (n=14) and preeclamptic (n=7) pregnancies. Up to 11 blood samples were collected between gestational age (GA) week 12 and 40, at monthly intervals during 1st and 2nd trimester and every other week during 3rd trimester. In addition, paired placental tissue (FFPE) was collected at delivery for the analysis of the local immune landscape using imaging mass cytometry (IMC). Increased CD44 expression by T cell subsets over gestation of PE-affected pregnancies suggests the presence of an activated and antigen-experienced systemic immune system while increased PD-L1 expression by innate and adaptive immune cells during late-second and early-third trimester (GA week 24-32) suggests concomitant immunoregulation. Moreover, increased CPT1a expression in T cells proposes increased fatty acid oxidation utilization during pregnancies affected by PE. Furthermore, we report on the development and optimization of a high-dimensional IMC assay that allows the simultaneous evaluation of 44 markers on intact tissue. This will support the assessment of local immune cell distribution, activation, and spatial organization at the feto-maternal interface. Coupling this with peripheral blood immune activity will provide an in-depth characterization of PE immune pathogenesis. Given the global impact of PE, this work is a key step towards defining mechanistic targets from blood-based early diagnosis of PE and guide potential therapeutic interventions for PE, thereby improving mother and child health.
E-cigarette Access and Age Verification Among Youth Under 21

Introduction
Several regulations aim to mitigate the adolescent and young adult e-cigarette epidemic, including raising the federal minimum age of tobacco sales to 21 years and improving age verification practices such as showing driving licenses or restricting home delivery. However, we do not know whether and how adolescents and young adults over and under age 21 are accessing e-cigarettes in light of these regulations.

Methods
An online, national cross-sectional survey of 13–40-year-olds was conducted in Nov-Dec 2021 (n=6,131; 59.7% under 21 years). We used quota sampling for age, sex, and race/ethnicity. Among those who had ever used any type of e-cigarette, we assessed: 1) source of obtaining and buying e-cigarette products; 2) whether age was verified at the time of purchase; and 3) how age was verified, by age group under 21 (U21) and 21 and above (21+).

Results
Our sample included 2,536 (41.7%) participants who had ever used a nicotine e-cigarette, including 1,334 U21 ever-users. Similar to e-cigarette sources among participants 21+, U21s reported getting products in retail stores (47.8%); from someone they know (35.1%); online (19.3%) home delivery apps (10.9%); social media including Snapchat, TikTok, and Instagram (10.3%); and other (3.6%). Participants U21 bought e-cigarettes from smoke shops (28.5%), vape shops (27.9%), gas stations (17.7%), convenience stores (14.2%) among other retail locations. Age was not verified for 24.6% of participants U21 and 14.6% of participants U21 did not know whether their age was verified. Age was verified for U21s in the following ways: 45% showed ID, 13.5% uploaded ID online, 8.0% provided an email login to verify ID, and 8.6% showed someone else’s ID, and 4.9% showed a fake ID.

Conclusions
Current e-cigarette age verification practices do not prevent e-cigarette sales to underage youth in retail stores, online, and social media, warranting more effective and enforced policies.
Abnormal activation in the frontal-parietal-basal ganglia systems in children with ADHD: meta-analytic research of attention, inhibition and working memory?

Cognitive control deficits are landmarks of childhood ADHD. Functional neuroimaging studies have highlighted dysfunction in the frontal-parietal-basal ganglia systems during cognitive performance in children with ADHD. However, there is lack of systematic investigation about whether these findings are consistent and replicable across independent studies. To address these challenges, we conducted a meta-analysis of functional neuroimaging studies in children with ADHD with focuses on three core cognitive domains: attention, inhibition and working memory (WM).

Literature search on PubMed, ScienceDirect, and Google Scholar have led 16 studies of attention (286 ADHD, 271 Controls), 23 studies of inhibition (567 ADHD, 519 Controls), and 9 studies of WM (279 ADHD, 307 Controls). Activation likelihood estimation are conducted using GingerALE.

Children with ADHD, in comparison to controls, showed increased activity in salience network for inhibition and WM, but no such effect for attention (p < 0.01, FWE corrected). We did not find significant activation increase in controls than ADHD for each domain after FWE correction, suggesting weak consistency across previous findings which is likely due to small number of studies and small sample size in early studies. So, a more lenient threshold was used below (p < 0.01, uncorrected). Children with ADHD showed increased activation than controls in sensory-motor and default-mode regions across three domains. Interestingly, this increase in ADHD showed different lateralization effects in the prefrontal cortex across domains, with bilateral activation for attention, left for WM, and right for inhibition. Children with ADHD showed reduced activation than controls in regions implicated in cognitive control, including salience, frontal-parietal, and default-mode network areas across three domains, suggesting under-development of cognitive control systems.

In conclusion, children with ADHD have consistent functional abnormalities in the cognitive control system encompassing the salience, frontal-parietal, and default-mode networks. Future research with larger sample size is needed to replicate these findings.
Achieving safer school drinking water: Considerations for school drinking water testing improvements based on a California case study

Context/Purpose: Reports of unsafe school drinking water across the United States, including the Flint water crisis, highlight the importance of ensuring the water provided to students is safe to drink for their health and development. This is the first comprehensive examination of drinking water quality in public schools, including lead at the American Academy of Pediatrics (AAP) standard (1ppb) and other common contaminants (copper, nitrate, arsenic, hexavalent chromium).

Methods: Eighty-three schools from a representative sample of 240 California public schools participated in water quality sampling. School administrators collected samples from three unique food service area drinking water sources after stagnation overnight and after running water for 45 seconds. Our analysis included these results and school demographic characteristics from publicly available databases.

Results: While our sampling found no state action level violations for arsenic or nitrate, 4% of schools exceeded either the proposed 10 parts per billion (ppb) action level for hexavalent chromium, or the 1300 ppb action level for copper. Of first draw lead samples, 4% of schools exceeded the California action level of 15 ppb, 18% exceeded the Food and Drug Administration’s bottled water standard of 5 ppb, and 75% exceeded the AAP 1 ppb standard. After running water, 2%, 10%, and 33% of schools exceeded the same standards, respectively.

Interpretation: Our sampling reports lead in drinking water findings similar to previous California studies and found limited problems for the other contaminants.

Conclusion: Although there is no safe level of lead exposure, enforcing stricter lead action levels lower than 5 ppb, would lead to a significantly larger proportion of schools and districts to balance the cost of remediation tradeoffs. Recent policy changes provide a critical opportunity to require universal implementation of a school drinking water testing database to better evaluate such tradeoffs nationally.
The Impact of a Water Promotion and Access Intervention on Elementary School Students in the Presence of Food and Water Insecurity

Context:
Implementation of school-based water access and promotion interventions have been associated with reductions in sugar sweetened beverage (SSB) intake and childhood obesity. Experiencing food insecurity or in-school water insecurity may impact adoption of healthy behaviors encouraged by drinking water interventions.

Methods
The Water First drinking water access and promotion clustered randomized controlled trial enrolled 26 San Francisco Bay Area elementary schools. Impacts of the intervention were monitored through student height, weight, and student-reported dietary intake via 24-hour recalls. Students (n=1057) reported experiences of food insecurity and water insecurity over the previous year. Mixed-effects linear and logistic regression evaluated the interaction between both food insecurity-intervention-time and water insecurity-intervention-time relative to weight status, and food and beverage intake.

Results
There was a trend for interaction among food insecurity and the drinking water promotion intervention with water intake over time (p=0.06). There was a statistically significant interaction among water insecurity, the intervention and time on overweight status (p=0.02). Trends were also present among water insecurity and the intervention over time with SSB intake (p=0.07) and water intake (p=0.08).

Interpretation:
Counter to anticipated results, higher levels of food insecurity were marginally associated with increased drinking water rates in students receiving the drinking water promotion intervention compared with controls over the study period. As hypothesized, students reporting no water insecurity were significantly more likely to have reduced rates of overweight status and marginally more likely to have reduced SSB intake as a result of the intervention.

Conclusion
Future investigations of nutrition interventions in the presence of food and water insecurity are needed to better understand this interaction and inform the design of interventions that comprehensively impact childhood obesity.
Investigating genes within the 22q11.2 deletion region as potential risk factors for Parkinson's disease in human iPSC models.

Adults with genomic deletions on chromosome 22q11.2, which include a 3 Megabase genomic region with approximately 41 protein-coding genes expressed in the human brain, have a higher risk of developing typical Parkinson’s disease (PD), however, it is unclear which of these genes contribute to the neurodegenerative process.

The goal is to identify genes in the 22q11 deletion region that contribute to causal molecular mechanisms of neuronal dysfunction and predispose to a neurodegenerative process reminiscent of PD due to diminished gene dosage. (i) Using human induced pluripotent stem cells (iPSCs), we investigate whether iPSC-derived dopaminergic neurons from 22q11DS patients replicate pathophysiological phenotypes associated with PD. (ii) Implementing CRISPR perturbation that will allow us to identify biological targets and pathways.

We show successful differentiation of human iPSCs from 22q11DS patients and controls to neurons that express genetic markers for midbrain dopaminergic identity and neuronal maturation. In iPSC-derived neuronal progenitors, we found no differences in reactive oxygen species (ROS), mitochondrial dysfunction, or endolysosomal activity in cultures from 22q11DS compared to controls. By contrast, we detected increased levels of ROS in neurons (30 days in vitro) derived from 22q11DS patients compared to controls. Both mitochondrial activity and density were altered in 22q11DS neurons. We further detected enhanced endolysosomal activity with Lysotracker in 22q11DS neurons. We currently optimize CRISPR perturbation in 22q11DS and control iPSCs to reversibly regulate gene expression and systematically increase protein levels of deleted genes in the 22q11.2 deletion in human iPSC-derived neurons to identify causative genes.

Mitochondrial phenotypes could only be detected in iPSC-derived post-mitotic dopaminergic neurons from patients with 22q11DS and not in corresponding neuronal progenitor cells. Our findings are consistent with data from 22q11DS mouse models indicating increased ROS levels in cortical projection neurons and replicate mitochondrial phenotypes found in human iPSC-derived dopaminergic neurons and animal models of PD.
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Adults with genomic deletions on chromosome 22q11.2, which include a 3 Megabase genomic region with approximately 41 protein-coding genes expressed in the human brain, have a higher risk of developing typical Parkinson’s disease (PD), however, it is unclear which of these genes contribute to the neurodegenerative process. The goal is to identify genes in the 22q11 deletion region that contribute to causal molecular mechanisms of neuronal dysfunction and predispose to a neurodegenerative process reminiscent of PD due to diminished gene dosage. (i) Using human induced pluripotent stem cells (iPSCs), we investigate whether iPSC-derived dopaminergic neurons from 22q11DS patients replicate pathophysiological phenotypes associated with PD. (ii) Implementing CRISPR perturbation that will allow us to identify biological targets and pathways.

We show successful differentiation of human iPSCs from 22q11DS patients and controls to neurons that express genetic markers for midbrain dopaminergic identity and neuronal maturation. In iPSC-derived neuronal progenitors, we found no differences in reactive oxygen species (ROS), mitochondrial dysfunction, or endolysosomal activity in cultures from 22q11DS compared to controls. By contrast, we detected increased levels of ROS in neurons (30 days in vitro) derived from 22q11DS patients compared to controls. Both mitochondrial activity and density were altered in 22q11DS neurons. We further detected enhanced endolysosomal activity with Lysotracker in 22q11DS neurons. We currently optimize CRISPR perturbation in 22q11DS and control iPSCs to reversibly regulate gene expression and systematically increase protein levels of deleted genes in the 22q11.2 deletion in human iPSC-derived neurons to identify causative genes.

Mitochondrial phenotypes could only be detected in iPSC-derived post-mitotic dopaminergic neurons from patients with 22q11DS and not in corresponding neuronal progenitor cells. Our findings are consistent with data from 22q11DS mouse models indicating increased ROS levels in cortical projection neurons and replicate mitochondrial phenotypes found in human iPSC-derived dopaminergic neurons and animal models of PD.
Objective
To assess cabergoline’s efficacy at decreasing lactation after second-trimester abortion or loss.

Methods
This is a double-blinded, block-randomized superiority trial (IRB approved, NCT04701333) comparing cabergoline 1mg once to placebo for preventing bothersome breast engorgement after second-trimester uterine evacuation. April 2021-June 2022, we enrolled pregnant people 18-28-weeks gestation, English- or Spanish-speaking, without contraindication to the study drug. Participants completed a validated, piloted, electronic survey at baseline and through two weeks post-procedure assessing breast symptoms, side-effects, and bother at each time point. Our primary outcome is breast symptoms on day 4; we planned to enroll 80 patients to show a 30% difference in breast symptoms (80% power, α = 0.049). A sub-group of participants returned for serum prolactin levels.

Results
After screening 150 patients, we enrolled 73 participants. Baseline demographics were balanced between groups: median gestational age 21 weeks (range: 18-26), 56% nulliparous, 35% self-identified as Hispanic, 37% with public insurance.

At baseline, reported breast symptoms were similar between groups. At day 4, significantly fewer participants receiving cabergoline reported any symptoms compared to placebo (27.8% vs 97.0%, p<0.0001) and fewer reported significant bother (2.8% vs 29.7%, p=0.002). These differences persisted through day 14.

Reported side-effects (p=0.31) were similar between groups: most common were constipation (44%), fatigue (32%), and headache (29%).

Serum prolactin was similar at baseline. On day 4, mean serum prolactin was 6.5ng/mL (std dev 2.2) for those receiving cabergoline and 18.0ng/mL (std dev 5.9) for placebo (p=0.04).

Conclusion
Cabergoline is an effective strategy to prevent breast symptoms following second-trimester abortion or loss.
Ped-BERT: Early detection of disease for pediatric care

Early detection of diseases has been shown to increase the quality and effectiveness of pediatric care, but there is still a high unmet need in this area. A unique healthcare database and the latest developments in bidirectional encoder representations from transformers (BERT) allow us to propose a model that accurately predicts the likelihood of a couple of conditions in a child’s next visit. Precisely, we link location and health information about the mother in the prenatal period to child hospital discharge data and emergency room visits for California hospitals between 1991 and 2017 for 765,561 mother-child pairs. We pre-train our Ped-BERT model specification using a masked language model (MLM). We then use the learned disease embeddings as features in a downstream task to predict the diagnosis code in the next visit with high accuracy (area under the receiver operator curve [AUROC] = 0.913 and average precision score [APS] = 0.223). Finally, we show that we can improve the model performance (AUROC) of our downstream task by 1.23 percent by adding information on levels of PM2.5 pollution exposure in the zip code at birth.
Misoprostol and Abortion in the Developing World: A meta-synthesis exploring the experiences and perspectives of women and healthcare providers using only misoprostol for abortion in the developing world

Unsafe abortion is a leading cause of maternal mortality, and nearly all unsafe abortions occur in developing countries. One opportunity to reduce morbidity and mortality is misoprostol, an abortion pill. Previous systematic reviews established misoprostol’s clinical efficacy, barriers, and facilitators of abortions in developed countries. This review attempted to bridge the gap in literature surrounding the experiences and perspectives of women and providers who use misoprostol for abortion in developing countries. A systematic search was conducted across five electronic databases and the grey literature, with 1436 records identified and screened. Fifteen primary qualitative or mixed-method studies met the predetermined inclusion criteria. They were critically appraised using the CASP checklist and data was extracted using a modified JBI qualitative data collection form. The data was synthesized using Noblit and Hare’s meta-ethnographic guidelines. Six third-order themes emerged following reciprocal translation and were grouped into three categories. Themes of improved agency and access was categorized under empowerment, with women reporting misoprostol as private and affordable. Social inequalities and socially informed perceptions were categorized under social influence, with marginalized women at risk of exploitation and women and providers basing expectations of the drug on personal encounters with friends and patients. Finally, the fact that information both mediated experiences and was impeded by traditional abortion barriers produced the category of information flow and showed that women and providers require more knowledge surrounding correct misoprostol use. In conclusion, misoprostol granted women privacy and reproductive autonomy from partners and physicians and enabled them to create a socially acceptable narrative about their abortion. It was affordable and accessible but marginalized women remained exploitable. Further, information about correct misoprostol use was not as accessible as the drug. Stigma and legislation continued to restrict such information, preventing misoprostol from reaching its full potential as a consistently safe abortion option.
Which Osteochondritis Dissecans Subjects Will Heal Nonoperatively? An Application of Machine Learning Methods to the ROCK Cohort

Osteochondritis dissecans (OCD) is a focal idiopathic alteration of subchondral bone and/or its precursor with a risk for instability and premature osteoarthritis. There are limited evidence-based guidelines to predict which lesions will heal with non-operative treatment. This study aims to train a classification algorithm to determine whether a patient with OCD of the knee will heal with nonoperative treatment from intake visit characteristics.

Methods:
Subjects from the Research in OsteoChondritis of the Knee (ROCK) prospective cohort were excluded from the study if data were missing. They were included if they met definitions for either success or failure of nonoperative treatment: failure of nonoperative management was defined as the crossover from to surgery at any point at or beyond three-month follow-up. Successful healing was defined as complete healing on imaging with full return to sports participation.

A suite of machine learning algorithms was tested, and the best-performing models were selected based on AUC and raw classification accuracy. Each model type was hyperparameter tuned with five-fold cross-validation repeated ten times.

Results:
The current study population includes 81 subjects. The logistic regression model developed by previous studies had a cross-validated accuracy of 65.3% and an AUC of 0.645. Our logistic regression model had a cross-validated accuracy of 71.2% and an AUC of 0.750. A generalized boosted classifier had the highest cross-validated accuracy and AUC of any model at 74.8% and 0.762, respectively. Normalized lesion width was the most important variable in the generalized boosted model, followed by lesion location in the posterior sagittal zone, age, the presence of mechanical symptoms, and normalized lesion length.

Conclusions:
Machine learning models can predict which OCD lesions will heal with nonoperative management. In the future, model performance should be tested on new, prospective patient data and compared against clinician judgment to validate its performance.
Background: Adolescent girls and young women (AGYW) face substantial barriers in seeking care for gender-based violence (GBV) worldwide. These barriers are greater in low-resource settings, where reporting and care options may be more limited. The COVID-19 pandemic and its associated pandemic spread control policies have led to global increases in GBV, as well as decreased access to resources and dissolution of pathways to care, especially in low-resource settings and for vulnerable groups like AGYW.

Methods: Using a community-partnered participatory research (CPPR) approach, focus group discussions (FGDs) with AGYW were completed to understand how COVID-19 affected experiences of AGYW, including experiences of diverse types of gender-based violence (GBV), healthcare services availability, mental healthcare, and economic and social outcomes. Opportunities for prevention and interventions to mitigate negative impacts of COVID-19 and violence were also investigated.

Results: 5 FGDs were completed in June-September 2021 with 46 AGYW. Girls were split into age-matched groups; average age for older groups was 20.9 and 17.4 for younger groups. AGYW described violence as both a cause and effect of poor economic, social and health outcomes related to the pandemic. AGYW described increases in all types of GBV discussed, particularly child sexual abuse, intimate partner violence (including observed parental violence and in their own relationships). Early marriage and subsistence transactional sex were also increased due to pandemic economic disruptions. COVID-19 also disrupted referrals to all violence-related services, with closure of services, as well as increased corruption within such services, such as experiences of authorities soliciting money or sexual favors during reporting processes. AGYW were asked about what types of interventions they believed to be feasible and acceptable in mitigating the effects of increased violence; girls were interested in economic interventions in order to prevent violence, and thought improving mental health services for trauma would best help intervene in pre-existing situations of violence.

Discussion: AGYW reported disturbing increases in numerous types of GBV, and decreased access to services during the COVID-19 pandemic. As there is no evidence that issues like violence and mental health challenges will quickly resolve, and the pandemic stretches on, there is an urgent need to
identify and implement interventions to mitigate these negative effects. Next steps of this work are to identify and secure funding to implement, and ideally research, an intervention with peer (or near-peer) led mental health services, and enhanced referral systems, in this population.
Maladaptive Myelination Promotes Generalized Epilepsy Progression

Activity-dependent myelination is a newly appreciated form of brain plasticity which influences neural network function, enabling adaptation. Myelin plasticity involves oligodendrogenesis (proliferation and maturation of oligodendrocyte precursor cells, OPCs). This requires brain-derived neurotrophic factor (BDNF) signaling through its receptor, TrkB, on OPCs as well as epigenetic changes that can be blocked with histone deacetylase inhibitors. The relationship between pathological neuronal activity (seizures) and myelination is unknown. We hypothesized that generalized absence seizures might induce aberrant activity-dependent myelination that contributes to pathological network change and epilepsy progression. We used Scn8a+/mut mice which develop absence seizures during well-defined periods of seizure progression (increasing seizure frequency). We used unbiased stereology, electron microscopy and MRI to assess oligodendrogenesis and myelin structure within the thalamocortical seizure network. We generated Scn8a+/mut mice with conditional and inducible deletion of TrkB specifically from OPCs (Scn8a+/mut; TrkBfl/fl; PDGFRA::Cre-ER mice, designated as Scn8a+/mut OPC cKO. In separate studies, Scn8a+/mut mice were treated with the histone deacetylase inhibitor trichostatin A (TSA). Seizures were quantified with EEG. Callosal oligodendrogenesis was increased in association with seizures in P45 Scn8a+/mut mice; these changes were absent at earlier time-points before seizure onset. Decreased callosal g-ratios, indicative of thicker myelin sheaths per axon diameter were observed in Scn8a+/mut mice with established seizures. Genetic blockade of activity-dependent myelination in Scn8a+/mut cKO mice prevented aberrant myelination, thalamocortical hypersynchrony assessed with EEG coherence, and significantly decreased seizure progression. Pharmacological blockade of activity-dependent myelination in Scn8a+/mut mice with TSA, initiated after seizure onset, prevented subsequently increased oligodendrogenesis and decreased seizure burden. A subset of these findings was recently published in Nature Neuroscience (Knowles et al, May 2022). Myelination induced by absence seizures can become maladaptive and contribute to epilepsy progression. Maladaptive myelin plasticity may be a novel therapeutic target to decrease seizure burden in some types of epilepsy.
Cross-linking of erythrocyte CD44 promotes host cell clustering and enhances Plasmodium falciparum invasion

Malaria is caused by Plasmodium parasites, with most cases of severe, multi-system disease caused by Plasmodium falciparum. Children and pregnant women are at highest risk, partly because they lack adequate immunity. P. falciparum invasion of red blood cells (RBCs) requires several host-parasite interactions, some of which have potential as therapeutic or vaccine targets. Previously, we identified CD44 as a new RBC host factor for invasion, yet its function is unclear. Through affinity purification, we identified EBA175 and EBA140 as parasite ligands for CD44. We generated CD44-null cultured RBCs (cRBCs) from primary hematopoietic stem cells using CRISPR/Cas9 and ex vivo erythropoiesis, and found that P. falciparum invasion was substantially reduced in these cells compared to isogenic wildtype (WT) cRBCs. We next tested monoclonal antibodies (mAbs) targeting CD44 for their impact on P. falciparum invasion. Surprisingly, we found that none inhibited invasion, including IM7, which blocks the interaction between CD44 and its canonical ligand hyaluronic acid. Instead, we identified one anti-CD44 mAb, BRIC 222, that significantly enhanced P. falciparum invasion in a dose-dependent manner. BRIC 222 F(ab) fragments had no impact on invasion, suggesting that the observed effect was due to CD44 cross-linking. These results are reminiscent of work by Paing et al. (2018) showing that shed EBA175 promotes P. falciparum invasion and induces RBC clustering through Glycophorin A (GYPA). We found that CD44 cross-linking by BRIC 222 induced a similar RBC clustering phenotype. However, clustering assays in WT versus CD44-null cRBCs showed that EBA175-induced clustering is CD44-independent. We found that both BRIC 222 and EBA175 led to increased RBC surface phosphatidylserine externalization, a hallmark of cryptoplasia. Based on our findings, we speculate that RBC CD44 may function as an environmental sensor during malaria infection that the parasite exploits to promote invasion and evade immunity, potentially in parallel to the EBA175-GYPA interaction.
RREB1 loss-of-function reduces expression of human adipocyte genes

Diabetes and obesity are global health concerns that can affect individuals as early as the neonatal period and mothers during pregnancy. Genetic variation in the Ras Responsive Element Binding Protein 1 (RREB1) locus has been associated with several glycemic and metabolic traits, including type 2 diabetes and waist-hip ratio. Previously, the transcription factor RREB1 has been shown to recruit epigenetic modifiers and activate expression of brown fat genes. However, the role of RREB1 and genetic variation at the RREB1 locus in white fat formation is unknown. We hypothesize that RREB1 transcriptionally regulates expression of adipogenic genes involved in white fat development. siRNA-mediated knockdown of RREB1 in pre-adipocyte Simpson-Golabi-Behmel Syndrome (SGBS) cells significantly decreased expression of adipocyte genes PPARG, ADIPOQ, and CEBPA. RNA-seq and differential expression analysis identified 383 upregulated and 204 downregulated genes following RREB1 knockdown in SGBS-derived adipocytes (fold change \(\geq 2\), \(\text{padj} < 0.05\)). Gene Ontology revealed that the downregulated genes were enriched for adipocyte-related pathways, including ‘lipid homeostasis’, ‘response to insulin’, and ‘developmental process’, consistent with RREB1 loss-of-function decreasing adipocyte differentiation. To address a potential developmental role for RREB1, we next differentiated genome edited RREB1 wildtype and homozygous knockout human induced pluripotent stem cells (hiPSC) to adipocytes in vitro. RREB1-/- hiPSC-derived adipocytes had significantly decreased expression of PPARG and CEBPA. Conversely, there was a four-fold increase in expression of the osteoblast gene SPP1 in RREB1-/- adipocytes, consistent with a de-repression of genes involved in bone formation. RREB1-/- hiPSC-derived osteoblast cells also had increased expression of SPP1. Our current data supports RREB1 as a positive regulator of adipocyte genes and suggests that in the absence of RREB1, genes of other cell lineages, such as bone, can be activated.
Delineating the Epilepsy Phenotype of NGLY1 Deficiency via a Natural History Study

Objective: To define the electroclinical phenotype of epilepsy in NGLY1 deficiency via prospective clinical international natural history study.

Background: N-Glycanase 1 (NGLY1) deficiency is a rare autosomal recessive disorder of deglycosylation. NGLY1 deficiency is characterized by global developmental delay, hypo- or alacrima, transient transaminitis, hyperkinetic movement disorder, and mixed polyneuropathy. In published cases, 48% (23/48) reported comorbid epilepsy with limited details. Early diagnosis and treatment of epilepsy can impact both development and the risk of adverse events.

Methods: We performed prospective phenotyping of 29 participants with NGLY1 deficiency via standardized clinician interviews every 4 months of medical, developmental and seizure history. Seizure and medication history were confirmed with prior records. 15 subjects also underwent in-person annual evaluations including EEG. Descriptive statistics are provided for the first two years this natural history study.

Results: 59% (17/29) of participants had a history of epilepsy, with mean seizure onset at 43 months. The most common seizure types included myoclonic (53%) and atonic (47%). 80% (12/15) of participants had EEG abnormalities during onsite evaluations. EEG background was otherwise normal without slowing. Commonly used antiseizure medications were valproate, levetiracetam, lamotrigine, and clobazam. 35% (6/17) of participants achieved complete seizure control. There were trends but no significant differences in neurodevelopmental assessment outcomes between participants with and without epilepsy.

Conclusions: We highlight a significant risk of epilepsy in NGLY1 deficiency. Seizure semiology is varied, with predominant myoclonic and atonic seizure types with onset commonly in early childhood. EEG abnormalities are non-specific and indicate a risk of epilepsy, but most patients do not have EEG slowing, which is a correlate of encephalopathy. Seizures often require treatment with multiple medications. Valproate and clobazam were hepatically tolerated. Providers should educate caregivers about varied seizure types to ensure prompt detection and treatment of epilepsy.
Impact of Fetal Sex on Associations Between Drugs and Adverse Pregnancy Outcomes

Purpose:
Sex differences play a role in adverse outcomes of pregnancy with higher numbers of males among spontaneous preterm births (PTBs) and with a higher mortality rate. The mechanisms that cause these disparities are not completely understood. More than 7 million medications are prescribed in pregnancy each year. While unexplored, fetal sex differences may be involved in responses to drug treatments during pregnancy, one example being antenatal glucocorticoid treatment. Whether a disparity by fetal sex exists in associations between adverse pregnancy outcomes and medication exposures is unknown.

Methods:
A singleton pregnancy cohort of linked moms, newborns and outpatient medication intake during pregnancy was established from IBM MarketScan® databases. Odds ratios (ORs) for risk of PTB and stratified by the newborn sex were evaluated. Multiple hypothesis testing and the Benjamini–Hochberg procedure that limited false discovery rate at 5% were used to identify significant associations that were then compared for pregnancies with boys versus girls.

Results:
Among 1,221,992 identified mom-baby pairs, 51.5% pregnancies had a male newborn. PTB prevalence was 6.5% with higher prevalence for boys (55.1%). Among 547 medications, 80 had statistically significant ORs. Five medications had the most significant sex differences: gabapentin (boys: OR [95% CI] = 1.22 [0.93, 1.61]; girls: 2.12 [1.66, 2.71]), oxycodone (boys: OR = 1.33 [1.24, 1.43]; girls: 1.56 [1.44, 1.68]), labetalol (boys: OR = 3.02 [2.85, 3.19]; girls: 3.56 [3.35, 3.77]), fluconazole (boys: OR = 1.07 [1.01, 1.12]; girls: 1.20 [1.14, 1.27]) and nystatin (boys: OR = 2.04 [1.91, 2.17]; girls: 1.06 [0.96, 1.16]).

Conclusions:
Data-driven approaches can effectively generate new hypotheses on sex differences in associations between medications and PTB. While unknown confounders may impact these findings, our results indicate that disparities exist for certain medications, as their odds ratios associated with PTB depend on the fetal sex and will be further investigated.
Macrophage lysosomal alkalinization drives invasive aspergillosis in a mouse cystic fibrosis model of airway transplantation

Pediatric Cystic fibrosis (CF) lung transplant recipients (LTRs) exhibit a disproportionally high rate of life-threatening invasive aspergillosis (IA). Loss of the cystic fibrosis transmembrane conductance regulator (CFTR-/-) in macrophages (mφs) has been associated with lysosomal alkalinization. We hypothesize that this alkalinization would persist in the iron-laden post-transplant microenvironment increasing the risk of IA. To investigate our hypothesis, we developed a murine CF orthotopic tracheal transplant (OTT) model. Iron levels were detected by immunofluorescence staining and colorimetric assays. Aspergillus fumigatus (Af) invasion was evaluated by Grocott methenamine silver staining. Phagocytosis and killing of Af conidia were examined by flow cytometry and confocal microscopy. pH and lysosomal acidification were measured by LysoSensor and Lysotracker respectively. Af was more invasive in the CF airway transplant recipient compared to the WT recipient (p<0.05). CFTR-/- mφs were alkaline at baseline, a characteristic that was increased with iron-overload. These CFTR-/- mφs were unable to phagocytose and kill Af conidia (p<0.001). Poly(lactic-co-glycolic acid) (PLGA) nanoparticles acidified lysosomes, re-storing the CFTR-/- mφs’ ability to clear conidia. Our results suggest that CFTR-/- mφs’ alkalinization interacts with the iron-loaded transplant microenvironment, decreasing the CF-mφs’ ability to kill Af conidia, which may explain the increased risk of IA. Therapeutic pH modulation after transplantation could decrease the risk of IA.
Impact of a one-session educational program on high schoolers’ e-cigarette knowledge, perceptions, and refusal skills

Purpose: Educational programs which promote knowledge of e-cigarette harms and addictiveness play an important role in preventing adolescent e-cigarette initiation and use. This study evaluates the impact of a school-based e-cigarette educational session on high school students’ e-cigarette knowledge, perceptions, and refusal skills as part of a broader effort to inform the development and evaluation of a school-based curriculum based on the Stanford Tobacco Prevention Toolkit.

Methods: Researchers at Stanford University partnered with a public health professional who implemented a 60-minute vaping prevention curriculum at a high school. The study was a one arm pre-post design in which students (N =322; Mage = 16.1) completed pre- and post-test assessments of e-cigarette knowledge, perceptions, and refusal skills. Matched pairs t-tests and McNemar tests of paired proportions were applied to assess changes in study outcomes.

Results: Following the curriculum, participants perceived e-cigarettes as more addictive (p < .001), more harmful to their lungs (p<.001), and less effective at reducing stress (p < .001). Participants also indicated it would be easier to say no to a friend if offered an e-cigarette (p <. 001). However, students showed improvement on only one out of three questions assessing e-cigarette knowledge.

Interpretation: Following the curriculum, high school students indicated changes in their perceptions of e-cigarettes and tobacco companies which generally align with an anti-e-cigarette profile. However, students did not demonstrate improved knowledge of the definition of addiction or how often nicotine must be used to become addicted, highlighting addiction as a topic which should be clarified in future implementations.

Conclusion: The e-cigarette curriculum was associated with positive changes in high school students’ perceptions of e-cigarettes. Future evaluations of the curriculum will apply more rigorous causal designs and also assess long-term changes in adolescents’ e-cigarette knowledge, attitudes, perceived control, intentions, and use.
Bioenergetic dysfunctions in neurons carrying the 22q11.2 deletion

The 22q11.2 microdeletion syndrome (22q11.2 DS) affect roughly 1:4000 individuals. Some of the most common clinical manifestations include congenital heart disease and immune system dysfunction. Later in life, neuropsychiatric diseases become apparent. Rapid medical advances have improved the survival of affected individuals through highly specialized prenatal and neonatal care. Still however, life-long neuropsychiatric morbidities remain common and a substantial challenge.

The 22q11.2 chromosomal region contains 9 genes associated with mitochondrial function. In vitro models derived from human induced pluripotent stem cells (hiPSC) showed mitochondrial dysfunction in monolayer human forebrain neurons, and calcium signaling defects and neuronal hyperexcitability in human cortical spheroids (hCS) carrying the 22q11.2 deletion.

In the current study we use human cortical spheroids (hCS) derived from hiPSC to identify metabolic and mitochondrial phenotypes in neural progenitors versus post-mitotic neurons. Using untargeted metabolomics we identified substantial metabolic differences in intracellular and secreted metabolites between 22q11.2 DS and controls. Functional analysis of the mitochondrial respiration using Seahorse assay and 13C based metabolic flux analysis revealed abnormal mitochondrial function and energy production, starting as early as the progenitor stage of development. Together, these data suggest early mitochondrial and metabolic phenotypes during brain development in neural cells carrying the 22q11.2 deletion.
Preterm twin gestation: The association of severity of small for gestational age and neonatal outcomes

Objective: Twin gestations account for a substantial proportion of preterm, small for gestational age (SGA) neonates. Evidence is limited on SGA severity and outcomes by twin status and birth order. We investigated the associations between SGA severity and birth outcomes among twins using a contemporary, population-based cohort.

Study Design: This is a prospective cohort study of the California Perinatal Quality Care Collaborative database of NICU patients from 2008-2018. We included early preterm (24-32 weeks), non-anomalous births with weight for gestational age (WGA) < 10th percentile. Based on fetal growth restriction criteria, we considered severe SGA as < 3rd percentile and moderate SGA as 3rd to < 10th percentile. The primary outcome was a composite of major neonatal morbidity or mortality. Secondary outcomes included: cesarean birth, intubation, Apgar score < 4 at 5 minutes, neonatal delivery room death, and infant death. We compared outcomes among twins by SGA severity, and estimated the associations between WGA z-score and the outcomes stratified by twin status and birth order.

Results: Among 3,524 neonates, 690 (20%) were twins, of which 154 were severe SGA and 536 were moderate SGA. Compared to neonates with moderate SGA, severe SGA neonates had significantly higher rates of major morbidity and mortality, infant death, delivery room death, and Apgar score < 4 (p-values ≤ 0.001, Table 1). An increase in WGA z-score was associated with a significant decrease in major morbidity or mortality for singletons, twin A and twin B (aRR 0.66, 0.61 and 0.69, respectively, Table 2). Within each stratified group, strong relationships existed between WGA z-score and major neonatal morbidity or mortality, delivery room death, and Apgar score < 4. An increase in WGA had a protective association with infant death for singletons and twin B, but not twin A (aRR=0.45, 95% CI 0.16-1.31, Table 2).

Conclusion: SGA severity was associated with a higher risk of morbidity and mortality among early preterm twin neonates. Neonatal outcomes in relation to SGA severity were similar despite twin status or birth order.
Does magnesium sulfate for hypertensive disease reduce the risk of hypoxic ischemic encephalopathy?

Objective: Maternal hypertension (HTN) is a known risk factor for neonatal hypoxic ischemic encephalopathy (HIE). We hypothesize that the use of magnesium for maternal seizure prophylaxis in HTN will reduce the risk of HIE in an at-risk population.

Study Design: Analysis of a prospective cohort within the California Perinatal Quality Care Collaborative (CPQCC) registry, which includes infants with qualifying conditions, and is linked with the California Department of Public Health Vital Statistics birth cohort. We included live births ≥36 weeks’ gestation, born 2012-2019, and excluded delivery room deaths or those with unknown HIE status. Normotensive patients were compared to patients with HTN alone or HTN who received magnesium sulfate treatment. Hierarchical logistic regression models were built for any HIE and moderate or severe HIE to explore if maternal HTN with and without magnesium sulfate treatment were independently associated with the outcomes.

Results: 51,062 unique infants met inclusion criteria. In the adjusted model, infants of hypertensive mothers without treatment with magnesium had a statistically significant increase in any severity of HIE (OR 1.26, 95% CI 1.01-1.57, p=0.04) and moderate or severe HIE (OR 1.27, 95% CI 1.05-1.54), compared to normotensive patients. In contrast, infants of hypertensive mothers treated with magnesium did not have increased rates of any severity of HIE, when compared to normotensive patients. In a second adjusted model, utilizing patients with HTN as the comparison, those with HTN treated with magnesium had no difference in any HIE or moderate or severe HIE.

Conclusion: Maternal HTN is associated with any HIE and moderate or severe HIE. However, treatment of maternal HTN with magnesium sulfate may reduce the risk of HIE, despite being a higher risk subset. Studies are needed to elucidate the causal role of magnesium sulfate in decreasing severity of HIE in high-risk neonates.
Exploring the Reproductive Black Market: Family Planning Medications on the Dark Web

OBJECTIVE: This study aims to characterize the illegal trading of family planning medications on the dark web—a segment of the Internet not accessible by routine web browsers.

MATERIALS AND METHODS: A database of reproductive medications was created using a list of medications created from Micromedex with the terms “female reproductive agents” (n=77) and “male reproductive agents.” (n=20). Veterinary reproductive medications were also included. Synonyms of terms (e.g., common, chemical, and trade names) were identified and included using PubChem. With a total of 1,193 terms, we conducted a word-matching search for family planning medications (i.e., estrogens, progesterone/progestins, misoprostol, and mifepristone) implementing a crawler through Python (a technique to index large volumes of dark web content) to solicit data from anonymous online marketplaces (n=10) and forums (n=6). From 2011-2015, we extracted data on vendor name, product, price, advertised origins, and acceptable shipping destinations.

RESULTS: Family planning medications were present in 3 anonymous marketplaces (i.e., Alphabay, Agora, Evolution). There were 25 family planning listings (20% included multiple medications, n=5/25) from 10 anonymous marketplaces listings and 15 family planning-related forum traces. There were 6 unique family planning suppliers identified. The most common class of family planning medications encountered were estrogens (n=22) followed by progesterone/progestins (n=14), and prostaglandins (i.e., “misoprostol” n=10, $6.9/mg). Notably, thirteen listings used the generic term “birth control”. Estrogens included “estradiol” (n=16, $0.6/mg) and “ethinyl estradiol” (n=6, $6/mg). Progesterone was listed four times ($1.92/mg) as well as the following progestins: “levonorgestrel” (n=4, $2.67/mg), “desogestrel” (n=4, $0.12/mg), and “dienogest” (n=2, $0.33/mg). One listing for “misoprostol” specifically mentioned the Combipack (i.e., combination of misoprostol and mifepristone). Most listings shipped to worldwide destinations from unknown origins.

CONCLUSIONS: Due to stigma, limited access, and increasingly restrictive legislation, patients will continue to resort to illegal means of obtaining family planning medications despite significant risks of using drugs without a prescription (e.g., counterfeit, contamination, and presence of untested substances).
Inequity of in-hospital lactation support for Spanish-prefering birthing parents: a qualitative needs assessment

Introduction: At Lucile Packard Children’s Hospital Stanford, roughly one-third of birthing parents are Hispanic, only 54% of whom exclusively human milk feed upon discharge as compared to 83% of White parents. This disparity is greater among Spanish-prefering parents. In 2021, the use of pasteurized donor human milk (PDHM) was also lower for Spanish speakers; 9% compared with 16% for English-prefering parents. We explored perceptions of lactation and experiences with in-hospital lactation support.

Methods: Qualitative needs assessment conducted March-May 2022 included a site visit where nurses, lactation consultants, clinicians, and parents were interviewed and observed (n=15). We also interviewed Hispanic birthing parents (English- and Spanish-prefering) (n=17) and pediatricians (n=5). Data were analyzed using thematic analysis.

Results: Parents expressed positive attitudes towards intention to breastfeed. Postpartum however, milk supply concerns often led parents to supplement, a cultural practice referred to as “las dos cosas”. Consistency of supplementation advice also varied, with many receiving little support on expressing their own milk. Notably, Spanish-prefering parents received less support/advice, particularly around PDHM. Ipad interpreters were inconsistently used with Spanish-prefering parents. When used, barriers remained, including difficulty understanding one another and accessing iPads.

Conclusion: When faced with milk supply concerns, Spanish-speaking patients opted for formula supplementation due to minimal lactation support and inconsistent offering of PDHM when supplementation was medically indicated. Improved interpreter use is needed to overcome language barriers. Additionally, expectation setting and education on PDHM use in lieu of formula should be incorporated prenatally.
Influence of climate and environment on the effectiveness of water, sanitation, and handwashing interventions on diarrheal disease in rural Bangladesh

Background: Climate change may influence the effectiveness of environmental interventions. We investigated if climate and environment modified the effect of low-cost, point-of-use water, sanitation, and handwashing (WASH) interventions on diarrhea.

Methods: We analyzed data from a cluster-randomized trial in rural Bangladesh that measured diarrhea prevalence in children 0-2 years from 2012-2016. We matched remote sensing data on temperature, precipitation, humidity, surface water, and land use to households by location and measurement date. We estimated prevalence ratios (PR) for any intervention vs. control stratified by environmental factors using generative additive models and targeted maximum likelihood estimation. We estimated intervention effects under predicted precipitation in the study region in 2050 for climate change scenarios from different Shared Socioeconomic Pathways (SSPs).

Findings: WASH interventions more effectively prevented childhood diarrhea under higher levels of total precipitation in the previous week and when there was heavy rain in the previous week (heavy rainfall PR = 0.38, 95% CI 0.23-0.62 vs. no heavy rainfall PR = 0.77, 0.6-0.98). We did not detect substantial effect modification by other variables. WASH intervention effectiveness increased under most climate change scenarios; in a fossil-fueled development scenario (SSP5-8.5), the PR was 0.46 (0.44-0.48) compared to the 0.67 (0.65-0.68) in the study.

Conclusion: WASH interventions had the strongest effect on early childhood diarrhea under higher precipitation, and effectiveness may increase under climate change without sustainable development. WASH interventions may improve population resilience to climate-related health risks.
Patient-Derived Colon Organoids Recapitulate Mitochondrial Dysfunction in Pediatric Ulcerative Colitis

Background: A lack of preclinical human models limits drug testing abilities targeted at sustaining remission via epithelial healing in UC. Epithelial mitochondrial dysfunction has been implicated in UC pathogenesis. Patient-derived organoids recapitulate several complexities of the parent tissue, but it is unclear if they can model mitochondrial functional impairment observed in the UC epithelium.

Objective: To determine whether mitochondrial impairment observed in pediatric UC is modeled in patient-derived colonoids.

Method: We generated colonoids from rectal biopsies obtained from pediatric UC patients with active disease (Mayo score>1) and non-IBD controls. Cryopreserved colonoids (P3-P5) were thawed, passaged once, and differentiated for 3 days. We assessed oxygen consumption rates (OCR) using the Seahorse (XFe96) MitoStress Test. Mitochondria Membrane Potential (MMP) was measured with JC-1 uptake flow Cytometry and ROS production was determined using Mitochondrial Superoxide (MitoSox) analysis.

Results: UC colonoids had a significantly higher Basal OCR compared to controls. This was associated with an 85% increase in proton leak (P =0.015) in UC colonoids compared to controls. ATP-linked respiration was slightly higher (27%) in UC colonoids compared to controls (P= 0.037). The percentage of OCR coupled to respiration (coupling efficiency) in UC colonoids was significantly lower than that in control colonoids (P=0.019). The bioenergetic health index (BHI), which estimates a patient’s composite mitochondrial health profile, was significantly lower in UC colonoids compared to controls (P=0.018). JC-1 uptake analysis showed 31% lower MMP in UC colonoids compared to controls (P=0.027). Kinetic MitoSox uptake analysis revealed a significant increase in ROS production in UC colonoids compared to the control (P=&lt;.001). These mitochondrial impairments in UC colonoids may be due to increased uncoupling activity, as we observed a significant increase in Ucp2 gene expression in UC colonoids compared to control, and this was supported by rectal bulk RNA-seq data from the PROTECT Pediatric UC inception cohort study.

Conclusion: Colonoids from active pediatric UC patients recapitulate the functional mitochondrial impairments reported in UC tissues. Future studies will enlarge the number of patient colonoid studied and determine if mitochondrial functional abnormalities persist with endoscopic healing. This
study provides preliminary evidence for metabolic parameters that could be targeted in interventions aimed at improving epithelial metabolism and healing in active pediatric UC.
Children with dyslexia do not show deficits in exogenous visuospatial attention.

Reading is a complex visual task which demands the shifting and focusing of attention to extract relevant information. Spatial attention can be directed to a location in space involuntarily (exogenously) due to the appearance of a peripheral stimulus (Ramamurthy, 2021). Previous studies have shown that children with dyslexia show deficits in utilizing exogenous, or reflexive, spatial cues in a task that has no relation to reading (Facoetti, 2000). In this study, we analyzed how children with dyslexia utilize exogenous spatial cues in a task relevant for reading compared to a general visual perception task. 40 children (6-16 yrs) participated in the study, completing our reading specific multi-letter processing task, reading non-specific dot detection task, and battery of standardized reading assessments (a standardized reading score below 85 is considered dyslexic). For both tasks, participants were required to fixate on a central cross while a pre-cue flashed before a stimulus. In the dot detection task, a dot flashes for a brief period on either side of the cross and participants report the dot position. In the multi-letter processing task, a string of letters appeared for an encoding period and participants reported the element in the post-cued position. Performance in the multi-letter processing task shows a moderate but significant correlation with reading ability, while the non-specific task shows no correlation. We analyzed the cue benefit, cue cost, and cue effect of valid, neutral, and invalid pre-cues to determine attentional effects. We replicated previously reported differences in visual encoding but, on two separate tasks that target exogenous, reflexive visual attention, there were no differences between groups. Accuracy and reaction time are also comparable across groups. Our findings show no deficit in the utilization of exogenous attentional cues in children with dyslexia compared to typically developing children for tasks either generic or specific for reading.
Leukocyte telomere length in the first trimester of pregnancy and its association with perinatal outcomes

INTRODUCTION: Short leukocyte telomere length (LTL) is a biomarker of cellular aging and morbidity in non-pregnant adults, but little is known about its predictive utility in pregnancy. To address this knowledge gap, we evaluated associations between first trimester LTL and adverse perinatal outcomes.

METHODS: This was a prospective cohort study of pregnant people who presented for care at Stanford between January 2020 and June 2021. Blood was sampled from 18-50 year old nulliparous patients with a viable pregnancy between 10 and 14 weeks 0 days. LTL was measured via quantitative PCR and reported in basepairs. In the primary analysis, the exposure was first trimester maternal LTL and the outcomes were perinatal complications, including gestational diabetes, hypertensive disorders, and spontaneous preterm birth, among others, that were abstracted from medical records. Secondary outcomes included serial responses to the Patient Health Questionnaire-9 (PHQ-9). Given the normal distribution of LTL, we used t-tests to compare LTL between people with and without each categorical outcome, and computed Pearson correlations between LTL and continuous outcomes such as gestational age at delivery.

RESULTS: 46 pregnant people were included. Mean ± SD maternal age at enrollment was 31.2 ± 3.4 years. Shorter first-trimester maternal LTL was significantly correlated with earlier gestational age at delivery (r=0.33, p=0.03). Although limited by small numbers, we found non-significant differences for shorter first-trimester LTL in people who developed preeclampsia (5511.4 ± 429.7 versus 5756.0 ± 249.7 basepairs, p=0.28) and spontaneous preterm birth (5488.8 ±262.9 versus 5745.3 ± 278.2 basepairs, p=0.23) but not for PHQ-9 scores.

CONCLUSION: We identified significant correlations between shorter first-trimester LTL and earlier gestational age at delivery. Our data also suggest possible associations between LTL and preeclampsia and spontaneous preterm birth that warrant further investigation in larger cohorts.
Causal Effects of Air Pollution on Pediatric Health Outcomes

In this study, we contribute to the healthcare literature by exploiting a unique database that allows us to investigate a causal relationship between air pollution exposure and adverse health outcomes for children 18 and below in California. Our current work adds to the existing knowledge of the harmful effects of pollution on children's respiratory health by investigating other health outcomes, such as behavioral and mental health conditions. Current studies show that suicide has become the second leading cause of death among children, and treatment for behavioral and cognitive conditions accounts for the largest share of health care spending for children in the U.S.

Primary health care data come from the California Department of Public Health (CDPH) and the California Office of Statewide Health Planning and Development (OSHPD), and includes emergency and overnight hospital visits between 1991 and 2017. Supporting data for pollution levels come from California AQI and PM2.5 measurements collected by the Environmental Protection Agency (EPA) and satellite images. For our primary analysis, we use an instrumental variables two-stage regression model to investigate causal determinants of this condition and other health outcomes in the young population. We conclude our investigation with a counterfactual (predictive) study aimed at quantifying the benefits of mitigating air pollution on the costs of hospital infrastructure.
Maternal and Child Health Research Symposium
Submitted Abstracts

**Category:** Poster Session  
**Submitter:** Caroline Pecos-Duarte  
**Department:** Community Health and Prevention Research  
**Research Area:** Advocacy & Community Health  
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**The Impact of Jacob’s Heart, a Community-Based Organization Serving Families of Children with Cancer: Perspectives from Organization Staff**

Context/Purpose: Jacob’s Heart Children’s Cancer Support Services is a nonprofit organization that supports families of children with cancer in the Salinas Valley, a region with low area socioeconomic status and a large Latinx farmworker population. Jacob’s Heart and Stanford collaborated to understand the needs of cancer survivors and families after completing cancer treatment. The purpose of this sub-analysis is to identify what Jacob’s Heart provides for families affected by childhood cancer and investigate drivers of the organization’s success.

Methods: Semi-structured interviews were conducted with current/former Jacob’s Heart staff members to explore their experiences working with cancer survivors and their families. Interview transcripts were analyzed qualitatively using iterative rounds of inductive coding, followed by analysis of emergent themes.

Results: Seven current/former staff members participated (all female, 5 Hispanic/Latinx, 6 speak Spanish/English). Two themes emerged from the data: services provided by Jacob’s Heart and contributors to Jacob’s Heart success. Jacob’s Heart provides resources to fulfill basic needs (transportation, grocery, financial assistance) as well as support groups, mental health services, and community events. Staff act as a liaison to other community resources and serve as language brokers and health navigators for Spanish-speaking families. The organization responds to community needs and establishes trust with families through long-term relationships.

Interpretation: Jacob’s Heart’s staff are familiar with the language and cultural values of the community they serve. Staff serve in multiple roles to meet families’ needs. The organization is firmly grounded in its mission of responding to community needs, and this contributes to their success through the connections that staff develop with families to provide consistent support.

Conclusion: Jacob’s Heart exemplifies a community-based organization that is mission driven with committed staff who are motivated to meet families’ needs in an effort to work toward achieving equity for Spanish-speaking immigrant families.
Targeted Digital Media Marketing to Black, Indigenous, and Youth of Color: A Community-Based Participatory Research Content Analysis of Alcohol, Marijuana, and Tobacco Instagram Advertisements

Purpose: The last five years has seen concerning increases in the use of alcohol, marijuana, and tobacco among Black, Indigenous, and Youth of Color (BIYOC). Marketing plays a key role in promoting substance use, especially among BIYOC. However, few studies have examined digital media marketing to BIYOC. Our objective is to identify key themes and messages used in alcohol, cannabis, and tobacco advertisements marketed through social media specifically to BIYOC.

Methods: A total of 21 official verified Instagram accounts were systematically selected using United States sales data and app rankings. Posted advertisements were captured from the selected accounts over a six-month period (January 2022-June 2022). Using a community-based participatory research (CBPR) approach, we collaborated with our youth action board (YAB) to conduct a qualitative content analysis. We developed a codebook and used an iterative process of applying codes to the data to develop a consistent coding framework. We are now conducting a thematic analysis with input from our YAB collaborators.

Results: A total of 2,028 Instagram posts are being analyzed, with the following preliminary themes: (1) many advertisements featured individuals of color, (2) celebrity sponsorships played a large role in alcohol advertisements and many were noted to be athletes and artists of color, (3) advertisements featured messages focusing on social activism including issues that predominantly affect marginalized communities, and (4) tobacco companies were noted to have minimal to no official presence on Instagram.

Conclusions: Alcohol and cannabis advertisements on Instagram feature themes, such as sponsorships with celebrities of color and messages focused on issues of social activism, that can particularly appeal to BIYOC. Understanding the targeted messages featured on digital media advertisements can inform the development of policies to limit such targeted marketing. A CBPR approach can improve research by providing views and lived experiences of BIYOC youth themselves.
Deep learning identifies sex-specific regions in pre- and post-adolescent brains

INTRODUCTION
Previous research has shown that psychiatric differences emerge between females and males during adolescence. Examining specific brain regions over the pubertal period may provide important insights into biological underpinnings for these developmentally-sensitive phenomena (1, 2). We aim to train machine learning models on structural brain images (sMRI) to identify the presence of sex-specific regions (SSRs) of interest in cohorts of pre-adolescent and post-adolescent youth.

METHODS
SMRI data derived from 5698 participants in the Philadelphia Neurodevelopmental Cohort was split into two groups: 3075 children <11 years (mean = 9.41, SD = 1.11) and 2623 adolescents >16 years (mean = 18.33, SD = 1.15). Images were processed via Freesurfer (3) and normalized using estimated total intracranial volume (eTIV) to account for individual variances in global brain size. We applied four different machine learning models to examine classification accuracy for sex in each age group. Average accuracy and feature importance of each model was compared to each other as well as accuracy across age groups.

RESULTS
When examining XGBoost, Random Forest, and Linear SVM’s classification accuracies, Deep Neural Network performed 3% worse when trained on the <11 cohort and 17% worse when trained on >16. Based on classification accuracies, we selected the XGBoost model (73% accuracy), and identified the top five significant brain features contributing to the classifier for the >16 cohort: R lingual gyrus, bilateral inferior parietal lobule, L postcentral gyrus and R fusiform gyrus. And the <11 cohort: R inferior parietal lobule, R pars orbitalis, L postcentral gyrus, R temporal pole, and L transverse temporal gyri.

INTERPRETATION
Our results demonstrate that certain SSRs are important in accurately classifying sex in pre-adolescent youth, and also in post-adolescence. Importantly, these SSRs appeared to contribute more strongly to classifier accuracy in older youth, in addition we found additional SSRs in the older cohort which were not identified in pre-adolescent brains.

CONCLUSION
Using sophisticated machine learning processes, we identified SSRs that classify sex differences in pre- and post-adolescent brains, as well as novel SSRs that appear to only emerge after adolescence.
REFERENCES:
Bioengineering Osteosarcoma

Osteosarcoma (OS) is the most common bone tumor in the pediatric population and has a poor 5-year survival rate of 60-70% for primary disease and 20% in relapsed, refractory or metastatic disease. Gold-standard chemotherapy was first introduced in the 1970’s, and despite efforts in creating molecular-targeted drugs, survival rates remain unchanged. A major limitation of developing effective new treatments is the lack of representative tumor models. Conventional in vitro models utilize 2-dimensional (2D) cultures in tissue culture plastic; however, these models lack the extracellular matrix (ECM), a major component of the OS tumor microenvironment. To recapitulate the native bone ECM, 3D collagen (COL) type 1 hydrogels were fabricated through a pH and thermo-sensitive reaction. Matrix mechanics were quantitatively characterized through shear rheology, and matrix architecture was qualitatively assessed through confocal reflectance microscopy. At a concentration of 4 mg/ml, COL hydrogels exhibited an average storage modulus of 800 Pascals. Confocal reflectance microscopy illustrated the disorganized fibrous architecture of the COL hydrogels. 143B OS cells were then cultured in 3D collagen hydrogels and compared to conventional 2D cultures. Live/Dead staining displayed high cell viability in both 2D and 3D culture conditions at days 1, 3, and 7. OS cells exhibited a significant alteration in their morphology in 3D cultures compared to 2D cultures whereby cells aggregated, elongated, and spread in 3D hydrogels but remained spherical in 2D cultures. This was independent of cell concentration as these findings were consistent at lower and higher seeding densities. These data verify the need to study OS cells within 3D biomimetic systems in order to capture cell-matrix interactions critical to the native tumor microenvironment. Future work will investigate OS response to standard chemotherapy within 3D COL hydrogels and the utilization of this model to investigate patient-derived cell lines to derive personalized medicine approaches to treatment.
Crosstalk between Circadian and Myelin Biology in Brain Development and Disorders

The causes of neurodevelopmental disorders are incompletely understood, hindering our ability to gain precise diagnoses and design effective therapeutics. While current models of neurodevelopmental disorders focus on changes in neuronal signaling, myelin is also integral to neural circuit function. Myelin, the multi-layered structure that surrounds neuronal axons, allows for efficient communication between neurons. Nearly half of the brain is composed of white matter tracts that consist of myelinated axons in which new myelin-forming oligodendrocyte production from oligodendrocyte precursor cells (OPCs) is critical to neurodevelopment and maintenance. Even though OPCs are a functionally, spatially, and temporally heterogenous precursor population, they have a remarkable ability to maintain a consistent homeostatic density throughout the brain. The molecular circadian system regulates cell proliferation of numerous neural precursors and stem cells. The potential role the circadian clock plays in regulating the homeodynamic nature of oligodendroglial lineage cells remains unknown. This is especially important given the observations that children with neurodevelopmental disorders like autism and ADHD exhibit both myelin and circadian and sleep disruptions. We posit that the molecular circadian system driven by BMAL1 regulates oligodendroglia and myelination, contributing to the maintenance of systems-level homeostatic processes such as sleep. By eliminating Bmal1 from OPCs, we show that the dynamic nature of oligodendroglia and myelination are regulated by the circadian factor BMAL1. Bmal1 knockdown in OPCs during development decreases OPC proliferation, density and morphology. BMAL1-associated OPC deficits are primarily related to decreased expression in genes linked with cytoskeletal regulation, cell cycle, and proliferation. These deficits translate into thinner myelin sheaths and decrements in motor and cognitive functions associated with white matter structures. Additionally, we found that the oligodendroglial dysregulation and dysmyelination since development leads to sleep fragmentation. These findings have broad mechanistic and therapeutic implications for numerous neurodevelopmental disorders that include both myelin and sleep phenotypes.
Elucidating Novel Binding Partners of Gibbin and their Orchestrated Contributions to Childhood Birth Defects

Every year, millions of infants worldwide are born with severe birth defects. The most common genetic abnormalities in infants, either inherited or acquired, are associated with craniofacial abnormalities, intellectual disabilities, learning delays, short stature, and seizures, which arise from early developmental defects in the growing embryo. Yet, remarkably, in over 50% of reported cases, the exact genetics and biological mechanisms underlying many birth defects remain largely unknown. Recently, exciting work from the Oro lab has shed much-needed light on how proper patterning of the human embryo is achieved. Specifically, it was revealed that a novel mesoderm regulator called Gibbin, which is encoded by disease-associated gene Xia-Gibbs AT-hook DNA Binding Motif Containing 1 (AHDC1), is a critical transcription factor that coordinates early epithelial morphogenesis (Collier A. et al., Nature, 2022).

Interestingly, these results hinted that solo binding of Gibbin is not sufficient for driving gene expression and requires interaction with other factors that are currently unknown. By utilizing a CRISPR-Cas9 approach to functionally interrogate the Gibbin interactome in human embryonic stem cells, coupled with differentiaional gene expression analysis, we seek to identify how Gibbin and its interacting regulatory factors sense positional information and orchestrate the expression of different genes in the growing embryo for proper organ development.

From our initial screening experiments, we have identified two promising candidate methyl-reading proteins that display downregulated gene expression patterns similar to AHDC1/Gibbin knockout phenotypes. Importantly, both of these proteins have been associated with human neurological and developmental disorders.

Given the profound effects that AHDC1/Gibbin mutations have on proper fetal and child development and our lack of knowledge regarding the complexities of transcriptional regulation patterns during early development, it will be key to decipher these factors and their functions in order to advance our abilities to diagnose and treat different child pathologies.
Redefining Mucosal Barrier Injury (MBI) Bloodstream Infections in Allogeneic Transplantation

Context/Purpose: Bloodstream infections (BSI) occur in ~20% of pediatric patients undergoing allogeneic hematopoietic cell transplantation (allo-HCT), accounting for ~18% of deaths and costing $40,000 to $70,000 per BSI incident. Being able to localize the source of the infection will allow us to characterize and subsequently target these microbes with the goal of decreasing BSI.

Methods: Patients enrolled in a Phase 2 clinical trial who developed BSI had their BSI isolates sequenced and compared on a genome-wide level to the serial stool metagenomes. BSI organisms were then classified as either MBI or non-MBI based on NHSN criteria (Jan. 2021) of the CDC.

Results: In 6 of 20 patients (30%) who developed a BSI, 9 total BSI episodes occurred. Of these, 5 of the 9 episodes had identical genomes between the BSI and stool metagenome prior to or around the time of the BSI. An additional 2 BSI were too low in abundance to do a genome-wide comparison, but have a temporal rise in abundance in the gut prior to the BSI. Notably, 2 BSIs caused by Staphylococcus spp. (a genus defined by NHSN as non-MBI organisms) were found at 35% and 61% relative abundance in the gut around the time of the BSI.

Interpretation: These data suggest, but do not prove, that 7 of 9 BSI in this study are traceable to the gut either temporally or via strain-specific analysis. Furthermore, non-MBI organisms such as Staphylococcus were found in high abundance around the time of the BSI.

Conclusion: We may need to redefine the MBI vs non-MBI BSI definition in the immunocompromised populations (such as oncology, allo-HCT, and neonatology) where the mucosal barrier and immune systems function differently from an immunocompetent host.
Asymptomatic dengue virus transmission among rural and urban populations in Western and Coastal Kenya in 2016

Dengue is an acute disease which has a wide range of clinical presentations in humans from asymptomatic infection or self-limiting febrile episodes to severe and life-threatening disease. The causative agent is the dengue virus (DENV), a flavivirus widespread throughout the tropical regions of the world, with recent studies estimating there to be 390 million infections yearly, of which three fourths are asymptomatic. To identify and estimate rates of asymptomatic dengue infection in Kenya, we performed RNA extraction and RT-qPCR using serum samples from a cohort of children (3 to 16 years old) from four different Kenyan sites: Ukunda (Urban), Kisumu (Urban), Msambweni (Rural), and Chulaimbo (Rural). Serum samples were selected based on the highest rates of seroconversion according to previous epidemic and serologic results from our active surveillance studies. A total of 1769 samples collected from April 2016 to September 2016 were pooled in batches of 9, with 30 microliters of each sample. RNA extraction was performed using Omega Bio-Tek kit, following manufacturer’s instructions. The purified RNA was used in a standardized RT-qPCR protocol, targeting part of the DENV virus genome. Among the tested samples, 50% were male. 71% reported seeking medical care in the last 6 months of the sample collection date. Our results showed one dengue positive sample, DENV serotype-2. This sample was from a 14-year-old male from Msambweni, who did not report fever and did not seek medical care in the previous 6 months. Our data highlights that a burden of asymptomatic infection exists in coastal Kenya that is not being captured by traditional surveillance mechanisms. The methods of pooling and testing used in this study are a cost-effective way to detect asymptomatic infection in low prevalence populations. Future studies could target asymptomatic screening in household of symptomatic infections, as asymptomatic cases are known to lead to further dengue virus transmission.
Single cell profiling of embryonic livers reveals insight into macrophage sub-populations with innate immune function

Macrophages, a key component of the innate immune system, originate from the embryonal yolk sac and fetal liver. F4/80HI yolk sac macrophages travel to multiple tissues, including the liver, where a second wave of myeloid development produces CD11bHI macrophages. These two populations of fetal macrophages have differential responses to the TLR4 agonist LPS, with CD11bHI macrophages having a pro-inflammatory phenotype. In comparison, F4/80HI macrophages also express the phagocytic component C1q and do not generate an innate immune response. These findings suggested that fetal macrophages arising from different origins have differential immune functions.

Here, we investigated the molecular trajectories of fetal macrophage populations by single-cell RNA sequencing (scRNA-Seq). CD45+ immune cells were isolated from the E15 livers of C57BL/6 mice and sequenced using the 10X platform. Following data processing and clustering, macrophage populations were re-clustered for differential analysis. RNA velocity was carried out using SCvelo. Within the E15 liver macrophage population, we used differential gene expression and RNA velocity to identify developing Kupfer cells, monocytes, inflammatory macrophages, and C1q-positive, potentially immunotolerant macrophages. C1q expression was highest in both older, likely yolk sac-derived macrophages and a second group of recently differentiated cells. RNA velocity also identified yolk sac erythromyeloid progenitors that gave rise to all other cell types. Interestingly, subpopulations within each macrophage category contained cells with active cell cycle progression. These data supported a novel hypothesis that each type of fetal macrophage has self-renewal potential after cell differentiation. Elucidating key differences in the multiple macrophage populations and their development will aid in understanding the developing immune system.
Cultivating knowledge, interest, and confidence in youth through a community-based dermatology education and mentorship program

Introduction: Children in under-resourced communities have lower access to career opportunities and mentorship. Community outreach focusing on mentorship and education has been cited as one of the best ways to increase the pipeline to health science careers and also improve dermatology workforce diversity. Through our unique hands-on program, Interactive Technology for Skin: Community Outreach, Research, and Education (iTS-CORE), we aimed to educate students about skin conditions and medical technology, and provide mentorship on health careers.

Methods: We developed a partnership with a community youth program with a commitment to diversity (Boys & Girls Clubs of the Peninsula). Based on community input, we designed and implemented an interactive curriculum, where students learned about skin physiology and conditions, had hands-on experience with dermatology diagnostic and procedural tools, and discussed dermatology health careers. Pre- and post-surveys were used to assess the program’s effectiveness.

Results: Students reported increased knowledge of skin cancer recognition (2.11 [SD=1.20] vs. 3.33 [SD=1.11], P=.005), health careers (2.72 [SD=1.19] vs. 3.61 [SD=0.83], P=.005), and medical technology (2.50 [SD=1.17] vs. 3.22 [SD=0.85], P=.023).

Conclusions: Our program demonstrated that interactive programs with community youth programs can be an effective way to educate youth in under-resourced areas on dermatologic conditions and careers. We believe similar programs can be expanded and feasibly implemented around the Bay area.
Fasting versus fed: A randomized trial on oral intake prior to the 1 hour oral glucose tolerance test

CONTEXT/PURPOSE: To evaluate the effect of fasting versus eating prior to the 1-hour oral glucose tolerance test (OGTT) on gestational diabetes mellitus (GDM) screening results.

METHODS: We performed a single-center prospective randomized trial. Participants were randomized between 18-22 weeks' gestation to: 1) Fasting for 6 or more hours, or 2) Oral intake (“fed”) within 2 hours of the 50-gm 1-hour OGTT. The 1-hour OGTT was administered after 24 weeks of gestation. A positive screen was defined as a serum glucose level ≥140 mg/dL. Protocol adherence was assessed by a survey administered immediately following the OGTT. Using an intention to treat analysis, we planned to enroll 100 participants in each group to detect an absolute difference of ≥ 20 percentage points on the 1-hour OGTT screen positive rate using Fisher’s exact test, assuming an incidence of 35% in the entire cohort and 10% attrition, with a two-sided α=0.05, power=0.8. The primary outcome was the 1-hour OGTT screen positive rate, and secondary outcomes included mean 1-hour OGTT glucose values, GDM diagnosis, maternal and neonatal outcomes, and patient perceptions regarding the 1-hour OGTT.

RESULTS: After randomizing 200 participants, 195 completed the 1-hour OGTT (97 fasting, 98 fed). Participant surveys confirmed 99% (n=96) adherence to the fasting and 94% (n=92) adherence to the fed groups. The screen positive rate was significantly higher in the fasting than the fed group (32.0% vs. 13.3%, respectively, P=.002), as was the mean glucose value (127.7 mg/dL vs. 113.3 mg/dL, P=.002). The incidence of GDM in the fasting group was 12.5% (n=12) and in the fed group was 5.1% (n=5) (P=.08). There were no significant differences in maternal or neonatal outcomes.

CONCLUSION: Fasting for 6 or more hours doubled the incidence of a positive 1-hour OGTT screen when compared with eating within 2 hours of the test.
A multi-omic, longitudinal approach to study the maternal term and preterm immunome

Context and Purpose
Recent insights into the chronology of maternal immune adaptations suggest that pregnancy complications are associated with immune deviations that precede clinical symptoms. Here, we aim to find early indicators of later pregnancy pathology by tracking the progression of maternal immune dynamics across gestation in healthy term (T) compared to preterm (PT) pregnancies. More than half of all cases of PT birth, the leading cause of childhood mortality world-wide, occur spontaneously without clinical indication. Despite major efforts to define biomarkers, currently available tools to clinically predict or prevent premature delivery remain inefficient.

Methods
Peripheral blood mononuclear cell and serum samples from a longitudinal cohort of PT (N=24) and demographically (maternal age, fetal sex, BMI, parity)-matched T (N=46) pregnancies (gestational age at delivery (median[IQR]: T 39.6 [38.8, 40.4], PT 35.7 [34.8, 36.5]) were collected once in each trimester (total sample n = 176). In parallel, single-cell immunome (high-dimensional mass cytometry), serum proteome (high-throughput proteomic platform), psychometric measures (PSS, EPDS), and T cell transcriptome (single-cell RNAseq, nested, paired cohort, T N=5; PT N=5) are determined and computationally integrated using cross-validated regression modeling with stable feature selection to identify immune trajectories predictive of PT birth.

Results
Pilot analyses of global second-trimester transcriptome signatures revealed differentially expressed TCR variable region genes and deviating cytokine and steroid hormone responses in PT CD8 memory T cells. Mass cytometry confirmed that these changes manifest functionally on the protein level – PT CD8 memory T cells show higher levels of HSP90ab, a chaperone for steroid responses, and increased
pro-inflammatory TNFα production compared to T. Further, first trimester IL-8 production in classical monocytes is enhanced in PT vs. T pregnancies, prior to clinical presentation.

Interpretation and Conclusion
Integrating multiple biological modalities promises to advance our pathobiological understanding, reveal biomarkers, and, ultimately, improve clinical management of preterm pregnancies.
CRISPR/Cas9 targeting of IL10 for the design of a novel humanized VEO-IBD mouse model

Very early onset inflammatory bowel disease (VEO-IBD) is an intractable form of IBD affecting children under the age of 6 years old. Loss-of-function (LOF) mutations of the genes encoding for the immunosuppressive cytokine IL-10 or its receptor result in the most severe form of VEO-IBD, characterized by excessive chronic inflammation of the gut while extra-intestinal manifestations are also observed. Our current mechanistic knowledge of IBD is mainly based on transgenic, adoptive transfer or chemically induced mouse models which do not fully recapitulate the human disease. Therefore, we established a method to disrupt the IL10 gene in both human CD4+ T cells and CD34+ hematopoietic stem and precursor cells (HSPCs) via CRISPR/Cas9 manipulation. Our multi guide approach resulted in a high knockout (KO) efficiency of the endogenous IL10 locus in both cell types and the insertion of a therapeutic cassette containing IL10 cDNA via rAAV6 transduction of CD4+ T cells restored IL-10 production. To create a more human-relevant and physiological mouse model of IL10 deficiency and VEO-IBD, we transplanted immunodeficient NSG-SGM3 mice using IL10-KO HSPCs. This approach showed high engraftment of all hematopoietic lineages across tissues and, in contrast to mice transplanted with wildtype HSPCs, splenomegaly was observed in mice engrafted with IL10-KO HSPCs. Furthermore, splenic CD4+ T cells showed a proinflammatory phenotype as demonstrated by a decrease in TIGIT expression and an increase in TNF-α and IL-17A secretion. In conclusion, we have designed a method to generate an IL10-deficient humanized mouse model that, upon further optimization, will be a valuable tool to both study VEO-IBD as well as serve as a pre-clinical platform for testing novel gene and cell therapies to ultimately avoid the use of toxic immunosuppressive drugs and allogeneic stem cell transplantations for these patients.
Explainable artificial intelligence reveals brain fingerprints of psychosis in 22q11.2 deletion syndrome resembling idiopathic early psychosis

Psychosis is among the most prominent feature of 22q11.2 deletion syndrome (22q11.2DS); yet the etiology of psychosis in 22q11.2DS is poorly understood, as most studies have relied on analytical approaches ill-equipped to capture robust neurobiological markers of psychosis and have focused on group differences despite evidence of high individual variability in 22q11.2DS. Here we leverage exciting recent advances in explainable artificial intelligence to develop a novel deep neural network (stDNN), which uses spatiotemporal convolution on fMRI data to extract robust functional brain features that are unique to a patient with 22q11.2DS, analogous to a fingerprint, and trace psychosis symptoms. We examined one of the largest resting-state fMRI and clinical datasets from 22q11.2DS patients with and without psychosis as well as patients at various stages of idiopathic psychosis (Total N = 622). stDNN revealed individualized brain fingerprints localized to the salience network, and these fingerprints mirrored the broader diagnostic discrimination of 22q11.2DS. Critically, intra-22q11.2DS with psychosis group distances were significantly shorter than distances with the 22q11.2DS group without psychosis, highlighting the distinctness of brain fingerprints associated with 22q11.2DS psychosis. Notably, distances between the 22q11.2DS with psychosis group and the idiopathic early psychosis group were significantly shorter than distances between the 22q11.2DS without psychosis group and the idiopathic early psychosis group, indicating overlap between brain fingerprints of 22q11.2DS psychosis and idiopathic early psychosis. In contrast, distances between the 22q11.2DS with psychosis group and the schizophrenia group were not significantly different from distances between the 22q11.2DS without psychosis group and the schizophrenia group. Our findings reveal distinct functional brain fingerprints associated with psychosis in 22q11.2DS and provide evidence that they overlap with idiopathic early psychosis rather than established schizophrenia. These findings contribute to the development of robust biomarkers for psychosis at a nascent stage and further establish 22q11.2DS as a model for investigating the neurobiology of psychosis and its progression.
Developing a Branching Web-Based Behavioral and Social Health Needs Screening Tool for Under-Resourced Adolescent Populations

Purpose:
For clinicians caring for adolescents, choosing among the many validated surveys and tools to screen patients for health-related behaviors and needs can be overwhelming, and patients simply could not complete all available questionnaires during one visit. With little agreement in the field on a publicly available, efficient, and clinically relevant assessment tool, a mobile clinic serving under-resourced adolescent populations began a quality improvement project aimed at better screening its under-resourced adolescent patient population (ages 12-25) for health-related behaviors, mental health needs, and social determinants of health.

Methods:
We compiled and reviewed validated health screening tools for adolescents and young adults, categorizing them into broad areas including food security, housing security, safety, mental health, sexuality/reproductive health, substance abuse, out-of-home experiences (foster care, juvenile/immigration detention), and adverse childhood experiences. Developed on Qualtrics using a combination of clinical experience and the HEADSS assessment model, a 10 question “Part A” designed to screen broadly for the risk behavior categories above branches into a individually curated “Part B”, consisting of clinically relevant, validated questionnaires. Clinicians and the social worker receive a results overview highlighting potential areas of concern and follow-up. Pre-testing and piloting with volunteer youth ensured comprehension, acceptability, and feasibility.

Results:
371 patients completed the Part A screening during the first year. The completion rate of a social needs screen increased from 35% (paper) to 76% (online survey) when comparing the years prior to and following implementation of the new survey. 95% of patients stated that they “Strongly Agree” or “Agree” to the questions, “I thought the questions made sense,” and “The survey was easy to take”.

Over 80% of patients screened had at least 1 psychosocial need, and 62% had more than 1. The inclusion of a question about personal strengths proved valuable both for patients’ views of themselves and for patient-clinician rapport.

Conclusions:
A brief, secure, online screening tool that branches to relevant, validated health behavior questionnaires has proven to be acceptable, feasible, and effective in increasing screening rates in our under-resourced adolescent patient population.
Human sperm TMEM95 binds eggs and facilitates membrane fusion

Tmem95 encodes a sperm acrosomal membrane protein, whose knockout has a male-specific sterility phenotype in mice. Tmem95 knockout murine sperm can bind to, but do not fuse with eggs. How TMEM95 plays a role in membrane fusion of sperm and eggs has remained elusive. Here, we utilize a sperm penetration assay as a model system to investigate the function of human TMEM95. We show that human TMEM95 binds to hamster egg membranes, providing evidence for a TMEM95 receptor on eggs. Using X-ray crystallography, we reveal an evolutionarily conserved, positively charged region of TMEM95 as a putative receptor-binding surface. Amino-acid substitutions within this region of TMEM95 ablate egg-binding activity. We identify monoclonal antibodies against TMEM95 that reduce the number of human sperm fused with hamster eggs in sperm penetration assays. Strikingly, these antibodies do not block binding of sperm to eggs. Taken together, these results provide strong evidence for a specific, receptor-mediated interaction of sperm TMEM95 with eggs and suggest that this interaction may have a role in facilitating membrane fusion during fertilization.
The Prognostic Value of Urinary Biomarkers in the Evaluation of Congenital Hydronephrosis

Hydronephrosis is the swelling of the kidney due to obstruction of urine outflow. Distinguishing true obstructive hydronephrosis – leading to pressure-induced renal damage – from non-obstructive dilation that presents no harm to the kidney remains challenging via radioisotope renography. Indeed, the diagnostic capacity of both ultrasound and renography to identify hydronephrosis are limited, and subjectivity in diagnosis introduces significant variability in the rates of surgical intervention. These diagnostic challenges underscore the need for a non-invasive metric to accurately predict renal decline in patients who would need surgical intervention. Several urine biomarkers have been reported to correlate with renal obstruction and damage in mice and humans. However, there are no longitudinal studies to date assessing the diagnostic potential of urinary biomarkers for obstructive hydronephrosis in children before mandatory surgical intervention. Ideal biomarkers would be predictive of future loss of renal function, differentiating patients with obstructive hydronephrosis from those with nonobstructive hydronephrosis. This could replace more invasive measurements of renal function like renograms and identify patients requiring surgery prior to the irreversible loss of renal function, while sparing patients whose biomarkers indicate stable renal function. We are actively constructing a urinary biobank to identify and evaluate the longitudinal correlation of candidate urinary biomarkers capable of identifying and distinguishing obstructive hydronephrosis from nonobstructive dilation. Urine samples from enrolled children (1 month to 17 years) with unilateral hydronephrosis are centrifuged and supernatants are collected for analysis of biomarkers NGAL, MCP1, and HCA19-9. Our preliminary data demonstrate that these biomarkers can differentiate between obstructive hydronephrosis and non-obstructive dilation (NGAL, p=0.0228; MCP1, p=0.0316; HCA19-9, p=0.0008). Presently, the collection of timepoint samples is ongoing and we will utilize these timepoint samples to validate our hypothesis that these urine biomarkers can accurately predict obstructive hydronephrosis that leads to future loss of renal function.
Examining racial/ethnic disparities in the trends of postpartum readmissions in California from 1997-2018

Context/Purpose: Substantial racial/ethnic disparities in postpartum readmission (PPR) exist, but little is known about temporal trend in this disparity and factors that contribute to it. Our objective was to explore whether trends in PPR vary by race/ethnicity.

Methods: We examined trends in PPR, defined as hospitalization within 42 days of childbirth, using fetal death and live birth certificates linked to delivery discharge records from 10,711,289 births in California from 1997-2018. We used multivariable logistic regression models stratified by race/ethnicity to estimate the annual change in PPR during the study period and report odds ratios (OR) and 95% confidence intervals (CI) that reflect the total change in odds of PPR from 1997 to 2018. For simplicity, we focus this abstract on comparing results for Black and White individuals. We adjusted models for prenatal (e.g. demographics) and clinical (e.g. comorbidities) factors. We included year-squared in models to allow for non-linearity of trends.

Results: The overall prevalence of PPR was 1% (1.7% for Black; 1% for White mothers). In our unadjusted models, the increase in odds of readmission from 2018 to 1997 was 44% for Black mothers (OR: 1.44; CI: 1.35-1.53), compared to 26% for white mothers (OR: 1.26, CI: 1.22-1.31). After adjustment for prenatal and clinical factors, White (OR: 1.13; CI: 1.09-1.18) and Black (OR: 1.12; CI: 1.05-1.20) mothers had similar increases in odds of readmission.

Interpretation: There was a large increase in PPR during the study period, and it increased the most for Black mothers. Racial/ethnic differences in the trend were largely explained by adjustment of prenatal and clinical factors.

Conclusion: Given that PPR varied by race/ethnicity, it is important to find ways to prevent further increases in PPR especially among groups at highest risk.
Hypertrophic cardiomyopathy is the most common inherited form of heart disease and the leading cause of sudden cardiac death in children and young adults. Approximately one third of known hypertrophic cardiomyopathy mutations are found in beta-cardiac myosin, the motor protein responsible for contraction in human ventricles. Given the known hypercontractile phenotype of HCM in patients, our biochemical studies have investigated how these mutations alter kinetic rates of myosin function and myosin regulation. We have previously reported profound differences in the effects of different pediatric-onset mutations on molecular metrics of myosin function, but our ongoing work aims to clarify how these mutations affect contraction in a cellular context. H251N, was edited onto a healthy background line with a GFP-tagged alpha actinin protein to allow for live cell tracking of cardiac differentiation, cell size, and sarcomeric contraction. H251N cells plated on patterned glass were imaged before, immediately after and 24 hours after treatment with 300 nM Omecamtiv Mercabil (OM), a myosin activator, and quantified patterns of sarcomere lengths during contraction. Initial sarcomere kymographs showed more coordinated motion and more distinct diastolic rest periods in control cells compared to H251N. After 24 hours, the magnitude of sarcomere movement along the myofibrils were significantly reduced in both cell lines, and we observed examples of cytoskeletal and myofibril reorganization. Analysis of myofibril and sarcomere dynamics in control and H251N cells before and after perturbing their homeostasis with OM treatment revealed differences in patterns of myofibril contraction that were exacerbated by acutely increased force. These differences may correspond to the myofibril disarray and adverse remodeling observed clinically in patients with hypertrophic cardiomyopathy. Future experiments will determine if inhibiting myosin activity with mavacamten in the H251N cells rescues sarcomeric organization and cell spreading, and will characterize cellular responses with different mutations.
Parental bladder health knowledge, beliefs, practices and barriers related to pediatric lower urinary tract symptoms: Initial thematic findings

Context/Purpose
Pediatric lower urinary tract symptoms (LUTS) remains a common childhood problem. Bladder health education programs can teach effective behavioral change habits to improve symptoms. To support program development, we asked parents about their knowledge, beliefs, practices and barriers related to pediatric bladder health.

Methods
We have coded 6 interviews of parents of children ages 5-10 who received pLUTS care at our institution (goal = 40) using a framework-based semi-structured moderator guide. Qualitative thematic analysis was conducted using an iterative approach between two independent coders. A preliminary codebook was developed using a hybrid deductive and inductive approach.

Results
All participants identified as female. We identified domains of bladder health knowledge, daily practices related to bladder health, potty training, knowledge seeking preferences in bladder health education and school-level barriers. Parents recognize the importance of healthy diet, hydration and avoiding constipation but are unfamiliar with the concepts of timed voiding and specific hydration requirements by age. Potty training advice and knowledge is widely available, but post potty-training recommendations are found in “mommy and me” groups and online resources. Parents feel comfortable making these changes at home. School is a significant barrier due to parents being unable to intervene or track behavior in this environment.

Interpretation
Gaps exist in the knowledge of key post-potty training bladder habits, specifically timed voiding (every 3 hours) and hydration. Knowledge seeking is community-based without an evidence-based resource. Effective implementation may involve addressing school-level barriers. Results are limited by the participation of mothers only.

Conclusion
Next steps include revising our moderator guide, conducting additional interviews and analyzing results using these themes. Final results will inform development and deployment of our bladder health education programs.
Human or machine? Detection of acute dengue virus infection, with and without concurrent malaria infection, at Kenyan clinics in a cohort of acutely febrile children, 2014-2019

Background. Poor access to diagnostic testing in resource limited settings restricts surveillance for emerging infections, such as dengue virus (DENV), to clinician suspicion, based on history and exam observations alone. We investigated the ability of machine learning to detect DENV based solely on data available at the clinic visit.

Methods. We extracted symptom and physical exam data from 6,208 pediatric febrile illness visits to Kenyan public health clinics from 2014–2019 and created a dataset with 113 clinical features. Malaria testing was available at the clinic site. DENV testing was performed afterwards. We randomly sampled 70% of the dataset to train DENV and malaria prediction models using boosted logistic regression, decision trees and random forests, support vector machines, naïve Bayes, and neural networks with 10-fold cross validation, tuned to maximize accuracy. 30% of the dataset was reserved to test the models.

Results. 485 subjects (7.8%) had DENV and 3,145 subjects (50.7%) had malaria. 220 (3.5%) subjects had co-infection with both DENV and malaria. In the reserved test data, clinician diagnosis of malaria outperformed all models (82.4% accuracy versus 53.4 – 68.7% for the models). In contrast, clinicians detected only 21 of 145 cases of DENV (79.7% accuracy, 14.5% sensitivity). Of the six models, only logistic regression identified any DENV case (8 cases, 91.1% accuracy, 5.5% sensitivity).

Conclusions. Without diagnostic testing, interpretation of clinical findings by humans or machines cannot detect DENV at 8% prevalence. Access to point-of-care diagnostic tests must be prioritized to address global inequities in emerging infections surveillance.
Starting to see a pattern: Longitudinal SSVEP signals reveal how growth in children's cortical sensitivity to statistical properties of letters within word forms is uniquely linked to two-year growth in reading fluency

Learning to read depends crucially on a novel form of configural processing whereby the precise way letters are combined conveys the critical information that distinguishes tens of thousands of visual word forms. Thus, one critical neural developmental process supporting fluent reading skill may be on one's ability to rewire visual cortical circuitry to take advantage of statistical constraints inherent in combining letters into visual word forms. To test this idea in early readers, we tracked the impact of two years of schooling on within-student longitudinal changes in cortical responses to visual word forms and growth in reading fluency. Three stimulus contrasts—words versus pseudofonts, words versus pseudowords, pseudowords versus nonwords—were presented while high-density EEG–Steady-State Visual Evoked Potentials (SSVEPs, n=31) were recorded.

Internalization of abstract sublexical orthographic pattern processing over two years of reading experience, resulting in a near doubling of SSVEP amplitude, with increasingly left lateralization. Cortical entrainment by such sublexical orthographic pattern information predicted the growth in reading fluency. However, no such changes were observed for lexical tuning, which stable across two years.
Novel pathways that mediate the therapeutic effects of metformin in treating type 2 diabetes

Childhood obesity and type 2 diabetes are a major and growing epidemic. 11.3% of the population in United States have diabetes, and 38.0% have prediabetes. Increasing numbers of children and adolescences are diagnosed with diabetes. In addition, nearly one third of pregnant women in United States are obese. To date, metformin is the only prescribed drug approved by FDA to treat diabetes in children and adolescences. Metformin treatment lowers blood glucose, and reduce body weight. The pleiotropic beneficial effect of metformin indicates that it may act on multiple organs with different cellular and molecular mechanisms. However, what molecules mediate the systematic beneficial effect of metformin remain controversial. Our laboratory has recently discovered N-lactoyl phenylalanine (Lac-Phe) as a circulating molecule that mediates beneficial effects of exercise by reducing food intake and leading to weight loss. Here we show that Lac-Phe levels in blood also increased when mice and human were treated with metformin, indicating that Lac-Phe might also mediate the anti-obesity effects of metformin. Current progress has been testing whether the anti-obesity effects of metformin depends on CNDP2, an enzyme that contributes to biosynthesis of Lac-Phe in vivo. Discovery of novel pathways that mediate metformin therapeutic effects may be useful for more precise development of anti-obesity and anti-diabetic therapeutics for childhood and material obesity and diabetes.
Cell-free DNA Sequencing for Diagnosis of Complex Pediatric Otolaryngologic Infections

CONTEXT/PURPOSE
Current clinical practices can have low yield at pathogen identification among pediatric patients with complex otolaryngologic infections. Some concerns include invasiveness, lack of specificity and objectivity, timeliness to diagnosis and treatment which implies broad-spectrum antibiotics usage as well as prolonged hospital stays. Recent research has shown promise using microbial cell-free DNA (mcfDNA) testing to detect pediatric endocarditis, complicated pneumonia, and other diseases in children. However, this assay is unstudied in pediatric patients with complex otolaryngologic infections.

METHODS
In this study, we analyzed and evaluated the diagnostic accuracy of a commercially available mcfDNA assay, the Karius test. Patients (n=28) were admitted into a quaternary children’s hospital with suspected complex otolaryngologic infections. Blood samples were obtained and analyzed using mcfDNA testing. We examined detected pathogens and the MPM (mcfDNA molecules/µL) values. We also obtained microbiological diagnostic tests (i.e. blood, surgical, viral, & PCR cultures) and imaging from patients. Clinical adjudication was used to qualify the data. In addition, a cohort of non-infected patients (n=99) was recruited during elective surgery.

RESULTS & INTERPRETATION
After clinical adjudication, 13 of 26 patients were found to have a true positive result on mcfDNA testing. The positive predictive value (PPV) among patients who tested positive and have the likelihood of infection was 81%. The sensitivity of mcfDNA testing in infected patients was 62%. In infected patients, there were 17 different pathogens detected, with 2 classified as likely contaminants resulting in false positive mcfDNA testing. In controls, there was detectable mcfDNA signal of similar pathogens, though most often at a lower MPM.

CONCLUSION
Overall, mcfDNA testing was found to have fairly strong positive predictive value but should be used to augment microbiologic diagnosis. More data is needed to understand the background signal of mcfDNA in healthy children with no clinical signs of illness.
Live chromosome imaging in mouse oocytes: a pilot study

Introduction: An estimated 20~80% of human oocytes (or eggs) have the 'wrong' number of chromosomes (or aneuploidy) with the rate increasing with maternal age. Identifying aneuploidy in oocytes can drastically decrease pregnancy losses as well as intellectual and developmental disabilities in children. To date, there is no test available to diagnose aneuploidy in an oocyte while maintaining its viability or reproductive competence. Here, we conduct a pilot study in mouse oocytes as the first step towards developing a live imaging method to detect aneuploidy in live oocytes.

Methods: The 4-week-old C57/BL6 female mice were superovulated with PMSG and hCG. The mature oocytes were collected. We deployed fluorescent ribonucleoprotein (fRNP) containing chemically synthesized fluorescent guide RNA (gRNA) probes (Atto647-crRNA) assembled with catalytically inactive deadCas9 (dCas9) protein to target and visualize chromosome 7. We used electroporation to deliver fRNP into the mouse oocytes. After 4-5 hours of incubation, the oocytes were imaged under the confocal fluorescent microscope. The fertilization rate and the blastocyst formation rate were compared between the experimental group and the control group which did not undergo probe delivery or imaging.

Results: Strong and clear signals of sister chromatids of chromosome 7 were observed in the metaphase II oocytes. The labeling efficiency, i.e. the percentage of the oocytes labeled with fRNP and showing clear signals, was 60%. Slightly lower fertilization rate (74% vs 81%) and blastocyst formation rate (69% vs 81%) were observed in the experimental group as compared to the control group. The morphology of the blastocysts were similar between the two groups.

Conclusions: In this pilot study, we have shown that a chromosome can be clearly imaged in live mouse oocytes with minimal impact on their reproductive competence. Further studies are ongoing to optimize our live imaging method and to image multiple chromosomes simultaneously.
Performance in a multi-element processing task varies as a function of position, but not encoding time, in skilled readers.

In a large-scale current study in our laboratory, we observe a moderate but significant correlation between reading ability and the ability to identify letters in a string during a multi-letter processing task. These findings suggest that this task could be used as a dyslexia screening tool. Ultimately, our goal is to replace letters with elements that are similar to letters in all their features but do not require formal reading instructions. A key feature of this task is that recognition accuracy varies as a function of position within the string (O’Regan, 1981). This is displayed by the serial position function (SPF) which is W-shaped for letters and an inverted U-shape for other symbols (Stevens and Grainger, 2003). In this study, we analyzed how performance on a multi-element processing task varied as a function of position and encoding time to better understand how different visual stimuli were processed when in a string. Twenty adults (18-35 yrs) with normal vision and reading ability (determined by standardized reading assessments) participated in the study. Participants were required to fixate on a central cross, while a string of 6 elements (letters, numbers, objects, or pseudo-fonts) appeared for an encoding period (60ms, 120ms, or 240ms). Participants then reported the element in the post-cued position. Eyes were tracked to ensure fixation during encoding periods. Performance varied based on stimulus type, but not based on encoding time. The SPFs for letters and numbers were W-shaped, while that of objects was an inverted U-shape. Notably, the SPF for pseudo-fonts was between that of letters and objects. These results provide a baseline with which to compare results from children. We are especially interested in observing the SPF of pseudo-fonts in children to better understand the predictive power of multi-element encoding in future reading ability.
The burden of asymptomatic dengue infection at two urban sites in Kenya

Dengue is a re-emerging human health threat worldwide. It is thought that Africans suffer less severe dengue infection and currently escape infection detection based on the WHO standard algorithms (Khan et al, 2022), but 75% of dengue is thought to be asymptomatic. In this study, we followed 4495 febrile and healthy adults and children in two urban cities in Coastal (Ukunda) and Western (Kisumu) Kenya for 15 months at 6 month intervals to detect dengue virus (DENV) transmission. Our study population included 1863 (41%) children and 2,773 (61%) females. We separated the study population into two groups: those with fever (body temperature=38°C or more) or reported history of fever 3 days prior to the study visit and those without fever. All samples were tested by anti-DENV IgG ELISA and all febrile samples were additionally tested by DENV PCR. Overall, the majority of our study population did not have fever (4130 (91.9%)) while 365 (8.1%) of our participants were febrile at the study visit. Of the febrile individuals, 2 (0.5%) tested positive for dengue infection by PCR and 7 (1.9%) seroconverted by the next visit. Of those without fever, 103 (2.5%) seroconverted by the next visit. Over the entire 15 month study period, 112 (3.1%) individuals had dengue diagnosed by PCR or seroconversion. Of these, only 9 (8.0 %) had fever and 103 (92%) were afebrile. Most dengue cases (102 (91.4%)) lived on the coast (p value&lt;0.01). These findings emphasize the potentially high burden of asymptomatic dengue infection in Kenya, and the need for more active surveillance to detect dengue transmission. The disease course of dengue in Kenya may differ from other parts of the world and needs further detailed study.
Objective: Current data suggests three in four patients undergo antenatal testing during their pregnancy. Utilizing non-stress tests (NST) and amniotic fluid (AFI) levels are currently accepted practice, with maternal vital signs and uterine contractions assessed simultaneously. In this quality improvement study, we aimed to quantify the value-add of assessing maternal wellbeing during antenatal testing.

Study Design: This retrospective cohort study from June-December 2020 compared patients with abnormal “fetal” testing, defined as non-reassuring NST/AFI, to those with abnormal “maternal” assessments, defined as abnormal vital signs (typically blood pressure) or uterine contractions. The primary outcome was delivery within 24-hours. Secondary outcomes included delivery within 72-hours, readmission, and delivery type. An a priori power analysis ensured detection of difference of at least 10%. X2 tests were used for demographic categorical variables, and logistic regressions were run for outcome variables.

Results: We reviewed 3,020 charts, of which 270 (8.9%) had abnormal antenatal results. The median gestational age was 36 weeks, with 58% of our population identifying as non-Hispanic. Common indications for testing included gestational diabetes (18%, n=49) and congenital fetal anomaly (8.8%, n=24). We excluded two patients with lethal anomalies. Our comparison groups were split evenly, with 52.5% abnormal maternal (n=107) and 47.5% fetal (n=97) findings. Non-reactive NST was the most common indication for prolonged monitoring (28.4%, n=77), followed by uterine contractions on tocometry (26.1%, n=71) and elevated maternal pressures (19.1%, n=52). Overall, more patients with abnormal fetal testing delivered within 24-hours (15% vs. 9.1%) compared with maternal findings, (p=0.03), with an OR of 1.88[1.06, 3.35]. Yet, the incidence of delivery within 72-hours was similar (7.8% vs 6.9%, p=0.49). There was no difference in delivery type (p=0.74).

Conclusions: In our cohort, abnormal fetal testing resulted in a higher incidence of delivery within 24 hours, but delivery rates between our two groups were similar by 72 hours. Assessing maternal wellbeing during antenatal testing may identify important maternal pathology contributing to pregnancy outcomes, specifically within 72 hours of initial testing. Further quality improvement research will explore the high re-admission rate among the population with abnormal maternal findings, particularly elevated blood pressures.
Stabilization of Large Genetic Payloads with Virus Like Particle Mediated Knockins

Synthetic biology and clinically relevant cell engineering require robust, efficient, and easy-to-implement technologies for knockin of large genetic payloads and their long-term stable expression. Retroviruses have been widely adopted for semi-random, large payload integration into primary cells but are hindered by silencing. Homology directed repair (HDR) could overcome this through targeted knockin of payloads into endogenous essential genes. But HDR methods using adeno-associated virus (AAV) or naked DNA templates are challenged by either limited payload size or high cytotoxicity in primary cells. Here we develop CLIP, CRISPR for Long-fragment Integration via Virus Like Particles (VLPs), enabling efficient large gene knockins in diverse cell types including primary human T cells and induced pluripotent stem cells. Using CLIP, we knockin large and difficult to express transgenes into essential genomic loci and demonstrate resistance to the silencing faced by lentivirus. Finally, we demonstrate the application of CLIP for engineering human T cells that stably express multiple SARS-CoV-2 antigens for displaying immunopeptides as a proof-of-concept cell-based vaccine. CLIP can be broadly utilized for diverse synthetic biology and clinical cell engineering applications that require stable expression of large or multiple genetic payloads.
Cellular Physiology and Modulation of Human Neurons in Acute and Cultured Brain Slices

Autism is a pervasive neurodevelopmental disorder that affects as much as 1-2% of the population, and typically manifests in the first 3-12 years of life. It is most commonly characterized by repetitive and restrictive behaviors, and deficits in social interactions. In addition, approximately 10-20% of autistic patients also have epilepsy. Pediatric epilepsy accounts for approximately 15% of active epilepsy cases, with approximately 1/3 of patients being drug resistant, and an even smaller fraction being good surgical candidates. Advances in genomics and imaging have revealed human-specific features of cellular physiology that are not conserved across species, making them inaccessible to investigation in rodent models. We investigate these specific features as they relate to the epileptic and autistic brain using human derived tissue from pediatric patients with drug-resistant epilepsy, a subset of which also have an autism diagnosis. We use a combination of electrophysiological recordings, next-generation transcriptomic analysis, and morphological reconstruction to investigate how genetics drive functionality of neuronal cell types, and how these may be different in the pediatric epileptic and/or autistic brain. We show here examples of how intrinsic properties of excitability of layer specific cortical neurons show variability within layers in a predictable manor, and interestingly, by patient age. We also demonstrate how transcriptomic data can be used to drive investigations of specific neuronal pathways that may be different across species, or in neuronal properties linked to co-morbidities commonly found with childhood epilepsy, such as oxytocin and autism. While studies with resected brain tissue from adult patients does provide access to human tissue, the value of studying the most severe cases in the pediatric patient is an opportunity for rare access to mechanistic insights not possible with adult, and may help resolve mechanistic unknowns for both disease etiology and drug resistance, which may lead to advances in treatment options.
Studying the role of MAP4K4 in pediatric high-grade glioma tumor cell migration using CRISPR-Cas9 screening approaches

Gliomas are the most common malignant brain tumors and the second-leading cause of cancer-related deaths in children in the United States. The prognosis for pediatric high-grade glioma (pHGG) remains grim, where there is overall survival of <20% at 5 years for patients with the disease. Tumor cell migration and invasion throughout the brain parenchyma are features of pHGG that render it difficult to treat. Previously, we performed a genome-wide CRISPR/Cas9 knock-out screen with the adult glioblastoma (GBM) line, U138, to identify regulators of tumor cell invasion. From the screen, MAP4K4 was identified as a key regulator of tumor cell invasion. For the current study, the role of MAP4K4 in tumor cell migration was investigated in a panel of pHGG cell lines. First, MAP4K4 expression for the pHGG cells was detected with western blot and immunocytochemistry. Next, the MAP4K4 inhibitor, PF06260933 dihydrochloride, was administered and cell migration was recorded with scratch assays. It was found that MAP4K4 inhibition decreased cell migration for pHGG cell lines. Future directions involve engineering a pHGG cell line to express Cas9 to conduct further screens and to knock out MAP4K4 and other proteins of interest thought to be implicated in tumor cell invasion and migration.
The End-of-Life Experience for Pediatric Ventricular Assist Device Patients: A Report from the ACTION Registry

Purpose: Although most pediatric VAD patients survive to transplantation, some die on device therapy. The end-of-life experience of pediatric VAD patients is not well characterized.

Methods: Retrospective review of pediatric VAD patients in the ACTION registry who died on device therapy between 3/2012-9/2021. Demographic and clinical data, including invasive interventions used at the end of life and the location of death, were analyzed.

Results: 107/721 (15%) of patients died on device at a median age of 5 years (IQR:1, 16) at 43 days (IQR: 17, 91) post implant. Goals of VAD therapy were bridge to candidacy for 50 patients (51%), bridge to transplant for 44 (37.6%), destination therapy for 2 (1.7%). The most common cause of death was multi-organ failure (n=35, 30%), followed by infection (n=12, 10.3%). Eighty-five of 92 patients (92.4%) died with a functioning device in place. Most patients were receiving invasive interventions (mechanical ventilation, 75%; vasoactive infusions, 62%) at the end of life (Table 1). Only 10 (9%) patients died at home.

Summary: Aggressive interventions are common at the end of life of pediatric patients with VADs. Dying at home is uncommon. These data can inform future practices to promote informed patient and provider decision-making to reduce suffering at the end of life.
Submitted Abstracts

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Division (if applicable): Interdisciplinary Brain Sciences
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An Optical Functional Neuroimaging Platform for Precision Mental Health

One in seven children in the U.S. suffer from an undiagnosed mental health disorder, reflecting poor management of mental health diagnosis during development. This is partly explained by lack of objective biomarkers for mental illnesses as modern clinical psychiatry practice still relies solely on structured interviews and patient’s reports to diagnose mental illnesses and monitor course of treatment. Over the past decades, National Institute of Mental Health has spent billions of dollars to fund large-scale, multi-site, functional MRI (fMRI) cohort studies to pursue reliable markers for mental illnesses. However, fMRI – gold-standard functional neuroimaging – still remains a primary tool for research and lacks clinical utility in mental health. Accumulating evidence suggests that large quantities of artifact-free, individual functional imaging data are critical for reliable detection of brain dysfunction in “individual” patients with neuropsychiatric conditions, and to establish clinical utility. However, collecting highly-sampled individual data is both too costly and impractical (patient’s compliance, etc.) using fMRI. Here, we describe an optical functional neuroimaging platform that we recently developed to bridge the gap in utilization of functional neuroimaging in psychiatric clinics.

The developed platform integrates (1) a multi-channel, consumer-grade, wearable, wireless optical imaging system; (2) an integrated tablet application that enables wireless recording of brain activity, guides patients throughout the data collection, presents a set of carefully designed cognitive tests to the patients for comprehensive assessment of cognitive functions, and helps patients with proper and reproducible placement of device; and (3) a HIPAA-compliant, cloud solution for clinicians to remotely manage patients’ brain response and cognitive outcomes. We have tested the reproducibility of measurements of the developed optical imaging device across different sessions, cognitive tasks, and settings (at-home, in-lab) and are currently testing the utility of this platform for stand-alone but reliable measurement of individual child’s brain activity at home using Attention Deficit Hyperactivity Disorder as a model illness. We believe that our platform provides a scalable solution for monitoring brain response in patients with neuropsychiatric conditions and paves the way for clinical utilization of functional neuroimaging for precision psychiatry.
Targeting the high-risk neuroblastoma: promoting differentiation with metabolic therapy

The Warburg effect is a major metabolic hallmark of cancer. According to Warburg himself, the consequence of the Warburg effect is cell dedifferentiation. However, it is unclear whether reversing the Warburg effect could restore cell differentiation. In this study, we use a mitochondrial uncoupler, niclosamide ethanolamine (NEN), to activate mitochondria respiration and induce neural differentiation in neuroblastoma cells. NEN treatment not only increases the NAD+/NADH ratio and pyruvate/lactate ratios but also increases the α-ketoglutarate (α-KG)/2-hydroxyglutarate (2-HG) ratio. Consequently, NEN treatment induces promoter CpG islands demethylation and epigenetic landscape remodeling. Globally, NEN treatment upregulates favorable prognosis genes and downregulates unfavorable prognosis genes that are defined from the neuroblastoma patient dataset. In addition, NEN treatment upregulates p53 but downregulates N-Myc and β-catenin signaling in neuroblastoma cells. Importantly, even under hypoxia, NEN treatment is still effective in inhibiting 2-HG generation, promoting DAN demethylation, and suppressing hypoxia-inducible factor (HIF) signaling. In an orthotopic neuroblastoma model, dietary NEN intervention reduces tumor growth rate, and 2-HG levels, N-Myc, and β-catenin expression in the tumor. Together, these results suggest that mitochondrial uncoupling is an effective metabolic and epigenetic therapy to reverse the Warburg effect and induce differentiation in neuroblastoma.
The Current State of Palliative Care in Pediatric Advanced Cardiac Disease: A Scoping Review

Title: The Current State of Palliative Care in Pediatric Advanced Cardiac Disease: A Scoping Review

Authors: Kate Johnson, RN MS, CPNP-AC*; Catherine Dietrich, MD*; Connie Wong; Kimberly Pyke-Grimm, PhD, RN; Seth Hollander, MD; Danton Char, MD

Introduction: Pediatric palliative care (PPC) is an approach to caring for pediatric patients with serious illnesses and their families. The National Consensus Project for Palliative Care broadly defines eight domains of interest: physical, structural, psychological, social, spiritual, ethical, and cultural aspects of care, as well as care of the imminently dying. While an often-discussed topic in the world of pediatric oncology, PPC is historically less-discussed within the context of pediatric congenital heart disease, despite life-long challenges facing these patients. A scoping review of the current literature of PPC in the pediatric advanced cardiac disease population was undertaken to better define ways to improve the intersection between these fields.

Methods: We conducted a scoping review of PPC publications in three electronic databases: Pubmed, CINHAL, and Embase. Topics included symptom management, goals of care discussions, and end-of-life experience. Covidence screening software was utilized to evaluate search results (Figure 1).

Figure 1: PRISMA Flow Diagram

Results: Thirty-seven studies were identified across several countries. The search yielded many surveys and retrospective chart reviews but very few prospective studies discussing PPC in patients with advanced cardiac disease. A common theme was the frequent consultation of palliative care only in the days leading up to a patient's death. One subset of articles discussed compassionate deactivation of ventricular assist devices; authors frequently concluded more research needed to be done on the subject. In the studies that did discuss consulting PPC at time of diagnosis, families and patients felt they had better lines of
communication with their providers, clearer delineation of goals of care, and improved symptom management.

Conclusions: Much of the published literature in PPC for pediatric advanced heart disease has been largely exploratory and descriptive in nature. However, the last five years has seen an increase in the number of studies looking at earlier involvement of PPC in the overall treatment plan when caring for children with advanced heart disease. While this is a promising start, significant opportunities still exist to identify and develop best practices for integrating palliative care into the standard of care for children with advanced congenital heart disease.
Screws or Sutures? A Pediatric Cadaveric Study of Tibial Spine Fracture Repair Techniques

Objectives:
Tibial spine fractures are common in the pediatric population because their subchondral bone is significantly weaker. Most studies in porcine or adult human bone suggest suture fixation is superior to screw fixation, but these tissue types may be poor approximates for pediatric bone. No prior study has evaluated fixation methods in human pediatric knees. This study aims to quantify the biomechanical properties of two-screw and two-suture tibial spine fracture repair in pediatric knees.

Methods:
Pediatric knee specimens were randomly assigned to either two-screw or two-suture fixation. An osteotome induced a standardized Meyers-McKeever Type III tibial spine fracture. Specimens were then mounted for biomechanical testing on a servohydraulic load frame at approximately thirty degrees of flexion to simulate typical ACL loading conditions. A cyclic loading protocol was applied to each specimen. This included 500 cycles between 5 and 75N at a crosshead speed of 100mm per minute, sampled at 20Hz. Upon the completion of cyclic loading, samples were allowed to recover for thirty minutes. Finally, a load-to-failure protocol was conducted at a rate of 0.5mm per second. The primary outcome was ultimate failure load in newtons (N).

Results:
Twelve age and laterality-matched pediatric cadaveric knees were tested. Ultimate failure load did not significantly differ between screw (Mean: 143.52N, SD: 41.97N) and suture (Mean: 135.35N, SD: 47.94N) fixations (p = 0.759). One screw construct and one suture fixation construct did not survive the cyclic loading protocol. Screw constructs demonstrated greater stiffness and lower amounts of elongation than suture constructs (21.79 N/mm vs. 13.83 N/mm; 5.02mm vs. 8.46mm). Both results approached statistical significance (p = 0.076; p = 0.069).

Conclusions:
Screw and suture fixation of tibial spine fractures in pediatric bone are biomechanically comparable, in sharp contrast with previous literature. Further investigation should be conducted into repair constructs that are more biomechanically sound in pediatric bone.
Sensitive multimodal profiling of native DNA by transposase-mediated single-molecule sequencing

We present SMRT-Tag: a multiplexable, PCR-free approach for constructing low-input, single-molecule Pacific Biosciences (PacBio) sequencing libraries through Tn5 transposition. As proof-of-concept, we apply SMRT-Tag to resolve human genetic and epigenetic variation in gold-standard human reference samples. SMRT-Tag requires 1-5% as much input material as existing protocols (15,000 – 50,000 human cell equivalents) and enables highly sensitive and simultaneous detection of single nucleotide variants, small insertions / deletions, and CpG methylation comparable to the current state-of-the-art. We further combine SMRT-Tag with in situ adenine methyltransferase footprinting of nuclei (SAMOSA-Tag) to facilitate joint analysis of nucleosome repeat length, CTCF occupancy, and CpG methylation on individual chromatin fibers in osteosarcoma cells. SMRT-Tag promises to enable basic and clinical research by offering scalable, sensitive, and multimodal single-molecule genomic and epigenomic analyses in rare cell populations.
Cervical Cancer Screening in Pregnancy at a University Obstetrics Clinic

Background:
Cervical cancer rates in the US are plateauing, despite wide availability of accurate screening tests. During pregnancy, patients are more engaged in health care and have the opportunity to undergo cervical cancer screening (CCS). We aimed to assess predictors of inadequate CCS during pregnancy in a university obstetrics population.

Methods:
A retrospective cohort study included pregnant patients aged ≥21 years old who were seen at least twice at the Stanford Obstetrics Clinic between January 1-December 31, 2021. Data was abstracted from the EMR through chart review and was entered into REDCap. Variables collected included demographic and clinicopathologic parameters, including the patient’s history of CCS. The primary outcome, adequate screening in pregnancy, was defined as having had a Papanicolaou smear (Pap) or high-risk human papillomavirus test at the recommended interval, and if the results were abnormal, having had cervical colposcopy for further evaluation. Descriptive statistics and logistic regression were performed using R.

Results:
Our study sample included 1,433 pregnant patients. To date, data was abstracted in 739 patients. The patients had a mean age of 33 (SD 5) years, were of Asian (37%), White (36%), or Other (22%) race, and the majority were partnered (92%), never smokers (92%), employed full time (85%), and had commercial insurance (89%). Thirty-four (4.6%) patients had inadequate screening during pregnancy. No variables were significantly associated with inadequate CCS in univariate analyses.

Conclusions:
Patients being seen for pregnancy care at Stanford Obstetrics Clinic have a low rate of inadequate CCS. We did not identify significant predictors of inadequate screening in our preliminary analysis. We plan to complete the study and rerun the analysis. Next, we will be performing in-depth interviews with patients who were not screened adequately, to understand better what some of the reasons were and to plan future interventions to improve screening adequacy.
Assessment of Patients' Perceptions Towards Embryo Disposition After Donation of Embryos to a Research Biobank

Purpose
To explore perceptions towards embryo disposition among patients who already donated excess embryos to a research biobank.

Methods
Cross-sectional study of survey responses collected as part of enrollment in a research biobank. At the conclusion of their fertility treatments, patients are asked questions regarding the difficulty of their disposition decision, their alternative disposition choice if donation to research was not available, quality of the counseling they received, and if additional counseling throughout their treatment would have been beneficial. Survey responses use 5-point Likert scales, with "1" being lowest/least and "5" being highest/most.

Results
157 men and 163 women enrolled in the biobank. Median scores for difficulty of disposition decision were 3 for females and 2 for males, and for quality of counseling were 4 for females and 3 for males. 70% of patients would have chosen to discard their excess embryos had donation to research not been an option. Statistical analyses showed no significant difference in responses based on variations in race, religion, sexual orientation, and infertility diagnoses. Concordance of responses within heterosexual couples was statistically evaluated and found to be poor to moderate.

Conclusions:
Assessing patients' perceptions towards embryo disposition after donation of their excess embryos to a research biobank affords a unique perspective. The difficulty of the disposition decision, the tendency to discard embryos in the absence of a means for donation to research, and the poor agreement between heterosexual partners highlight the importance of donation to research as an accessible disposition option and the need for a personalized approach to counseling and consenting for embryo disposition.
Breastfeeding disparities between White and Hispanic birthing parents recovering in the obstetrics units at Stanford Medicine Children’s Health

Context/Purpose: Disparities in rates of any breastfeeding are pronounced at 3 months post-birth; locally, 9 of 10 White infants receive any breastmilk whereas 7 of 10 Hispanic infants do in San Mateo and Santa Clara counties. We evaluated whether these disparities are present in the obstetrics units at Stanford Medicine Children’s Health where breastfeeding practices begin.

Methods: Feeding intentions and practices for White (n=458) and Hispanic (n=594) during their delivery and recovery hospitalization were captured from the electronic health record from January 1 – June 30, 2022. Differences between White and Hispanic parents and English- and Spanish-language-preferring Hispanic patients were evaluated with Fisher’s Exact Test or chi square tests.

Results: Almost all (≥99%) birthing parents reported they intended to provide some breastmilk to their infant and did begin breastfeeding in the obstetrics ward (≥97%). Disparities were present in exclusive use of parental human milk in the obstetrics ward: 86% of White parents exclusively fed parental milk to their infant whereas 75% of Hispanic parents (P<.0001). Within the sample of Hispanic birthing parents, 80% of English-language-preferring and 71% of Spanish-language-preferring birthing parents did (P<.0001). Among the sub-sample of parents intending to exclusively breastfeed, similar disparities between White and Hispanic (88% vs. 77%; P<.0001) and English- and Spanish-preferring Hispanics (83% vs. 73%; P=.01) where found.

Interpretation: These results indicate that nationally and locally observed disparities in breastfeeding begin earlier during recovery in local obstetrics units and may be exacerbated by language barriers. These disparities exist even among birthing parents who intended to exclusively breastfeed at admission.

Conclusion: In a collaboration between OB/GYN and the Evaluation Sciences Unit (ESU), a qualitative needs assessment was conducted to inform targeted efforts needed to address these disparities during recovery in the hospital. Insights from this assessment will be discussed.
Neurobiological Mechanisms Linking Early Experience Unpredictability and Child Behavioral Health: Implications for Intervention and Clinical Practices

Unpredictability is increasingly recognized as a core construct of adverse early experiences that negatively affects child health and development outcomes. In the current socio-cultural contexts of the global pandemic, intense climate-related disasters, and geo-political conflicts, families of young children are facing drastically increasing unpredictability, which poses significant risks for child mental and behavioral health. For example, in one study conducted during the pandemic, we found that the unpredictability of family material hardship status (i.e., difficulty paying for basic needs) increased young children’s internalizing and externalizing symptoms (β=.04, p<.05) through disrupting family routines (α*β=.01, p<.05). The current study aimed to synthesize animal- and human-based empirical research and propose a conceptual model to delineate the neurobiological underpinnings of early experience unpredictability in relation to child behavioral health.

Following a bio-ecological framework, this conceptual model illustrated three main neurobiological processes through which unpredictability in multiple social contexts (i.e., family, neighborhood/community, & socio-cultural events) negatively affected child behavioral and mental health: 1) the accelerated maturation of the corticolimbic neural circuitry, 2) physiological stress response mediated via the neuroendocrine and autonomic nervous systems, and 3) systemic inflammation and pro-inflammatory tendencies in the immune system. Young children’s typical neurobiological development largely depends on caregivers’ predictable signals as an external regulatory source. Thus, we further highlighted unpredictable parental signals and practices (i.e., caregiving unpredictability) as the key mediator through which unpredictability factors in distal social contexts (e.g., community, socio-cultural events) exert effects on child neurobiological and subsequent behavioral health. Although unpredictability in broader social contexts is often difficult to alleviate directly, parenting practices are relatively malleable processes. Intervention and clinical practices may incorporate the identification and intervention of caregiving unpredictability as an effective way to mitigate the negative health consequences of unpredictable early experiences in broader social contexts and facilitate optimal child health and development.
Peripartum risk factors contribute to trends in neonatal hypoxic ischemic encephalopathy

Objective: Analyze the temporal trends in hypoxic ischemic encephalopathy and peripartum risk factors from 2010-2019.
Study Design: This is a secondary analysis of prospectively collected data in the California Perinatal Quality Care Collaborative, linked to the Vital Statistics birth cohort. Records were analyzed that met inclusion criteria of live births ≥ 36 weeks, admitted to the NICU with qualifying conditions and born 2010-2019. Trend analysis analyzed the rate of peripartum risk factors and severity of hypoxic ischemic encephalopathy. Unadjusted and multivariable hierarchical logistic regression were performed to determine factors associated with hypoxic ischemic encephalopathy stratified by grade.
Results: 62,888 infants met inclusion criteria and 4,193 were diagnosed with hypoxic ischemic encephalopathy. Over a 10-year period, the incidence of hypoxic ischemic encephalopathy more than doubled. The greatest increase occurred in moderate hypoxic ischemic encephalopathy. In the trend analysis, rates of chorioamnionitis, diabetes, hypertension, obesity, maternal bleeding, fetal malpresentation, fetal distress, prolonged rupture of membranes, operative vaginal birth, vaginal birth after cesarean birth and vaginal birth increased; the rate of cesarean delivery decreased over time. Logistic regression demonstrated an increased odds of moderate/severe hypoxic ischemic encephalopathy with: more recent birth year, small for gestational age, higher maternal educational attainment, non-California Children’s Services level of care, hypertension, bleeding, operative vaginal delivery and cesarean delivery.
Conclusion: In this high-risk neonatal cohort, the diagnosis of hypoxic ischemic encephalopathy, peripartum risk factors and maternal co-morbidities have increased. Peripartum factors, availability of therapeutic hypothermia, maternal co-morbidities and delivery location likely contribute to the rise in hypoxic ischemic encephalopathy.
Lessons learned in maternal cardiopulmonary arrest simulation: where should educational efforts be concentrated?

Objective:
Deficiencies exist in the management of maternal cardiac arrest (MCA). Given the rarity of the event, simulation is vital to determine best practices. We analyzed action-based performance in multidisciplinary, simulated MCA scenarios to determine characteristics of teams that can initiate delivery by 4 minutes, as recommended by the American Heart Association (AHA).

Study design:
This is a prospective cohort from 2/2018-3/2021 of monthly sessions with didactics and four, high-fidelity, MCA training scenarios based on AHA recommendations. Nurses, medical students, residents and fellows from Ob/Gyn, Emergency Medicine, OB and General Anesthesia and Maternal Fetal Medicine were consented. Rhythm interpretation was required: half of the cases had pulseless electrical activity and half had ventricular fibrillation. Time to key events in the AHA directives were prospectively collected. The primary outcome was time of incision, a proxy for time of delivery. Secondary outcomes included additional key events in management. A sub-analysis compared time to events for teams that initiated delivery at 4 minutes (rounded to the nearest minute) and those that did not. Statistical analysis included an unpaired, two-tailed t-test.

Results:
111 scenarios were analyzed. Mean time (minutes:seconds) to events included: 00:35 to left uterine displacement, 00:53 to first ventilation, 1:53 to first rhythm check, 2:52 to advanced airway and 4:39 to incision. 47.5% of teams initiated resuscitative hysterotomy by 4 minutes and 52.5% by 5 minutes or later. Teams that initiated delivery by 4 minutes, were statistically significantly more likely to perform first and second rhythm analysis and administer a fluid bolus earlier (Table 1). Of all teams, 97% initiated delivery within 5:50.

Conclusion: We demonstrate the feasibility of a multidisciplinary simulation program that adheres to the AHA guidelines on MCA. Future educational efforts should prioritize teaching efficiency in rhythm analysis and fluid management, as these were rate limiting steps to initiating delivery.
The Role of Certified Diabetes Care and Education Specialists in the Development of the 4T Program

Objectives: To describe the role of Certified Diabetes Care and Education Specialists (CDCES) in the development and implementation of the 4T Program (Teamwork, Targets, Technology and Tight control) at Stanford Children’s Diabetes Clinic.

Methods: Youth with newly diagnosed Type 1 diabetes (T1D) were started on a continuous glucose monitor (CGM) within the first month of diagnosis as part of the 4T Program. A subset received weekly CGM data review by a CDCES and messages with education and dose changes were sent via the electronic medical record. Plan, Do, Study, Act (PDSA) cycles were utilized to determine the best workflow for the team and for families to develop a scalable process for CGM review.

Results: During the 4T pilot study, a total of 135 youth were started on CGM. The team developed a workflow for CGM initiation, follow up, and education as well as handouts for patients. The CDCES team reviewed the CGM tracings of a subset of participants (n = 89) weekly in their first year and sent messages to each family each week if changes to insulin dosing were needed. The CDCES team helped co-develop a population health dashboard to facilitate CGM data review. CDCES input helped engineering colleagues define a workflow that allowed the growth of this program without increasing the number of CDCES on the team.
Exogenous attentional deficits in children with dyslexia are comparable in tasks generic or specific for reading.

Reading is a complex visual task which demands the shifting and focusing of attention to extract relevant information. Spatial attention can be directed to a location in space involuntarily (exogenously) due to the appearance of a peripheral stimulus (Ramamurthy, 2021). Previous studies have shown that children with dyslexia show deficits in utilizing exogenous, or reflexive, spatial cues in a task that has no relation to reading (Facoetti, 2000). In this study, we analyzed how children with dyslexia utilize exogenous spatial cues in a task relevant for reading compared to a general visual perception task. 40 children (6-16 yrs) participated in the study, completing our reading specific multi-letter processing task, reading non-specific dot detection task, and battery of standardized reading assessments (a standardized reading score below 85 is considered dyslexic). For both tasks, participants were required to fixate on a central cross while a precue flashed before a stimulus. In the dot detection task, a dot flashes for a brief period on either side of the cross and participants report the dot position. In the multi-letter processing task, a string of letters appeared for an encoding period and participants reported the element in the post-cued position. Performance in the multi-letter processing task shows a moderate but significant correlation with reading ability, while the non-specific task shows no correlation. We analyzed the cue benefit, cue cost, and cue effect of valid, neutral, and invalid precues to determine attentional effects. We show comparable exogenous cue effects between typically developing children and children with dyslexia in both tasks, with the dot detection task showing lower effects. Accuracy and reaction time are also comparable across groups. Our findings show no deficit in the utilization of exogenous attentional cues in children with dyslexia compared to typically developing children for tasks either generic or specific for reading.
Global DNA Methylation of Human Placenta-Derived Trophoblast Stem-like Cells

The human placenta is the first organ established during development functioning as a site of nutrient exchange between mother and baby. Trophoblasts are the stem cell population that build the architecture of the placenta and their dysfunction is attributed to adverse pregnancy outcomes. Recent work by Okae and others has established the generation of trophoblast stem-like cells (TSC) from human placenta chorionic villi. Epigenetic regulation is essential to gene expression, and the placenta is known for its distinct DNA methylation profile which can be used to identify trophoblast subtypes. However, global DNA methylation of TSCs compared to widely used trophoblast cell lines, HTR-8/SVneo, and JEG-3 choriocarcinoma, has not been accomplished. This study investigates the global DNA methylation profile of TSCs isolated from first-trimester human placenta chorionic villous tissue. We hypothesize TSCs are a high quality model of early human placenta trophoblasts compared to standard trophoblast lines. We performed global DNA Methylation using an Infinium Methylation EPIC array on three TSC lines, and two standard trophoblast cell lines to examine over 185,000 methylation sites. Our results show a considerable variation in Cytosine Guanine (CpG) site differential methylation between TSC and standard trophoblast lines. Promoter methylation assessment by gene confirmed all three lines as trophoblasts based on EIF5 and CK7 lineage markers, with two of the lines possessing hallmarks of TSCs, including GATA3 and TFAP2α. Altogether, we derived TSCs from first-trimester whole placenta tissue which can be used as an important in vitro model of early development and contribute to future therapies to improve maternal-fetal medicine.
Location, location, location: the association between neighborhood factors and very low birthweight infant care processes and outcomes

Purpose: National organizations call for including social factors in neonatal intensive care unit (NICU) performance assessments. We aim to understand the contributions of maternal neighborhood factors on four quality-sensitive outcomes for very low birth weight (VLBW) infants.

Methods: Analysis of 119 NICUs in the California Perinatal Quality Care Collaborative included all VLBW infants born from 2008-2011; maternal addresses were geocoded and linked to 2010 census tract data on social and built environment attributes from the California Neighborhoods Data System. We used multivariable hierarchical regression including infant, maternal, hospital, and neighborhood factors; outcomes were high growth velocity (GV), healthcare-associated infections (HAI), chronic lung disease (CLD), and in-hospital mortality.

Results: Among 17,782 infants, 53% had high GV, 12% HAI, 21% CLD, and 6% died. Univariate models showed neighborhoods with high socioeconomic status were associated with decreased odds of HAI, CLD, and mortality; neighborhoods with high proportions of minorities or foreign-born people were associated with slower GV, higher HAI, and higher CLD. In multivariable models, population density and non-urban neighborhoods were associated with high GV; greater street connectivity was marginally associated with increased odds of CLD; and neighborhood factors were no longer associated with HAI or mortality.

Interpretation: Reduction of significant associations in fully-adjusted models indicates that maternal and infant factors may mediate relationships between neighborhood factors and clinical outcomes. Associations between population density and street connectivity with clinical outcomes require further investigation. The lack of significant associations of neighborhood factors with HAI supports its use as a measure of NICU care quality. Similarly, the lack of associations with mortality suggests that the NICU environment may buffer the impact of neighborhood influences.

Conclusion: Neighborhood characteristics are associated with some aspects of quality; further research is needed to better understand the underlying pathways.
Selection of Novel Blood-Brain Barrier Penetrating Adeno-Associated Virus Capsids with Bias for Neurons or Astrocytes

Research Statement: We sought to select novel capsids from an adeno-associated virus (AAV) library with enhanced ability to cross the human blood-brain barrier (BBB) and transduce brain cells.

Background and Relevance: Currently, FDA approved gene therapies target either the retinal pigment epithelium or spinal motor neurons for congenital blindness and spinal muscular atrophy, respectively. However, the brain is difficult to target because of the BBB. One way to resolve this impediment is to develop new AAV capsids with the ability to transcytose across the BBB and enter the brain. Design and Methods: Human cells were used in conjunction with a transwell system to select new AAV capsids using an ex vivo BBB model. Human cerebral microvascular endothelial cells (hCMEC/D3) were seeded on the apical side of the transwell and human astrocytes were seeded on the basolateral side. Capsids were selected from the library over the course of three rounds. RNA was harvested from the astrocytes to determine which capsid variants had crossed the endothelial cell layer. This process was repeated using neurons rather than astrocytes. Results: Six novel capsids transduced astrocytes 37 to 91-times better than AAV9. Four novel capsids selected using neurons crossed the transwell BBB 4 to 20-times better than AAV9. After systemic injections into mice, none of these capsids performed better than AAV9 at crossing the BBB, indicating that their BBB-penetrating properties may apply to human, but not mouse cells. Overall, a variety of novel AAV capsid variants were identified that have increased ability to cross a human ex vivo BBB compared to AAV9, the current gold standard being used in human clinical trials. Conclusions: The capsids developed here show promise in delivering genes to human neurons and astrocytes, cells that have historically been elusive for gene therapy. Future directions include testing these capsids in non-human primates.
Patient Centered Pathology for Family Planning Services: Procedures, Reporting, and Leveraging the EMR

Context: Histopathologic examination and reporting of fetal remains, when desired, can be helpful and therapeutic. When examination and reporting is not desired, these procedures cause unnecessary distress in addition to generating patient charges and using hospital and lab resources. At Stanford, we have implemented an opt-in system of internal tracking without reporting of such specimens in cases without a clinical question in which the patient does not wish to have the tissue examined.

Methods: Later this year, the Stanford Pathology Department will transition to EPIC Beaker as our primary laboratory information system (LIS). This will improve many aspects of care, and interface directly with the electronic medical record. We have leveraged this change to develop a new ordering format for fetal remains that will give the patient the option of waiving pathology examination, reporting, and charges. The specimen will be stored for 30 days before disposal, giving patients time to make arrangements if desired. The clinician also has the option of having the placenta, the fetus, or both examined if clinically relevant.

Results: This workflow adjustment will reduce the number of specimens received in the pathology department, reduce unnecessary report generation, and empower patients to make decisions that are appropriate for their reproductive lives and ensure patient privacy.

Interpretation: Our aim is to minimize the emotional and financial burden on patients experiencing a pregnancy loss or those receiving abortion care by decreasing unnecessary testing and reporting. We offer a simple workflow alteration which places decision-making around specimen examination back into the hands of the patient.

Conclusion: This alteration is a step toward patient-centered pathology reporting within a reproductive justice framework, in a political moment when reproductive self-determination is under threat. Here we outline our workflow as a roadmap for other institutions.
The Role of Emotion Regulation in the Relationship Between Self-Compassion and Psychological Well-Being: A Study among First-time Pregnant Women in Kerala

Women go through multiple physiological and psychosocial changes during the unique life experience of pregnancy. This makes pregnant women and new mothers vulnerable to a wide spectrum of mental disorders. Perinatal research so far, has almost exclusively focused on epidemiology and treatment of psychopathologies. But from a positive psychological perspective, pregnancy is a mature crisis with great potential for positive change and fortunately, now there is a slow shift of attention in similar lines. In terms of healthcare indicators, Kerala has achieved the UN’s SDG target for IMR reduction and commendable reduction in MMR and birth rate. But, when it comes to mental health and its promotion, the status is not quite laudable. Set against this backdrop, a positive psychological construct, self-compassion shows evidence of correlation with psychological well-being and with factors influencing well-being. There is growing interest in self-compassion as a possible intervention to enhance psychological well-being. However, a range of mediating factors affects and determine the extent to which well-being can be enhanced through self-compassion. But surprisingly, little research has examined these pathways in pregnant women. Research suggests emotion regulation as a plausible pathway. Hence, the study examines how self-compassion is responsive in enhancement of well-being through the emotion regulation strategies. Given that this research is first of its kind, in exploring elements involved in the buffering aspect of self-compassion in pregnant women, this study would possibly make significant theoretical and clinical implications. The findings of the research would ascertain the effectiveness of self-compassion as an intervention for mental health promotion. This research would steer deliberations on strength of mediating effect of emotion regulation on the relationship between self-compassion and well-being. This study will thereby provide an insight in framing the micro and macro strategies for intervention programmes for pregnant women.
Multiplex mass-tag cell barcoding assay for the high-throughput pharmacological assessment of candidate compounds to prevent pregnancy complications

While multiple biological and exogenous factors contribute to pregnancy complications such as preterm birth and preeclampsia, increasing evidence suggests that dysfunctional women’s immune adaptations during pregnancy play a key role in their pathogenesis. We adapted a multiplex Mass-tagged Cell Barcoding (MCB) mass cytometry assay for high-throughput, in vitro immune monitoring of promising pharmacological compounds for the prevention of PTB and PE, which were previously identified using a computational drug repurposing pipeline. The goal of this investigation is to identify promising immunomodulatory drugs that can effectively influence pathologically altered immune profiles.

We have finalized the technical implementation of our in vitro drug screening assay and validated the accuracy and reproducibility of our whole system’s immunology analysis (37 marker antibody panel) with a reduced volume of only 100µl of whole blood per condition. This allows us to screen a 10x higher number of drugs of interest compared to the standardly used volume of blood. We have generated time course data to optimize the timepoint for the detection of immunomodulatory effects. We are currently screening candidate drugs that were selected by a disease-drug effect gene-matching algorithm in a previous publication. Preliminary data show that our assay is extremely sensitive to detect signaling activity changes induced by stimulation and mediated by in vitro drug exposure.

After the initial screen of approximately 30 preselected candidate drugs in healthy volunteers, our analysis will identify the drug(s) with the best influence profile on pathology-related immunome features for further in vitro and in vivo testing.
Siglec-6 signaling uses Src kinase tyrosine phosphorylation and SHP-2 recruitment

Purpose- Preeclampsia is a pregnancy-specific disorder involving placental abnormalities. Elevated placental Sialic acid immunoglobulin-like lectin (Siglec)-6 expression has been correlated with preeclampsia. Siglec-6 is a transmembrane receptor, expressed predominantly by the trophoblast cells in the human placenta. It interacts with sialyl glycans such as sialyl-TN glycans as well as binds leptin. Siglec-6 overexpression has been shown to influence proliferation, apoptosis, and invasion in the trophoblast (BeWo) cell model. However, there is no direct evidence that Siglec-6 plays a role in preeclampsia pathogenesis and it’s signaling potential is still largely unexplored. Siglec-6 contains an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an ITIM-like motif in its cytoplasmic tail suggesting a signaling function. This study was conducted to determine if Siglec-6 possesses intracellular signaling capabilities.

Methods- We employed site-directed mutagenesis to create a series a series of Siglec-6 expressing HTR-8/SVneo cell lines to determine the contribution of each of these residues to Siglec-6 intracellular signaling capabilities. Co-immunoprecipitation and inhibitory assays were utilized to investigate the role of Src-kinases and SH-2 domain-containing phosphatases with Siglec-6.

Results- We show that Siglec-6 is phosphorylated at both the ITIM and ITIM-like domains by Src family kinases. Phosphorylation of both ITIM and ITIM-like motifs is essential for the recruitment of phosphatases like Src homology region 2 containing protein tyrosine phosphatase 2 (SHP-2), which has downstream signaling capabilities.

Interpretation- This work demonstrates the signaling potential of the uniquely expressed placental Siglec-6 suggesting it plays a functional role in normal trophoblast biology.

Conclusion- Siglec-6 acts as a signaling molecule in human trophoblasts. In addition, further investigation is warranted to determine which signaling pathways are activated downstream to SHP-2 recruitment and how over-expression of Siglec-6 in placentas from preeclamptic pregnancies impacts trophoblast function and preeclampsia pathogenesis.
Targeting Nemo-Like Kinase with small molecules for treatment of Diamond Blackfan Anemia

Context: Diamond Blackfan Anemia (DBA) is a rare inherited bone marrow failure syndrome characterized by a hypoproliferative anemia. Current therapies for DBA, including red cell transfusions, corticosteroid use, or stem cell transplantation, are associated with significant morbidity. Our previous work demonstrated that the serine threonine kinase, Nemo-Like Kinase (NLK) is hyper-activated in erythroid progenitors from the ribosomal protein S19 (RPS19)-deficient DBA models.

Methods: Cord blood CD34+ hematopoietic stem and progenitor cells (HSPCs) were transduced with lentivirus co-expressing GFP and shRNA against RPS19, RPL11 or luciferase control, and were differentiated in erythroid media in the presence of small molecule NLK inhibitors. The percentage of CD71+ erythroid cells was measured by flow cytometry and the absolute number of CD71+ erythroid population was normalized to the total viable cell number.

Results: After screening small molecule compounds that inhibit NLK as an off-target from previously approved or clinically advanced drugs, we found that a known MELK (maternal embryonic leucine zipper kinase) inhibitor, OTS167, improved erythropoiesis by 31% and 27% at 50nM concentration in RPS19- and RPL11-knockdown HSPCs, respectively. OTS167 was developed as an antitumor drug and is currently being tested in Phase II clinical trials for treatment of leukemias and advanced myelodysplastic syndromes.

Interpretation: The goal in treating DBA patients with NLK inhibitors is to sufficiently raise the hemoglobin to minimize the need for chronic red cell transfusions or treatment with steroids. Our in vitro results predict pharmacologic inhibition of NLK with OTS167 will achieve this goal. We have initiated studies testing the efficacy of OTS167 in vivo using a novel Rpl11-haploinsufficient mouse model that we recently developed. This model is superior to existing models, faithfully mimicking the macrocytic anemia and 50% reduction of RPL11 found in DBA patients.

Conclusion: Small molecule NLK inhibitors have the potential to treat anemia in DBA patients.
Ketonuria is associated with a positive 1-hour oral glucose tolerance test

Context/purpose: Fasting prior to the 1-hour oral glucose tolerance test (OGTT) may increase the probability of a positive screen result. Because ketonuria may be a surrogate marker for prolonged fasting, we examined the association between ketonuria and a positive OGTT screen.

Study Design: This is a secondary analysis of a single-center prospective randomized trial comparing the effects of a prolonged fast for 6 or more hours versus eating within 2 hours of the 1-hr OGTT. A positive screen was defined as a glucose ≥140 mg/dL. Ketonuria data were collected for participants enrolled in the trial when their obstetrics visit coincided with the OGTT. Rates of ketonuria in the fasting versus the fed group were ascertained. Fisher’s exact test was then used to analyze ketonuria and the rate of a screen positive result. Secondarily, we analyzed mean glucose values in those with and without ketonuria. We then calculated mean glucose values based on the ketonuria level (none, trace, 1+, 2+/3+) followed by the mean fasting times prior to the OGTT based on these levels of ketonuria.

Results:
Of 200 participants, 195 completed the 1-hour OGTT. Urine ketone levels were available in 168 patients, with an OGTT screen positive rate of 28.2% (n=37). 20 participants (11.9%) had evidence of ketonuria. Among those with ketonuria, the screen positive rate was 50.0% (n=10), as compared to 18.2% (n=27) in those without ketonuria (p-value = 0.003). The mean glucose value in the ketonuria group was 154.4 mg/dL versus 115.7 mg/dL in those without (p-value = <0.001). In participants with ketonuria, mean glucose values remained relatively similar and were not dependent on the amount of ketones present (153-155 mg/dL) (Table 2). However, the amount of ketonuria was dependent on the length of fasting prior to the OGTT ranging from 5.7 hours in those without ketonuria to 10.0 hours in those with 2+/3+ ketonuria (Table 2).

Conclusion: Women with ketonuria at the time of the 1-hr OGTT screen for GDM had higher mean glucose levels and were more likely to screen positive, suggesting a prolonged fast may be associated with a higher screen positive rate.
Patient preferences, beliefs, and experiences regarding oral intake and the 1-hour oral glucose tolerance test

Context/Purpose: The 1-hour oral glucose tolerance test (OGTT) is the primary test used for gestational diabetes mellitus (GDM) screening in the US and was designed to be administered without regard to time of day or last meal. We examined patient preferences, beliefs, and experiences regarding oral intake and their perceived effect of oral intake on the 1-hr OGTT.

Study Design: This was a secondary analysis of a single-center randomized trial that investigated the effect of oral intake on the 1-hour OGTT. Following the OGTT, participants completed an electronic survey querying their oral intake, and how they believed fasting versus eating within 2 hours of the OGTT would affect screening results.

Results: All 195 participants who completed the 1-hr OGTT completed the survey. Among participants, 19.8% (n= 38) believed fasting would increase the glucose level, 49.2% (n=95) believed fasting would decrease the glucose level, and 31.1% (n=60) believed fasting would not affect the glucose level (Figure 1). If given a choice, 71.0% (n=137) of participants would prefer to eat without restriction prior to the OGTT and 29.0% (n= 56) would prefer to fast for 6 or more hours prior. 89 (45.6%) respondents had taken the OGTT in a prior pregnancy, with 19.1% (n=17) reporting that a provider gave recommendations about oral intake before the test. Seven (41.2%) of these participants were told to fast or not eat for 8 hours or more, 3 (17.6%) were told to not eat for 1-2 hours prior, 3 (17.6%) were told to limit carbohydrates, and 4 (23.5%) did not specify the recommendation given (Figure 2).

Conclusion: While most respondents would prefer to eat within 2 hours of the 1-hr OGTT, the majority believed fasting prior to the 1-hr OGTT would lead to a lower glucose value compared to eating within 2 hours of the test. Objective data on the effect of oral intake prior to the OGTT are warranted to inform appropriate recommendations.
**Barriers, Facilitators, and Practice Patterns of Pediatricians Treating Lower Urinary Tract Symptoms: Initial Thematic Findings**

**Context/Purpose**
Pediatric lower urinary tract symptoms (LUTS) can significantly impact a child’s physical and emotional well-being. These negative consequences can be significantly reduced through early intervention, yet little knowledge exists about treatment of LUTS at the primary care level. To support early treatment of LUTS, we aim to understand pediatricians’ barriers, facilitators, and practice patterns in LUTS treatment.

**Methods**
We conducted two focus groups of pediatricians from California. A framework-based semi-structured moderator guide was developed based on the primary investigator’s clinical experience, previous literature, and feedback from providers. Focus groups were audio recorded and transcribed. The first two focus groups were analyzed by three independent coders using inductive and deductive methods to develop a codebook.

**Results**
Seven pediatricians aged 30-69 (male=1) were interviewed. Practice types included general outpatient setting, hospital-based/inpatient, and subspecialty. Preliminary codebook noted six domains: facilitators, barriers, practice patterns, knowledge, physician training, and potty training. Pediatricians vary in delivery of bladder health education, often prompted by family request. They discuss frequent voiding but do not obtain bladder diaries. Hygiene and potty training are considered topics of importance. Barriers to care include limited clinic visit time and knowledge of comprehensive LUTS treatment. Pediatricians acknowledged a need for resources for parents and providers in non-English languages.

**Interpretation**
There is a need for an evidence-based education program that is well publicized and accessible. Topics that fall outside recommendations, such as hygiene and potty training, emerged as areas to clarify and define as part of our provider education programs. Cultural barriers exist in practice. These findings were used to refine our moderator guide.
Conclusion
Next steps include analysis of the next three focus groups that were conducted with the revised moderator guide. Final data will inform bladder health programs aimed at delivering LUTS treatment at the primary care level.
A Novel Causal Mapping Approach to Define a System Dynamics Model for Prioritizing Strategies to Increase Low-dose Aspirin Adherence amongst Women at High Risk of Pre-eclampsia

Context: Pre-eclampsia (PE) is a leading cause of maternal mortality and morbidity which may be prevented by antenatal adherence to aspirin prophylaxis.

Purpose: The study aimed to 1) understand factors influencing pregnant women’s decision to take low-dose aspirin (LDA) from a systems perspective, and 2) design a prioritization strategy to achieve the highest gain in LDA uptake rate.

Methods: We conducted a literature review to identify causal factors and the frequency of causal relationships between variables and macro-variables, and synthesized the data into a causal map based on the Theory of Planned Behavior (TPB). Using the causal map to define the boundary of a system dynamics (SD) simulation model, simulation results were generated to identify effective intervention points and prioritization strategies.

Results: Provider, patient, community and systemic factors (n=65) were found to coexist in health systems and influence a pregnant woman’s attitude, perceived difficulty, and social pressures for LDA intake and adherence. Simulation modeling using our causal map of inter-relationships among these factors suggests that fostering communications between women who experienced PE, had taken aspirin, or experienced preterm birth with women at-risk for PE, alongside disseminating unified messaging and information from providers to at-risk women about aspirin may yield the greatest gains in LDA uptake - about 1,700% cumulative gain within five years. The second-best strategy identified was prioritizing word-of-mouth influence in the community, followed by disseminating information and messaging, producing a cumulative gain of 60%. Addressing individual factors yielded no substantial benefits.

Conclusion: We introduce a methodology to 1) capture the dynamics that arise from multi-level, multi-sectoral system components, and 2) identify priority intervention points that could optimize gains in LDA uptake. Our results favor combined interventions that include patient and community-level engagement as well as information dissemination by providers that improves attitudes and norms regarding aspirin uptake.
The Effects of Long-term Food Oral Immunotherapy on Compliance, Quality of Life, and Perceived Burden of Care

Rationale: The safety and efficacy of food oral immunotherapy (OIT) has been extensively studied, however long-term maintenance OIT studies are limited. To better understand the durability of OIT long-term, larger studies on compliance, quality of life (QOL), and burden of treatment (BOT) are needed.

Methods: Participants and caregivers who participated in food OIT clinical trials at Stanford University completed three IRB approved surveys: food allergy long-term follow up questionnaire (FALTFU), food allergy quality of life questionnaire (FAQOL), and burden of treatment questionnaire (BOT). FAQOL scores at baseline and study completion were compared using a Wilcoxon signed rank test. BOT was measured on a 1 to 7 scale (1=extremely positive, 7=extremely negative).

Results: 186 participants completed FALTFU questionnaires. 120 (69%) were children (0-12 years). Compliance rates were 79% for multi-food OIT (n=77) and 61% for single-food OIT (n=109, p=0.016). Younger children (0-12 years) were more compliant vs older children and adults (80% vs 52%, p<0.001). 104 (65%) continued peanut, 40 (78%) continued cashew, and 36 (69%) continued walnut. Multi-food OIT participants reported worse baseline FAQOL vs single-food OIT participants (p=0.034). Multi-food OIT participants reported significant improvement of FAQOL after study completion (p<0.001) but single-food OIT participants did not (p=0.055). Younger children reported positive or extremely positive more frequently on BOT vs older children and adults (82% vs 57%, p=0.001).

Conclusion: This is the largest, long-term OIT follow up study to date, to assess compliance, QOL, and BOT. The majority of participants continued OIT long-term. Positive treatment perception and improved QOL were seen among all participants.
The Effects of Turner’s Syndrome Aneuploidy on Human Trophoblast Stem-like Cell Differentiation

Turner’s Syndrome occurs when one chromosome is wholly or partially absent, with 99% of these pregnancies ending in spontaneous miscarriage within the first trimester.1 Although this type of aneuploidy occurs in 3% of all pregnancies, previous studies associate these adverse outcomes with abnormal trophoblast differentiation. Recently a method established the generation of trophoblast stem-like cells (TSCs) from human placental chorionic villi.2 Here, we use a novel model to determine if Turner’s Syndrome TSCs possess dysfunctional differentiation compared to TSCs from euploids, a complete number of chromosomes. We utilize live imaging methods along with immunofluorescent staining to assess phenotypic and functional differences under both stem cell maintenance and differentiation conditions. Our live imaging results identified Turner’s syndrome TSCs differ in morphology and cell proliferation capacities. Furthermore, protein expression data in aneuploid TSC is inconsistent with euploid differentiated into extravillus trophoblast-specific markers expression patterns. In conclusion, Turner’s Syndrome TSCs display phenotypic differences requiring additional experiments to validate abnormal differentiation. This study is the first to show the ability to generate aneuploid TSCs and thus produce a model to improve health outcomes for Turner’s syndrome pregnancies.

Variations in telehealth practice patterns in maternal fetal medicine providers by patient insurance status

Objective: Telehealth has the potential to improve access to care and decrease health disparities. The use of telehealth has increased following the Covid-19 pandemic. However, little is known on the variations in telehealth utilization by Maternal Fetal Medicine (MFM) providers and its relationship to patient insurance status. We aimed to assess differences in maternal fetal medicine physician telehealth usage by patient insurance status.

Study Design: We conducted a web-based, anonymous survey of Society for Maternal-Fetal Medicine membership in December 2020. Users of telehealth were defined by reported use of live video teleconferencing before or after the start of the Covid-19 pandemic. Comparisons by percentage of Medicaid-covered patients were performed using Fisher’s exact test and a p-value of <0.05 was considered significant.

Results: Out of 373 completed surveys, 330 (88%) were users of telehealth. Respondents practiced in the South (31%), West (28%), Northeast (21%), and Midwest (20%). Of the total respondents, 52% reported practicing in a university hospital setting. 50% of total respondents reported patient access to internet/data plans as a barrier to telehealth visits, independent of the percentage of publicly insured patients. Software/hardware usability (44%), the patients desire for in-person visits (41%), and reimbursement/insurance coverage (40%) were other more frequently selected barriers.

Conclusions: A majority of MFM providers offer telehealth services, with providers facing similar barriers, independent of patient insurance status. These results suggest an opportunity to improve overall uptake of telehealth for providers across diverse patient demographics.
Objective: Delivery of prenatal care via telehealth has the potential to improve access to and decrease health disparities. The Covid-19 pandemic precipitated changes to healthcare service provision including increased use of telehealth. The experience of Maternal Fetal Medicine providers can help identify general barriers encountered in the implementation of telehealth. We aimed to identify differences in barriers experienced by MFM providers by US region and urbanicity.

Study Design: We conducted a web-based, anonymous survey of the Society for Maternal-Fetal Medicine membership in December 2020, assessing telehealth use during the Covid-19 pandemic. Users of telehealth were defined by reported use of live video before or after the start of the pandemic. Comparisons by region and urbanicity were performed using Fisher’s exact test and a p-value of \(<0.05\) considered significant.

Results: The survey response rate was 16%. Out of 373 completed surveys, 330 (88%) were users of telehealth. Respondents practiced in the South (31%), West (28%), Northeast (21%), and Midwest (20%). Practice location was 65% urban, 21% suburban, and 14% rural or small town and. There was no difference by user status and region (p=0.29) or practice location (p=0.13). 52% reported practicing in a university hospital setting. 54% of respondents cited patient access to internet / data plans as a barrier to telehealth usage, with providers from rural practice settings more likely to report this barrier. 42% cited reimbursement / insurance coverage as a barrier. Of the total respondents, less than 20% of providers across all regions note patient privacy concerns or telehealth set up costs as barriers to telehealth in their practice.

Conclusions: Providers face similar barriers to telehealth implementation, independent of US region or practice. These results highlight opportunities to improve the use of telehealth, in effort to improve access and overall perinatal health.
**Practice patterns and telehealth usage of maternal fetal medicine providers by US region and urbanicity**

Objective: The Covid-19 pandemic precipitated changes to healthcare service provision. Little is known about Maternal Fetal Medicine (MFM) providers’ practice patterns and use of telehealth during this time. We assessed differences in MFM physician practice patterns and telehealth use by US region and urbanicity. Study Design: We conducted a web-based, anonymous survey of the Society for Maternal-Fetal Medicine membership in December 2020, amid the Covid-19 pandemic. Comparisons of clinical activities, provider preferences, and telehealth use by region and urbanicity were performed using Fisher’s exact test with a p-value of <0.05 considered significant. Results: The survey response rate was 16% and 342 out of 422 surveys had complete data for analysis. Respondents had a median age of 46 (interquartile range 39-58) and were 65% female. Practice locations were mostly in an urban/large city (65%) and distributed across all US regions (Table 1). Live video visits were used by 30% of participants before and 80% after the start of the Covid-19 pandemic. Providers in the South had the lowest percent of respondents providing comprehensive care, were more likely to care for majority Medicaid patients, and practice in a rural area or small town. Providers across all regions agreed that telehealth improved patient access to healthcare services (87%) and significantly improved the lives of patients (68%). Urban providers were more likely to provide comprehensive prenatal care, practice in a university setting, and less likely to provide telehealth before the pandemic compared to rural/small town providers (Table 2). Conclusions: Telehealth usage by MFM specialists has increased during the Covid-19 pandemic. Practice patterns vary by US region as well as within the urban vs rural practice setting. These results highlight changes in maternal fetal medicine distribution of care and suggest opportunity to improve access to care via long-term implementation of telehealth.
Organism-wide secretome mapping uncovers pathways of tissue crosstalk in exercise

There has been growing interest in identifying blood-borne factors that mediate tissue crosstalk and function as molecular effectors of physical activity. Although past studies have focused on an individual molecule or cell type, the organism-wide secretome response to physical activity has not been evaluated. Here, we use a cell type-specific proteomic approach to generate a 21-cell type, 10-tissue map of exercise-regulated secretomes in mice. Our dataset identifies >200 exercise-regulated cell type-secreted protein pairs, the majority of which have not been previously reported. Pdgfra-cre-labeled secretomes were the most responsive to exercise training. Elevated lactate levels can directly regulate protein secretion. Lastly, we establish an anti-obesity and antidiabetic role for a proteoform of an intracellular carboxylesterase whose secretion from the liver is induced by exercise training.
Activation of Nemo-Like Kinase in Diamond Blackfan Anemia is through aberrant localization of a ribosome-associated kinase

Diamond Blackfan anemia (DBA) is the clinical manifestation of mutations in one of over 28 different genes, over 90% are genes that encode ribosomal genes. Consequently, DBA patients have ribosome haploinsufficiency. Although ribosome insufficiency occurs in all cells, a failure of erythroid progenitors to expand underlies the anemia that typically presents within the first year of life. We recently characterized the activation of Nemo-like kinase (NLK) in erythroid progenitors and contributes to disease pathogenesis, but understanding the link to ribosome insufficiency and NLK activation has proven elusive.

Here we document that ribosome insufficiency causes a redistribution of the ribosome-associated kinase Protein Kinase R (PKR) into the cytoplasm. PKR expression and activity remains unaltered, but the increased cytoplasmic pool of PKR increases phosphorylation of cytoplasmic Transforming Growth Factor-beta activated kinase (TAK1). The kinase activity of TAK1 is stimulated by phosphorylation of threonine residues at sites 184 and 187 during normal hematopoiesis, but cytosolic PKR phosphorylates an additional TAK1 residue (serine 412). The additional phosphorylation on TAK1 increases affinity for, and results in direct phosphorylation of NLK. Although TAK1 activity is required for hematopoiesis, PKR is an actionable kinase with a number of well tolerated inhibitory compounds in clinical trials. The identification of PKR in DBA erythroid progenitors illuminates an additional therapeutic target for the treatment of DBA.
Development of Ex Vivo Lentiviral Hematopoietic Stem Cell Gene Therapy in Combination with Non-Genotoxic Anti-CD117 Antibody Conditioning for the Treatment of Hematolymphoid Diseases

Hematopoietic cell transplantation (HCT) is currently the standard of care for various blood and immune disorders. Allogeneic hematopoietic cell transplantation (allo-HCT) utilizes cells from a healthy donor to replace the diseased cells in the recipient. Unfortunately, there are serious limitations to this approach, such as lack of matched donors and graft versus host disease (GvHD) post transplantation. Given this, gene-corrected autologous HCT strategies using lentivirus (LV) and other methods have been proposed as alternatives; however, these forms of HCT still require genotoxic chemotherapy/irradiation conditioning to enable successful HSC engraftment. These conditioning agents can cause various short- and long-term toxicities including multi-organ damage, infertility, and secondary malignancies. We have previously developed several CD117 antibody (mAb) conditioning strategies to address these limitations, such as antagonistic anti-CD117 mAbs and CD117 antibody-drug-conjugates (ADC). In this study, we aim to combine these non-genotoxic conditioning strategies with autologous LV-corrected HCT in mice to enable efficient blood and immune reconstitution. Optimizing this combination is critical to enabling non-genotoxic autologous transplantation to become the new standard of care for treatment of blood and immune disorders.
Submitted Abstracts

Category: Submitted
Submitter: Yi Zhang
Department: Pediatrics
Division (if applicable): 
Research Area: Global Health / Infectious Disease
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Understanding and Improving Discharge Antimicrobial Prescriptions in LPCH

A recent analysis of discharge antimicrobial prescriptions at Lucile Packard Children’s Hospital (LPCH) Stanford found that 1 in 5 were suboptimal based on the antimicrobial choice, dose, frequency, formulation, route, and/or duration. Prescriptions are tagged as ‘suboptimal’ based on discrepancies between the order and guideline recommendations. Prescription discrepancies may significantly impact patient outcomes, including treatment failure and readmission. This project focused on understanding the drivers of discrepancies by analyzing historical prescription data and engaging with stakeholders at the hospitals. Upon identifying drivers that can be eliminated through process automation, we developed a solution to provide real-time clinical decision support to prescribers to improve prescription efficiency and accuracy.