Underrepresented Minorities in Research Summer Internship

2019 Summary Report
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Introduction

Fewer than 5% of non-employee students at Seattle Children’s Research Institute identify as a race or ethnicity other than white. Similarly, national data from the National Science Foundation reported that African American, Latino, Indigenous American, Alaskan Native, Native Hawaiian, and other Pacific Islander individuals were underrepresented in the biomedical workforce. (See the report, Women, Minorities, and Persons with Disabilities in Science and Engineering, 2015).

Currently, Seattle Children’s Research Institute does not have any programs designed to provide research opportunities for underrepresented minority undergraduate students. Additionally, the Office for Teaching, Education and Research (OTER) learned through anecdotal evidence that many underrepresented minority students were not able to work as volunteers at Seattle Children’s during the summer due to financial, geographical, and other financial and logistical barriers. In the spring of 2017, the Center for Diversity and Health Equity (CDHE) and OTER collaborated to create an Underrepresented Minorities in Research Summer Internship program for undergraduates, the first of its kind at the research institute.

The goal of this pilot program is to provide underrepresented minority (URM) students an opportunity to engage in research during the summer at the research institute under the mentorship of experienced Seattle Children’s researchers and their teams. During the summer of 2019, 36 interns joined the research institute and were engaged with a research lab focused on basic, clinical, translational, behavioral, or public health research. Interns learned new research methodologies, became experienced with lab equipment, worked with data, enrolled research participants, built mentor relationships, gained valuable research experience, attended career and professional training session, and much more.

The URM Summer Internship Program

The Underrepresented Minority Summer Internship program and application were posted to Seattle Children’s public facing website, and announcements were sent out to the local area colleges including the University of Washington, Bellevue College, Seattle Pacific University, Seattle University, Highline College, Evergreen Community College, and more. We received over 700 applications for the program. Additionally, we requested participation from the 354 principal investigators at the research institute, with 39 Principal Investigators expressing interest across 6 of the 7 centers. After closely reviewing over 200 applications, we were successfully able to place 36 Underrepresented Minority Students with 29 Principal Investigators. Some investigators mentored more than one student.
The students staggered their start dates throughout the end of June 2019 through early July 2019. A kick-off event was held in early July to officially commence the internship, which was attended by Dr. Jim Hendricks, Seattle Children’s Research Institute President and Dr. Leslie Walker-Harding, Chief Academic Officer of Seattle Children’s Hospital.

Interns spent 30 hours a week toward the research project agreed upon with their faculty mentor and 10 hours a week on professional and career development. Attendance of the required weekly professional skills/training events averaged about 85%. Additionally, each student submitted an abstract at the conclusion of their internship summarizing the research they conducted over the summer (see Appendix 2).

The sessions were designed to supplement their research skill development, and train them in professional skills that naturally built upon each other.

• **Session 1:** Reading Scholarly Articles, Dr. Julie Brown, Seattle Children’s Principal Investigator
• **Session 2:** Writing an Abstract, Dr. Susan Ferguson, Seattle Children’s Principal Investigator
• **Session 3:** Writing Your Individual Development Plan (IDP), Christina Riley, OTER Program Manager
• **Session 4:** Resume Writing Workshop, Markus Smith, Research Human Resources Recruiter and the Seattle Children’s Talent Acquisitions Team
• **Session 5:** Career Panel, Drs. Tumaini Coker, Yolanda Evans, Jason Mendoza, and Davene Wright
• **Session 6/7:** Elevator Speeches, Lindsay Kurs and Lyra Fontaine, Seattle Children’s Public Relations Specialists
• **Session 6/7:** LinkedIn Profiles and Networking Workshop, Kim Wilson, Director of Career Services at UW Bothell
• **Session 8 (optional):** Med School Application Info Session, Daniel Poux, Career Coach at University of Washington Career & Internship Center

Thus, for example, students were able to utilize the skills they learned in the IDP workshop to develop their resumes, and the information from the resume workshop to write and deliver better elevator speeches, as well as build their LinkedIn profiles. The internship culminated in a celebration event where students came together and practiced the networking skills they learned in their final training session.
RESULTS

An anonymous survey was fielded before and after the internship. Students were asked to rate their confidence in their abilities in specific professional skills on an incremental scale from “Not Confident at All” (1), “Slightly Confident” (2), “Somewhat Confident” (3), “Confident” (4), and “Very Confident” (5). On average, students reported higher confidence levels in all areas of professional skills in the post-internship surveys compared to the pre-internship surveys.

PROGRAM ASSESSMENT

We also asked students to describe the impact that the internship had on their career path, if any. The majority of respondents to the post-internship survey reported that they were positively impacted by the internship.

One student put it succinctly, “[The program] solidified my decision to enter [the] field of public health.” These positive comments correlate with the overall program rating in which 94% of respondents (n=31) rated the program as good or excellent (Fig. 1), 88% (n=29) reported that they would recommend the program to a friend or another student (Fig. 2), and 85% would come back for a second summer if offered the opportunity (n=23) (Fig. 3).

“The area of medicine I was introduced to blew my mind away. It’s definitely one I will explore in medical school. That path aligns with what I want later in life and I am glad I was able to experience it this summer. I learned a lot about what it takes to be in that setting and I am glad because [the internship] gave me more motivation to continue this career path.” – Summer Intern
Fig. 1

How would you rate the program overall?

- Poor: 3% (n=1)
- Fair: 0% (n=0)
- Neutral: 3% (n=1)
- Good: 39% (n=13)
- Excellent: 55% (n=18)

Fig. 2

How likely is it that you would recommend this internship to a friend or another student?

- Very Unlikely: 3% (n=1)
- Unlikely: 6% (n=2)
- Neutral: 3% (n=1)
- Likely: 21% (n=7)
- Very Likely: 67% (n=22)
Principal Investigators completed a survey at the conclusion of the internship program to give feedback on improving the program. This survey received 12 respondents out of 29 PIs who participated in the program. Of these respondents, 11 reported the experience as “good” or “excellent,” none reported the program as being neutral or fair, and only one person reported the internship as “poor.” We saw a mean of 4.25, and these results correspond to our student survey results (Fig 4.)
At the end of the summer program, five students were hired into permanent positions at Seattle Children’s with their faculty mentors (14%). An additional 4 students remained on during the 2019-2020 academic year as Research Students for credit (11%); thus 25% of the students in the internship stayed on at Children’s in some capacity. This was an unexpected but exciting outcome of the program—the ability of the Summer Internship Program to contribute to the diversity of Seattle Children’s research staff as well as to encourage students to pursue careers in the health and biomedical sciences.

**RECOMMENDATIONS**

With over 700 applicants for the program, we found the process of selecting candidates to be very difficult. We faced problems in discerning which of the applicants identified as a member of an underrepresented minority racial/ethnic group, as many students applied who were not eligible for the program. In the future, it will be helpful to include the following as part of our application process:

- A direct question on race/ethnicity, if allowed by legal standards
- An earlier application process to allow a longer period of time for matching students with PIs
- An earlier assessment of participating PIs for the summer
Increase the Target Audience
The target audience for this first year of the program was local undergraduate students. We believe that by expanding the program to out-of-state students, we may be able to expand our reach and thus program impact. In addition, we can expand our focus on other areas outside of Seattle in the greater WAMI region, to ensure access to URM students throughout the region.

Update the Programming
We also will change the programming slightly taking into account the feedback received from students and include more community building social time for the students to practice their networking skills amongst one another and with the PIs in the program.

Develop a Secondary Internship
We propose, as well, that a secondary internship opportunity be developed that would further the professional and academic development of the students who participated in this year’s internship, with the goal of retaining this talent for the long term. Students going into their senior year of college who also participated in the 2019 internship would be afforded the opportunity to return for a second internship, and then at the conclusion of their undergraduate degree, be hired on as a staff member at Seattle Children’s.

Develop a Follow-Up Survey for Medium- and Long-Term Outcomes
Finally, we propose that a follow-up survey be developed to track the progress of the students in the 2019 cohort to understand the longer term impacts our program has had on their career and professional development. All future surveys will be non-anonymous to allow us to match surveys over time and conduct valid comparisons.
Looking toward a sustainable program with long term outcomes, we surmise the following logic map would apply to the URM Summer Internship Program.

We assert that the long-term investments of the program will ultimately continue to benefit URM students, and Seattle Children’s generally. We believe this program will continue to 1) encourage URM students to pursue careers in science, medicine and public health, and 2) add to our efforts to increase the diversity of our faculty and staff at Seattle Children’s. By enhancing diversity, we can more fully meet our mission of providing hope, care and cures to help every child live the healthiest life possible.
Generating Plasmid Vectors for the Deletion of Plasmodium Falciparum Genes Essential for Sex Specific Gametocyte Formation

Malaria is a deadly disease contracted by hundreds of millions of people every year. In 2017 there were an estimated 435,000 deaths from the disease, mostly in children under the age of five in sub-Saharan Africa. Human malaria is caused by species of Plasmodium parasites, including P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi. Parasites replicate asexually and sexually in human host red blood cells and complete their life cycle in mosquito vectors. Currently there is no effective vaccine against human malaria and the emergence of drug resistance poses one of the greatest threats to malaria control. RTS,S is a vaccine that will soon be licensed but has shown only moderate efficacy in young children.

The aim of the study is to create P. falciparum gene deletion that leads to the creation of single sex gametocytes. This will allow for detailed studies to understand sexual stage biology which could lead to the development of novel drugs and vaccines.

To delete genes, we adopted a CRISPR/Cas9 approach. In Plasmodium, we use a single plasmid delivery system that encodes the Cas9, the specific guide RNA, a drug selectable marker and templates for recombination to generate efficient gene deletion. DNA templates for recombination were amplified by polymerase chain reaction (PCR). Two templates, upstream and downstream of the gene of interest were amplified and the amplified products were electrophoresed on an agarose gel to confirm the correct band size. PCR products were then digested with specific restriction enzymes and ligated into the plasmid cut with the same restriction enzymes within its multiple cloning site. The ligation reaction using T4 DNA ligase and appropriate ratios of plasmid vector and template insert were used in the ligation. The ligation reactions were transformed into chemically competent Escherichia coli cells and plated on LB agar plates containing ampicillin to allow for selection of transformed cells. Bacterial colonies thus obtained were inoculated in LB broth, grown up and plasmid DNA was then purified. A similar strategy was used to clone the specific guide RNA into the plasmid.

Plasmids were digested and sequenced to confirm ligation success. Confirmed plasmids were transfected into P. falciparum infected red blood cells by electroporation. These parasites were then subjected to drug pressure to select for parasites that had undergone gene deletion. Parasites obtained after drug selection were genotyped to confirm the removal of specific gene loci.

Excitingly, preliminary results suggest that we have deleted the specific genes of interest after drug selection. These parasites will now be phenotyped for their role in sex-specific P. falciparum gametocytogenensis.
A Library of Lentivirus-delivered Fluorescent Reporters

Recent advances in quantitative live cell imaging provide new opportunities to address unsolved cellular mechanisms in a variety of contexts including the study of infectious disease. Yet, it is currently difficult to efficiently label and observe the subcellular dynamics of primary cells. Immune cells are especially difficult as they are activated in response to most genetic engineering techniques, confounding results. Fluorescent organic dyes have been developed to specifically label subcellular compartments. Unfortunately, these dyes are often toxic to cells and ineffective at selectively labeling cells of interest; and there are subcellular compartments for which no dyes exist, e.g., peroxisomes. To address these issues, we are assembling a lentiviral library of genetically encoded fluorescent proteins and biosensors that target specific organelles. Lentivirus delivery has been demonstrated to be an effective strategy for introducing genetic material into primary immune cells without activating the innate immune response. We selected four fluorescent proteins that have non overlapping fluorescence spectra at various wavelengths across the visible and into the infrared wavelengths of light. These include mCerulean3, mEmerald, Tag-RFP-T, and miRFP670. We also selected four biosensors that respond to relevant bio-physical properties including pH (pHlourin2), cellular redox state (roGFP2-GRX1), hydrogen peroxide levels (HyPer3), and calcium levels (Twitch-2B). We started by adding organelle-specific targeting sequences to each of the reporters using PCR. These organelle targeting sequences function like a molecular “zip code” to target each subcellular location in the cell. We then ligated the reporters into a modified lentiviral plasmid for cloning and expression, which was then transformed into E. coli cells for amplification. To verify proper insertion of the fluorescent reporters into this lentiviral plasmid, we did a diagnostic restriction digest of plasmid DNA extracted from E. coli. The DNA will then be transformed into HEK293 cells to produce the lentivirus encoded by our designer constructs and eventually these will be transduced into primary macrophage cells. Even though we were unable to complete the cloning of all planned constructs, we were able to create stocks of amplified PCR reporters to be cloned in the future. While this project remains a work in progress, we anticipate that completion of this library will enable us to better understand the subcellular dynamics of primary immune cells and how different organelles contribute to the outcome of an infectious disease.
Difficult Intubation Patients

The “Downs project” is a clinical research project on airway intubation in down syndrome children/patients. The purpose for an airway intubation during a surgical procedure is to allow air to pass in and out of the lungs and to make sure the body maintains homeostasis. When the facial anatomy of a person is abnormal it tends to make the intubation procedure more difficult. Down syndrome patients are an example of patients that are difficult to intubate, due to their large tongue structure, and smaller windpipe and other facial anatomical differences they tend to have a higher prevalence and complicating factors for airway management. The aim of this project is to measure the front teeth to carina (FTC) and the nares to carina (NC) distance in children with down syndrome and examine whether the height based Morgan formula is as useful in measuring the depth of oral and nasal intubation in patients with Down syndrome as it is with non-syndromic patients. To accomplish this children with down syndrome undergoing cardiac surgery were enrolled into the study, then using radiographic images of the chest, the front teeth-to-carina (FTC) and nares-to-carina (NC) distance was measured using the marking on the endotracheal tube (ETT) in centimeter, the FTC and NC distances was then compared to previously studies control patients without down syndrome. The result of the study showed that the Morgan formula and the modified Morgan formula are useful in predicting ETT depth in children with down syndrome, however the Morgan formula may predict a depth to deep for oral intubation, whereas the modified Morgan formula may predict a depth to shallow in nasal intubation. To conclude the results of the study shows that NC and FTC distance correlates best with the height of the patient in both children with and without down syndrome, however, further confirmation of depth is necessary to ensure correct ETT placement.
Health Communication Preferences Among Families of Low Income Minority Patients: A Cross-Sectional Study

Introduction: Family centered care is recognized as the standard for pediatric inpatient care; however, little is known about the needs and preferences of parents of low income minority children in the inpatient setting. In an effort to diversify existing research and address the unmet social needs of low income minority patients, this research focuses on the health communication preferences of the subjects presented in this study.

Methods: This cross sectional study was embedded within a prospective study of a patient navigator intervention. The patient navigator interviewed enrolled families about communication preferences within the first three days of admission, and documented pertinent positive and negative aspects relating to their visit, in the medical record. Of the 60 enrolled families, 52 of them had documented preferences related to communication with the medical team. These notes underwent a content analysis, in which categories and themes were identified, then later quantified. Two trained coders independently coded each transcript; any disagreements were resolved through discussion. The data derived was then descriptively analyzed.

Results: Parents were primarily mothers (87%), born outside the US (60%), with a high school degree or less (60%). Sixty-three percent preferred English, 33% Spanish, and 3% Somali. Half reported annual incomes of <$30,000. We found that 59.6% of caregivers reported having a good understanding of the diagnosis and treatment plan, while 36.5% reported having significant questions or needing clarification. Over a quarter of parents reported having limited health literacy (26.9%), and an additional 34.6% reported needing explanation of medical terminology for proper understanding. Almost 29% of parents preferred communication via Spanish interpreter; 15.4% reported needing an interpreter for some but not all caregivers, and a separate 15.4% reported wanting interpretation under certain circumstances only. Just over one third of parents preferred large group discussions (34.6%), while 15.4% preferred small group discussions. The top two self-reported learning styles were demonstration, endorsed by 80.7% of parents, and written material endorsed by 65.3% of parents.

Conclusions: In this study of parents of hospitalized low income minority children, we found that many parents still had unanswered questions about the diagnosis, and at least a quarter reported limited health literacy. Parents expressed a wide range of preferences for communication with the medical team, which may not be elicited during routine care. Assessing and acting on these preferences has the potential to improve communication with vulnerable families.
Exploring the Use of Cell-Free DNA in the Diagnosis of PIK3CA Varients in Patients with PROS

**Background:** PIK3CA Related Overgrowth Spectrum (PROS) is caused by a mosaic, post-zygotic gain-of-function mutation in the PIK3CA gene which causes disorders such as MCAP syndrome and CLOVES syndrome. Due to the mosaic nature of PROS, molecular diagnosis is not only invasive, but challenging and may require multiple biopsies of affected tissue.

**Objective:** The purpose of this investigation is to find a less invasive molecular diagnostic assay for patients with PROs using cell-free DNA (cfDNA) from cyst fluid within the lesion and plasma from whole blood.

**Design/Methods:** Whole blood was spun down and plasma was extracted. cfDNA was isolated from frozen cyst fluid from two patients and plasma from two patients. Digital droplet polymerase chain reaction (ddPCR) was used to analyze the cyst fluid and plasma cfDNA contents from patients with PROS.

**Results:** Variants in PIK3CA were detected in 100% of patients’ cyst fluid derived cfDNA samples at levels of up to 2.2%. In one patient, the % mutation in the cyst fluid cfDNA exceeded that of their respective tissue, both analyzed using ddPCR. In plasma cfDNA, 50% of the tested samples detected the variants at up to 0.77%.

**Conclusion:** ddPCR allows for highly sensitive rare-event-detection for PIK3CA variants, potentially providing an alternative diagnostic tool for specimens with low concentrations of the variant present. Additionally, cyst fluid cfDNA is a candidate biomarker for patients with PROS and may reflect the overall affected tissue compared to a biopsy of a specific tissue. Plasma is less invasive than both tissue biopsies and cyst fluid extractions, and may provide an alternative for patients with non biopsiable overgrowths or for the diagnosis of PROS in vitro, using the mother’s cfDNA.
Predictors of Parental Academic Involvement in Parents of Children with ADHD

Many children with ADHD experience symptoms that lead to challenges in school such as disorganization, difficulty focusing, and forgetfulness (Center for Pediatric Behavioral Health). Because of this, many parents of children with ADHD involve themselves in their child’s academics and engage in practices like overseeing the child’s completion of homework and studying habits and checking the child’s grades (Gonzalez-DeHass, Willems & Holbein, 2005). Parental academic involvement practices like monitoring or helping with homework, have been proven to increase a child’s academic achievements, motivation, and confidence, though most of the studies are on parents of children without ADHD (Rogers, et al, 2009). Other literature has shown that reliable predictors of parental academic involvement include parent education level, SES, gender, and mental health status, though again, this has not been tested in an ADHD sample (Yotyodying & Wild, 2016).

To investigate if these predictors hold true for parents of children with ADHD, we integrated data from four randomized clinical trials of psychosocial treatments for adolescents which included a total of 854 youth ages 11 to 17. Data was pulled from the parent and child demographic and PAMS questionnaires. Parental academic involvement was measured via parent response to the following survey questions: “In a typical week, how many hours do you spend on activities related to supporting the teen’s academics?” and “How many days during the week did you do some of your child’s homework for him/her?” Based on answers, parents were placed into one of three groups: low involvement, medium involvement and high involvement. Medium involvement, classified as 1-7 hours of support, was set as the reference group. Low parental involvement was considered to be 0 hours of support, while high involvement was classified as 8+ hours of support. Parents were also categories based on self-report of completion of child’s assignments at least once a week (yes = 1, no = 0).

We ran a logistic multinomial regression analysis, with parental predictors set as education level, language, number of children, and parental marital status. Child predictors were set as IQ, medication status, ODD diagnosis, class placement, gender and age. When the low parental academic involvement group was compared to the reference group, only the presence of ODD diagnosis (b = 0.858, SE = 0.306 p = 0.005, OR = 2.359) and lower parental education level (b = 1.263, SE = 0.503, p = 0.012, OR = 3.535) were found to be statistically significant predictors. When comparing the high involvement group to the comparison group, only age was significant (b = -0.200, SE = 0.073, p = 0.006, OR = 0.819); the higher the age of the child, the lower the chance of parental involvement. A second analysis was run using binary logistic regression and found that the only significant predictor of parental homework-doing was the number of siblings; as siblings increased, the likelihood of parents doing their child’s homework for them decreased (b = -0.162 , SE = 0.072 , p = 0.025, OR = 0.851).

It is important to consider reasons why the only effective predictor of high parental involvement was age because as well as the fact that there were no significant predictors of parental homework completion. Further research should consider exploring the difference between predictors of low parental involvement and high parental involvement.
Addiction is defined as a chronic relapsing disorder characterized by compulsive binge-like behavior, sensitivity to drug cues, and continued use and seeking of the drug, despite negative consequences. Although it is very prevalent, little is known about the circuitry underlying drug addiction. Cocaine and other extremely addictive psychostimulants heavily target cortical regions which innervate striatum. Striatum is a critical region of the brain that has been implicated in addiction, along with many psychiatric disorders. This region is involved in encoding and processing of many reward, learning, and motivated behaviors. Within striatum is the IT/PT circuitry that makes connections with cortex and pons. The goal of this study was to determine if shutting down one of the cell types within this circuitry affected the behavior associated with cocaine administration through many sensitization sessions. There were seven sensitization sessions, with one every other day, followed by two weeks of abstinence prior to a challenge injection of cocaine. Subjects were divided into four treatment groups. Prior to the sessions, all subjects were given viral injections through stereo-tax surgery. These injections included DREADDs used to target IT neurons. This allowed injection of clozapine-N-oxide (CNO) to activate the DREADDs, thus inhibiting the target cell type. The treatment groups were as follows: an injection of CNO paired with an injection of cocaine, CNO paired with saline, vehicle paired with cocaine, and vehicle paired with saline. For each session, the rats were allowed a habituation period of 30 minutes before being injected with either CNO or vehicle, after which there was another 30-minute period before the next injection. The next was a 15 mg/kg intraperitoneal injection of either cocaine or saline. When comparing the two treatment groups given cocaine, there appears to be very little difference in average activity level. This difference was assessed by using locomotor chambers that recorded crossings for a measure of ambulations. Subjects demonstrated increased activity following cocaine treatment between the initial and final session, but not saline. Preliminary evidence suggests transient increases in locomotor activity following IT inhibition in cocaine-treated versus saline-treated groups, that is largely abolished in subsequent sessions., all subjects were pretreated with vehicle injections prior to a challenge injection of 10 mg/kg of cocaine. No differences were found in the total ambulations with repeated inhibition of IT activity versus vehicle-treated controls, but cocaine-treated animals did exhibit sensitized responding compared to saline-treated groups. While all groups exhibited increased ambulations following treatment with cocaine, no differences were found between treatment groups.
**Background:** There are many strict Cardiopulmonary Resuscitation (CPR) guidelines related to rate and depth of chest compressions, as well as ventilations. However, CPR guidelines for children have been developed by expert clinical consensus, which is limited to data from adult, animal, mechanical modeling, and pediatric radiological studies. There is minuscule data collected from children themselves in cardiac arrest, resulting in small knowledge surrounding pediatric resuscitation. There is also insufficient data on the association of post-cardiac arrest care (PCAC) and patient outcome after cardiac arrest.

**Methods:** Quantitative chest compression, post cardiac arrest care therapies, and pediatric survival outcome data are all being observed. The research design is an observational, multi-center cohort study of pediatric cardiac arrest management. This study anticipates approximately 250 pediatric cardiac arrest events (gestational age 37wk- 18y). Recruitment of qualified participants for this study includes pediatric cardiac arrests requiring chest compressions for less than or equal to one minute, managed at clinical centers identified as a component of standard clinical operations. 15 sites are anticipated to be participating in this project.

**Objectives & Anticipated Results:** This project will create a clinical CPR Learning Laboratory by helping characterize the quality of CPR quality measures delivered to children, such as: depth, rate, compression release, flow fraction, and survival to hospital discharge. It will also help determine the association between quantitative CPR quality measures (the list is as previously mentioned) and survival to hospital discharge. Finally this will all be used to determine the association of survival with site-specific post cardiac arrest care.
Spanish Speaking vs. English Speaking Parent Engagements During WIC Visits

**Background:** To deliver high-quality pediatric primary care to low-income populations, effective, equitable, understandable, and respectful quality communication between children’s parents and medical team is essential. Currently, a knowledge gap exists regarding communication engagement patterns of Spanish speaking parents with their child’s primary care providers during WCC visits. 

**Objective:** To determine if parent engagement during WCC visits differs between Spanish speaking parents and English speaking parents.

**Methods:** Audio recordings of well-child-care visits (2m-24m) at ten FQHCs in two major US cities (n = 21 English samples; n = 18 Spanish samples) were analyzed using Dedoose Software. Parent-provider engagement patterns were coded to identify four types of communication exchange: Clinician Guidance (information provided by the clinician to the parent) and Clinician Data-Gathering (question asked by the clinician); Parent Information-Giving (information provided by parent beyond yes/no response to a clinician data-gathering question); Parent Data-Gathering (question asked by parent). Additionally, communication interactions were coded for missed opportunities to provide important information to parents by the clinician. We examined 1) the frequency of visits inclusive of parent information giving in relation to specific WCC content (e.g., safe sleep, behavior management), during visits with English and Spanish speaking parents.

**Results:** Spanish speaking parents demonstrated Information Giving (11 of 18 visits) most often in relation to Concerns About Child’s Health; this occurrence was similar to English speaking parents (12 of 21 visits). Parent information giving for Food and Nutrition was less frequent in visits with Spanish speaking parents (8 of 18 visits) than visits with English speaking parents (15 of 21 visits). Missed guidance from clinician occurred 3 out of 18 in Spanish samples and 2 out of 21 in English samples.

**Conclusion:** Spanish speaking parents expressed their child's health concerns at a similar frequency to English speaking parents. However, in relation to most other WCC visits Spanish speaking parents rarely initiated conversations compared to English speaking parents. This communication pattern was present even when clinicians spoke Spanish or an interpreter was used. For Spanish speaking parents, having visits in Spanish either directly from their pediatrician or interpreter is not enough to meet parent communication satisfaction because critical information often gets lost or missed during these WCC visits. Structuring WCC visits to include additional time and personnel with appropriate linguistic, cultural and clinical knowledge can potentially address the gaps in quality WCC services Spanish-speaking Latino parents currently face.
Limit of Detection for ddPCR to Titer Lentiviral Particles

**Background:** Lentiviral Vectors (LV) are created by transfecting a producer line with plasmids encoding the major lentiviral ORFs including: Gag (encoding structural proteins), Pol (encoding reverse transcriptase) and Env (vector surface glycoprotein). We explored a new method to quantify LV, digital droplet PCR (ddPCR). This method uses the same primers and probes used in quantitative PCR (i.e TaqMan assays). ddPCR partitions 20 μL samples into 22,000 nanoliter sized droplets in which individual PCR reactions are carried out. Depending on PCR target concentration in the starting material, not all droplets contain a single template molecule. Poisson statistics along with internal algorithms quantify signal frequency and amplitude in each droplet, to precisely determine concentration of target DNA.

**Objective:** To optimize ddPCR LV quantification and determine the lower limit of detection (LOD) of ddPCR in our assay.

**Methods:** The targets for ddPCR were WPRE (vector encoded) and ALB (host genome encoded). Samples studied were either a plasmid encoding WPRE and ALB (pALB) or DNA samples from cells transduced with differing dilutions of supernatant containing LV, as used in LV titration assays. Target DNA, primers, probes and BIO-Rad ddPCR supermix were combined, droplets generated using a QX 200 BIO-Rad automated droplet generator, PCR amplified, and droplets counted and measured using a QX 200TM Bio-Rad droplet reader. In some assays, genomic DNA from the HT1080 cell was included to determine the background signal in cellular lysates. HT1080 is used in our lab as the target cell for the LV titer determination.

**Results:** ddPCR output changes with assay conditions. The copy number for pALB as determined by ddPCR using primers and probe specific for WPRE was inversely proportional to the dilution factor when pALB plasmid was serially diluted in dH2O in the reaction mix. When background DNA (HT1080) was added to the ddPCR reaction, there was no effect on the ability to detect WPRE. Thus, a standard curve could be created, where y=1.4675x+371.54. Where y is the copy number of pALB, and x is the concentration of DNA, ng/μL.

To determine the limit of detection, we analyzed pALB copy number when diluted in HT1080 carrier DNA. At 1x106 copies of pALB or greater the assay was at saturation and nonlinear. The linear range of the assay was determined by including values that differed (as pALB was serially diluted) greater than three times standard deviation at each dilution (with confidence interval of 97%), from the mean of the individual ddPCR assays. We determined that 43-62 copies of WPRE was the lower limit and 140,000-153,000 copies of WPRE was the upper limit of the linear range.

**Conclusion:** Though ddPCR is an optimal method for determining the LV titer, some parameters should be considered. First, the dilutions used for titer calculations must be in the linear range, as the reaction can be saturated. Second, below 62 WPRE copies, ddPCR loses its sensitivity. Nonetheless, this is a robust and dynamic copy number range that is suitable for LV copy number determination, even in the presence of background genomic DNA from HT1080.
The Effects of Replacing Patient Chart Review Diagnoses with ICD10 Code Diagnosis

Background: Abstracting chronic illness conditions from the medical record is time-consuming and error-prone. This analysis compared how accurately ICD-10 discharge codes identify chronic illness compared to clinical research coordinators’ chart reviews.

Objective: R statistical software used to determine accuracy and sensitivity of ICD-10 codes compared to chart reviews in determining presence of chronic illness.

Design/Methods: Data was de-identified, transformed to yes/no variables for each chronic illness condition, and restricted to conditions where > 5 patients had ICD-10 diagnoses. Data were analyzed by condition using the confusion matrix. The confusion matrix uses the True Positive, True Negative, False Positive, and False Negative results to generate 16 different values comparing the relationship between the ICD-10 codes and chart reviews. The primary outcome was F1, the accuracy of the entire confusion matrix when there is a large number of True Negatives. Secondary outcomes include kappa, the proportion of actual and predicted agreements that did not occur by chance; sensitivity, the proportion of patients with the condition who are correctly diagnosed; specificity, the proportion of patients without the condition who are correctly diagnosed; and negative predictive value, the probability that patients who have tested negatively for a condition have the correct result.

Results: The final sample included 309 patients. Mean F1 value across all conditions was 0.906 (Q1: 0.873, Q3: 0.953) indicating the ICD10 code diagnoses accurately compared to those of the chart reviews. The mean kappa value was 0.590 (Q1: 0.367, Q3: 0.761), indicating moderate agreement. For individual conditions, kappas ranged from <0.400 for allergic conditions and GERD to 1.00 for down syndrome. The mean sensitivity value was 0.923 (Q1: 0.894, Q3: 0.995). The sensitivity for GERD was an outlier at 0.733. The mean specificity value was 0.651 (Q1: 0.468, Q3: 0.843). Specificity ranged from <0.27 for allergic conditions prematurity to 1.00 for down syndrome. The average negative predictive value was 0.784 (Q1: 0.661, Q3: 0.917). Negative predictive values for down syndrome and prematurity were 1.00.

Conclusion: Chart review and ICD-10 codes were almost equally as good at identifying chronic illness. Future research should use this statistical model to test a dataset of >1,000 patients to robustly assess diagnoses of all conditions. Results from this model suggest ICD-10 codes can be used to identify patients with chronic allergic conditions, apnea, asthma, down syndrome, GERD, prematurity, and seizure disorder instead of chart reviews to maximize efficiency without adversely impacting accuracy.
The Epidemiology of Foreign Bodies at a Pediatric Hospital

Background: Foreign bodies are a common hazard to children and frequent complaint in the pediatric Emergency Department, notably in the younger age group. Foreign bodies include any foreign object anywhere in the body, and can cause discomfort or harm if not removed through medical intervention.

Objective: To describe the variety of locations of foreign body cases for patients seeking care at Seattle Children’s Hospital (SCH).

Methods: We studied patients ages 0-20 years treated for foreign bodies at SCH (main campus or any of three satellite urgent cares), from 1/1/2017 through 5/31/2018. Cerner Clinical Information System was used to review electronic medical record (EMR) information from patients presenting to SCH with any type of foreign body. All patient visits with ICD-9 and -10 diagnosis codes indicative of a foreign body were reviewed from 1/1/2017 through 5/31/2018. This included ICD-9 codes 931 - 939.9, E912 and E915, and ICD-10 codes T16.1-T19.9. Foreign body case encounters were categorized into 12 major categories, and 40 sub-categories, as follows: No Foreign Bodies, Upper Extremities, Lower Extremities, Gastrointestinal, Oropharyngeal, Genitourinary, Subcutaneous, Neck and chest, Respiratory, Ears and nose, Eyes, and Other. Each category was further divided into subcategories, e.g., lower extremity = toes, foot/midfoot, leg/thigh, shin, calf, or knee and upper extremity = fingers/nails, hand/ palm/wrist, forearm/arm/rest of arm/elbow.

Results: We retrieved EMR for 2,933 patient encounters, with duplicates. After analyzing all the patient records and different categories, 171 of the 2,933 patients encounters did not have any foreign bodies present. The most common location for foreign bodies were ears and/or nose (37%), followed by gastrointestinal tract (26.1%) and lower and upper extremities (16%). Rarer foreign body locations included eyes (6%), subcutaneous (5%), oropharyngeal (2.4%), respiratory (0.7%), neck and chest (0.4%), genitourinary (0.3%), Subcutaneous (5%), Neck and chest (0.4%), Respiratory (0.7%), Eyes (6%), and other locations (0.3%). The study also found that 54% of patients encounters were white race, 78% were Non-Hispanic, 90% were English speakers, and 91% of patient families declined to use an interpreter.

Conclusion: Most foreign bodies were found in easier to access locations such as ears, noses, superficial skin surfaces, and gastrointestinal tract. Because pediatric patients with foreign bodies may be asymptomatic or have a delay between exposure and symptoms, physicians and parents need to maintain a high index of suspicion. Education is needed to protect children from foreign body injuries. Parents and caregivers should be advised to keep small objects, batteries, magnets and other hazardous objects away from small children. Prevention could be the difference between a healthy child and a visit to the hospital.
Timing of Myc in CD8 T-cell Differentiation

**Background:** When a T-cell comes into contact with an APC (antigen presenting cell) the T-cell normally proliferates. Following the initial cell division the proximal daughter cell becomes an effector T-cell while the distal daughter cell ends up a memory T-cell. This process has been shown to be heavily dependent on Myc. Myc is asymmetrically distributed into the proximal daughter cells. The T-cells with higher levels of Myc have been shown to become effector cells with upregulation of certain genes and proteins that create a positive feedback and increasing glycolysis and glutaminolysis.

**Objective:** The specific questions we addressed were (1) during which stages of T-cell activation and proliferation is cMyc required for terminal effector T-cell differentiation, (2) can the T-cell be rescued from terminal effector fate by regulating cMyc expression?

**Design/Methods:** To achieve our objective we used CRISPR/Cas9 technology to delete the specific Myc gene from antigen specific mouse CD8 T-Cells at certain time points, and followed their differentiation. We measured the expression of cMyc to quantify how successful the CRISPR uptake was. CD44, PD-1 and CD25 expression was measured to assess the activation state of the T-cells. Based on the critical role of Myc in regulating T-cell metabolism we also assessed metabolic functions of the cell in the presence or absence of Myc. We measured glucose uptake, fatty acid content, and pS6 (measures mTOR activity). In order to achieve our goal of studying T-cell fate after deleting Myc at certain time points, we used p14 mice with the genotype Thy1.1/1.1(Homo) that received CRISPR crRNA targeting our gene of interest cMyc (reducing its expression), and Thy1.1/1.2 (Het) as Wild Type that received the Atto CRISPR as a control( gene of interest was intact). We deleted the cMyc gene either before activation (naive) or after 24 hours of activation (activated).

**Results:** The knockdown of the cMyc gene using CRISPR was successful. We were able to see the decrease in cMyc expression in naive cells by day 1 post CRISPR. We also observed a decrease in cMyc in activated cells by day 1 post CRISPR. The downstream effect of deleting Myc wasn’t observed to affect T cell activation compared to Wild Type, as the levels of CD44, PD-1, and CD25 did not drop for the naive or activated cells after following them 2 days post CRISPR. Myc did not appear to alter effector differentiation as assessed by the level of Granzyme B expression in Wild Type CD8 T-cells or CD8 T-cells deleted for Myc at Day 0, and Day 1 post-activation. However mitochondrial metabolism was modestly compromised in the absence of Myc, with both naive and activated Myc ablation leading to reduced oxygen consumption.

**Conclusion:** This first round of in-vitro analysis showed that Myc is important for CD8 T-cell metabolism at Days 0 and Day 1 post activation. Our next step is to investigate the biological relevance of Myc in in-vivo CD8 differentiation during infection. We will perform CRISPR again at D0, D1, D2 post activation, and follow their differentiation in-vivo.
The PEACE Project Initiative and Improving the Effectiveness of Therapy at the PBMU

As troubled mental health increasingly becomes a palpable concern for adults and youth alike across the US, the need for evidence-based therapy is high in demand. Crisis events spurred by aggression or suicidality may necessitate acute psychiatric care for stabilization. The Psychiatry and Behavioral Medical Unit at the Seattle Children’s Hospital admits youth for such care.

“The Peace Project” initiative provides a basis of comparison between the established therapy, and new integrated therapeutic technology to assess the strengths and weaknesses of each method. The two technologies trialed are Ripple Effects and emWave. Ripple Effects is a personalized learning tool, with a wealth of social scenarios for the kids to maneuver. emWave is a biofeedback toolkit that trains patients to attend to their breathing while monitoring HRV (Heart-rate variability) feedback. Many children are resistant to the standard therapy, especially those who are repeatedly admitted. The objective is to make the time the kids spend at the PBMU as efficient and effective as possible.

This project is being conducted by Drs. Carol Rockhill and Annie Chen with three aims in mind, concluding with survey comparison regarding behavior and satisfaction during hospitalization and 6 months after discharge:

1. Applying the new therapy to individual patient sessions.
2. Implementing these technologies in a group setting.
3. Allowing patients to take home mobile versions of the technologies.

During the duration of the internship, the first aim was ongoing, the second aim was completed, and the third aim was prepared. This study includes the ages 7-15. Aims 1 and 3 focused on youth with aggression; Aim 2 focused on youth participating in groups based on age (10-12, and 13-15).

Evaluation of the pilot is based on survey data from the coaches on the unit who run the groups alongside therapists. The focus of this internship was quantitative data assessing change in HRV from the first emWave session to the last. The analysis program was my own responsibility, alongside entering satisfaction data, which showed some increase in satisfaction and engagement with the children in the PBMU.

This intern supported the group therapy portion of this initiative, and observed that satisfaction was higher, but further quantification is in process. This intern’s project will allow analysis of emWave data, which be the first analysis of such data on an inpatient unit, and which may show youth building biofeedback skill.
**Discussion Frequency and Parent Engagement of Psychosocial Topics at Well-child Care Visits for Infants and Toddlers**

**Background:** Low-income families are at greater risk of having unmet psychosocial needs, including support for maternal depression, food, and housing. Further, existing disparities in child preventive healthcare for low-income families may lead to poor developmental outcomes. Few studies have examined parent-provider engagement dynamics during well-child-care (WCC) visits. We hypothesize that the extent of parent engagement during WCC visits is one of the key components of assessing the quality of care.

**Research Objective:** To assess discussion frequency and parent-provider engagement patterns of psychosocial topics during WCC visits.

**Study Design:** Audio recordings of 87 WCC visits (n= 44 English visits; n= 43 Spanish visits) for infants and toddlers 2-24 months old, across ten federally-qualified health clinics, were analyzed using Dedoose software. Recordings were coded for WCC topics and communication engagement patterns for parents in relation to their child's clinician. The psychosocial needs and risks parent code encompassed a total of 12 child codes including: parent mental health, financial needs, and home life (e.g., parent employment/education, sibling needs). Parent engagement codes included parent's information giving (information provided by the parent beyond a yes/no response to a clinician question) and parent's data gathering (questions asked to the clinician by a parent). Specifically, we examined the co-occurrence of psychosocial topics and parent engagement types.

**Results:** Psychosocial topics were not discussed at all by the parent or physician during 16% (n=14) of WCC visits analyzed. Psychosocial topics were most often mentioned towards the end of visits. Psychosocial topics and parent information-giving co-occurred 112 times, while parent data-gathering co-occurred 11 times. Home Life was the subtopic parents gave information about most often (56 out of 112 parent information giving codes). Intimate partner violence and child abuse/neglect were the subtopics parents gave information about least often, with one co-occurrence each.

**Conclusions:** The higher number of parent's information giving occurrences compared with parent's data-gathering suggests that WCC visit conversations are strongly clinician-led. Parent-provider exchanges regarding home life are less sensitive than other psychosocial topics, and thus may be why home life was the subtopic with higher levels of parent-clinician engagement. Future studies should determine what factors lead to more sensitive psychosocial topics being discussed during WCC visits, such as financial needs, particularly in low-income populations. Additionally, future work should examine differences in psychosocial discussion topics and parent engagement between English- and Spanish-speaking parents at WCC visits.
Predictors of Parental Academic Involvement Among Adolescents with ADHD

**Background:** Parental involvement is thought to have a positive influence on educational outcomes. Previous studies have shown, when parents see a child as difficult, they tend to be less involved. (Grolnick, Benjet, Kurowski, & Apostoleris, 1997) When a parent is educated and efficacious, they are likely to be academically involved, as they understand the material and can be a good resource. (Grolnick, Benjet, Kurowski, & Apostoleris, 1997) As children transition into middle school or higher, parents tend to be less involved. This uninvolved is perhaps due to the parents’ lack of understanding of the material (Balli, Sandra, 1997). In this study, we considered various predictors that may influence how much time parents spend in academic activities, with their ADHD (Attention Deficit Hyperactivity Disorder) diagnosed adolescents, and whether they do their children’s homework. Predictors included: parent’s language, marital status, and education, as well as, child’s gender, age, number of siblings, IQ, their class placement, ODD (Oppositional Defiant Disorder) diagnosis, and whether they take ADHD medication.

**Methods:** In order to test these predictors, the ADHD TIDAL Study gave parents a PAMS (Parent Academic Management Scale) questionnaire, which consists of a 16-item checklist used to measure the frequency with which parents monitor their child, assist with academics, and reinforce the child’s Organization, Time Management, and Planning (OTP) skills. (Sibley & Coxe, 2019) In a sample of 854 adolescent participants, predominantly male (72.5%), aged 11-17, all of which had an ADHD diagnosis. Parental involvement was measured and classified as follows: no involvement (group1=0 hours per week), medium involvement (group0=1-7 hours per week), and excessive involvement (group2=8+ hours per week). Multinomial and logistic regression tools were used to run the statistics.

**Results:** Our results showed that parental education level \( b=1.263, p=.012, OR=3.535 \), and ODD diagnosis on a child \( b=.858, p=.005, OR=2.359 \), influences the amount of time parents spend with their kids on academic activities. That is, specifically for group 1, the lower the parents’ education, the less time they spend and teens with higher levels of ODD get less involvement. Using group 0 as the comparison group, lower age was associated with high frequency homework help in group 2 \( b=-.002, p=0.006, OR=0.819 \). Additionally, the number of siblings a child has influences the parents’ likelihood of doing their homework \( b=-.162, p=.025, OR=.851 \).

**Discussion:** We found three maladaptive strategies that have their own predictors: no involvement, doing the homework for the children, and spending excessive time to help them. While we considered various predictors, we found that the predictors of maladaptive parenting included ODD diagnosis, the number of siblings, and parental education. These findings are in accord with previous research (Grolnick et al, 1997), however, perhaps the most surprising finding is that the fewer siblings a child has, the higher the likelihood their parents will go as far as completing their teen’s homework for them. On the other hand, the more siblings a child has, the less likely their parents will be academically involved. Future studies should examine the effects of maladaptive parental involvement on children.
Creating Dengue Virus Stocks for Continued Research

Dengue is a tropical mosquito-borne virus that infects between 50-100 million people annually, causing febrile symptoms known as dengue fever. Since the 1960s, there has been an increase in cases and a broadening of its geographic distribution due to urbanization, population growth, increased global travel, and climate change. There are four dengue virus serotypes, DENV 1-4, which have 60-80% homology with each other. Viruses require host cells to provide the necessary factors for completing their life cycle. Yet we know little about which host factors are required for dengue replication and even less about how well conserved the requirement for these host factors is across the dengue serotypes and strains. We therefore set out to cultivate and establish infectious viral cultures for several dengue strains including DENV-1 Hawaii, DENV-2 MON601, DENV-2 K0049, DENV-2 IQT2913, DENV-3 H87, and DENV-4 H241. In order to study dengue virus capable of producing relevant disease symptoms we began by establishing its sylvatic cycle in vitro as the resulting virus is slightly different when produced by mosquito cells versus mammalian cells. An immortalized monkey kidney cell line (Vero) and mosquito cell line (C6/36) were used to amplify the dengue virus and viral production was tracked over a two-week period. Titers were assessed by flow cytometry. MON601 harvested from Vero cells had an average titer of 3.29E06 infectious particles/ml. The titers of all the virus harvested from C6/36 cells varied greatly both by strain and over time from initial infection. Notable results were high titers from H87 with an average titer of 1.47E07 infectious particles/ml and K0049 with 1.21E07 infectious particles/ml. With these new stocks of infectious virus, our long-term goal will be to investigate how these different virus serotypes and strains utilize and influence host factors during infection. In particular we aim to gain insight into alterations made by dengue on mTOR signaling, which we previously implicated as being important during dengue infection.
Comparison of Direct Laryngoscopy and Video Laryngoscopy in Infants

Background: Video Laryngoscopy (VL) is becoming increasingly popular for tracheal intubation (TI) of children and infants, and it is often used as an alternative to Direct Laryngoscopy (DI) not only in patients with a history or features of a difficult airway, but also in patients with normal airways. Due to the small size of their airways, their increased oxygen consumption and their limited respiratory reserve, infants represent a vulnerable population during TI. More than two attempts at TI is associated with an increased rate of complications. No prospective, randomized, controlled trials comparing success rate, complications, and speed of DL and have been conducted in infants.

Objectives: The primary objective was to determine the first attempt success rate of TI with DL vs. VL in infants <12 months of age. Secondary objectives were the number of attempts to achieve successful TI, frequency of failed TI attempts, and complications of TI with device assigned.

Methods: This study was a prospective, randomized, a multi-center, controlled trial utilizing Storz C-MAC VL and DL. Eligible patients were under 12 months of age, presenting for non-cardiac surgery under general anesthesia requiring TI. Patients with history and features of a difficult airway were excluded. Informed consent was obtained from the patient's guardian and from the clinician assigned to the intubation of the infant. Patients were randomized to be intubated with DL or VL. The conduct of the anesthetic was to the discretion of the anesthesiologist, but the protocol required the use of muscle relaxant to achieve a train-of-four of 1 or less. Data collected were from devices for each attempt. First, TI details included time from insertion of device to the removal of the device, the number of maneuvers, and complications. Statistical analysis is performed at the primary site. For a significant clinical effect, a 10% improvement in a first attempt success rate for VL compared to DL must be shown. Based on an 84% baseline success rate, a sample size of 540 provides 80% power.

Results: Total number of evaluable patients from the five participating sites was 550. Our site contributed 30 subjects. Data analysis is currently ongoing. Logistic regression is used to determine the first attempt success rate. Adjustments to correct for the level of training of the clinician intubating is made. Multivariate linear models were used to analyze secondary outcome data. Based on my observation I predict that VL would be more successful than with DL.

Conclusion: This internship exposed me to clinical research while interacting with patients and their guardians, which gave me insight into a different medical profession that I had not given thought to.
Ears, Balance and Hearing: Interactions of a Vestibular-Cochlear Implant

Background: Patients who undergo chemotherapy or are administered antibiotics can experience vertigo, chronic imbalance, disorientation, and hearing loss. This is caused by damage to vestibular and cochlear hair cells of the inner ear. Vestibular and cochlear hair cells send balance and hearing information, respectively, to the brain. Because there is no treatment for restoring hair cell loss, a vestibular-cochlear implant is being developed that may benefit many patients who suffer from chronic imbalance and disorientation. The implant works by sending electrical stimulation to the vestibular sensory organs of the ear to reduce vertigo and restore balance. Individuals would also benefit from restored hearing provided by the cochlear portion of the implant.

Objective: The vestibular-cochlear implant was developed to restore balance, orientation and hearing to patients, however, interactions between combined vestibular and cochlear stimulation may be problematic. Here we aim explore those interactions. We evaluated the effect of sounds elicited by cochlear stimulation alone compared to sounds elicited by combined cochlear-vestibular stimulation. This prospective design evaluated the safety and efficacy of combined cochlear-vestibular stimulation and also assessed subjects’ perception of pitch and volume for the two conditions.

Design: Three subjects suffering from vestibular loss obtained the vestibular-cochlear implant. When we activated the device, we elicit sound percepts and eye movements from the cochlear and vestibular stimulation sites respectively. We performed psychophysical tests where subjects estimate sound pitch and loudness for cochlear stimulation alone and compare that to combined vestibular-cochlear stimulation. Subjects were given a Likert scale to measure these differences; a psychometric scale used to tell us if they were able to hear the differences for pitch or volume.

Results: Each subject reacted differently to the combined stimulation. One subject showed little or no interaction. The other two subjects showed large and relatively inconsistent interactions for both pitch and volume. The second subject reported louder sounds, but no change in pitch for combined vestibular-cochlear stimulation. The third subject reported changes for both pitch and volume for combined stimulation.

Conclusion: Interactions between hearing and balance stimulation, when present, could reduce the efficacy and therapeutic value of the vestibular-cochlear implant. Our studies warrant future investigations to develop innovative stimulation parameters in order to benefit patients so that they might regain hearing and balance function.
Parental Education Regarding the Disposal of Unconsumed Opioids Following Day Surgery

Background: A significant amount of patients in the U.S are prescribed opioid medications for pain management following surgical procedures with remaining opioid medicines after patients have completed using the medicine creating the potential for diversion or misuse. Informed opioid prescribing is key to decreasing the number of overall opioids remaining after completion of pain treatment, one aspect of which is education about what patients should do with remaining opioids. Some states, including Washington, have mandated education about medication disposal at the time of prescription of opioids (HB1427). Currently, there are gaps in knowledge regarding proper means of opioid disposal amongst parents and variation in the way clinicians are distributing education on opioid disposal.

Objectives: 1) Determine the type and amount of education parents receive regarding the disposal of unused opioid medications following day surgery procedures. 2) Quantify the amount of unused opioid medications remaining after patients stop taking prescribed pain medicine following these procedures. 3) Identify current disposal practices of parents for leftover opioid medicines after these procedures.

Methods: A list of day surgery procedures in which opioids are frequently prescribed was compiled. A mixed-methods approach was used with consent/assent from patients and parents, using quantitative “in-person” and telephone surveys, at the time of discharge from surgery and 10-21 days after surgery. Attempts were made to reach parents by phone 10 days after their surgery. If they were not reached, a voicemail was left and repeated attempts were made.

Results: A total of 19 patient/parent dyads were approached. 13 dyads completed both discharge and follow up surveys, 19 completed the discharge survey only. 74% of parents did not receive any opioid disposal instructions prior to leaving the hospital. 26% of parents did receive opioid disposal instruction. In the follow-up survey, all parents reported filling their opioid prescription. At the time of follow up, none of them were still giving their child the opioid medicine for pain, and all of them had stopped giving it because it was no longer needed for pain. 16% of parents had disposed of the opioids and 83% of parents had not disposed of the opioids. Since leaving the hospital one parent reported receiving additional instructions on how to dispose of leftover medications; the rest did not. Of the 2 parents that reported disposing the medicine, 1 had received instruction, 1 had not. Of the 10 parents that had not disposed of medicines, 6 reported receiving no instruction about disposal, and 4 reported receiving instruction regarding disposal.

Conclusion: The results show that many patients and parents are not receiving education about how to dispose of opioids that remain after use for pain following a variety of day surgery procedures, and that medicines are not being disposed of in a prompt manner. Further research is needed on understanding effective educational interventions to increase disposal, with development of preoperative counseling informing both parent and patient about the risks of opioid medications. Additional research is also needed to assess standardized educational materials currently being used, including assessment of potential barriers to effective education such as language, reading literacy, computer literacy, access to drop off locations, and transportation.
Background: A majority of adolescents and young adults (AYAs) with type 1 diabetes (T1D) are not meeting recommended targets for glycemic control. One barrier to optimal glycemic control is the presence of psychosocial comorbidities, including depression, anxiety and diabetes distress (DD) that commonly affect AYAs with diabetes and may complicate disease management.

Objective: To describe depression, anxiety, and DD rates in AYAs with T1D and examine if depression, anxiety, and DD are associated with high-risk glycemic control.

Design/Methods: Eligible participants included AYAs with T1D who received diabetes care in the Seattle Children’s AYA Diabetes Transition Clinic designed to assist AYA patients transition to adult diabetes care in 2017-2019. 103 AYAs with T1D were assessed for depression, anxiety and DD using the Patient Health Questionnaire (PHQ-9>10), Generalized Anxiety Disorder scale (GAD-7>10) and the Problem Areas in Diabetes survey (PAID-T>44), respectively. High-risk glycemic control was defined as a HbA1c >9.0%. Chi-square tests were used to assess the associations between categorical variables.

Results: Depression, anxiety and diabetes distress rates among participants were 18.4%, 17.5%, and 14.0% respectively. While participants with depression and generalized anxiety were not found to have poorer glycemic control, AYAs with T1D with DD were more likely to have a HbA1c level >9.0% (p=0.01).

Conclusion: Our findings demonstrate that a sizeable percentage of AYAs with T1D experience depression, anxiety, and diabetes distress, highlighting the need for mental health evaluation and support for AYAs with diabetes. Further, the finding that AYAs with DD were more likely to have high-risk glycemic control underscores the potential value in screening youth for psychosocial comorbidities with T1D in clinical settings. Identifying AYAs at increased risk for poor diabetes outcomes may allow diabetes care team members to provide tailored diabetes support for this vulnerable population.
**Thyroid Receptor Beta 1 and its Antibodies**

**Background:** Thyroid hormone controls a vast array of biological functions, such as metabolism, growth and neurotransmitters. Thyroid hormone actions are primarily mediated by thyroid hormone receptors (TRs) alpha and beta. Patients with mutations in the receptor genes display a range of disorders, including disruptions in cardiac functions. Thyroid hormone receptors are nuclear transcription factors that regulate the gene expression of a vast array of genes. It is the overall goal of our lab to understand the mechanisms involved in how TRs, especially TRβ1, regulate genes during different thyroid hormone disease states.

**Objective:** The utilization of antibodies is an essential tool in scientific research. However, the quality of the antibodies needs to be confirmed prior to use. Our lab investigates changes in the thyroid hormone beta 1 (TRβ1) receptor binding to DNA during thyroid hormone imbalance. Therefore, the antibody required needs to be highly specific and sensitive. TRβ1 has several commercially available antibodies produced in various species. In this study we investigated the characteristics from 4 to identify the best antibody to use.

**Methods:** Four commercially available antibodies were tested by western blot: 1) antibody GTX22744, a mouse monoclonal, 2) antibody GTX16393, a rabbit polyclonal, 3) antibody SC-737, mouse monoclonal and 4) antibody AB5622, a rabbit polyclonal. The samples tested were CV-1 cells, heart tissue and purified TRβ1 protein. The CV-1 cells were derived from green monkey kidneys and lack the TRβ1 protein, hence acted as the negative control in this experiment. The purified TRβ1 protein was the positive control.

**Results:** Each of the antibodies tested reacted with the purified TRβ1 protein. Antibody GTX22744 yielded the strongest signal after 5 seconds of imaging exposure time. Antibodies SC-737 and AB5622 identified a band at the correct size for TRβ1 in heart tissue. However, they also showed cross reactivity with unknown proteins. Additionally, the strong signal observed for antibody GTX22744 at 5 seconds may have been masking a signal from the heart tissue. Antibody GTX16393 failed to yield a signal at the correct size in the heart tissue.

**Conclusion:** Our study demonstrates the necessity of testing different antibodies because the different antibodies had varying levels of effectiveness, with differences in specificity and sensitivity. Presently, the lab is pursuing antibody GTX22744 as its antibody of choice for downstream experiments.
Identifying the Function of VSIG4 as Seen in the Tumor Microenvironment of Glioblastoma

**Background:** Glioblastoma (GBM) is the most aggressive primary brain tumor with a five-year survival rate of less than 10%. The tumor is impossible to completely remove with surgery and due to intrinsic and extrinsic properties of the tumor, immunotherapies have failed to provide a survival benefit. Of the immune cells present in the tumor microenvironment (TME) of GBM, tumor-associated macrophages (TAMs) are the most abundant and these cells play a key role in initiating and maintaining the immunosuppressive TME. Previous assays have shown protein VSIG4 to be overexpressed in TAMs.

**Objective:** To understand the molecular function of VSIG4, we sought to determine how VSIG4 overexpression altered the gene expression in TAMs by mRNA analysis.

**Design/Methods:** Using a VSIG4 lentivirus, we overexpressed VSIG4 in GmCSF-differentiated donor macrophages (n=3) and collected total RNA from control and VSIG4-overexpressing macrophages. RNA was purified by Qiagen RNeasy, and 25 ng run on Nanostring SPRINT instrument with the Metabolic Pathways Panel, using manufacturer’s protocols. Data was analyzed in nSolver and Microsoft Excel.

**Results:** VSIG4 overexpression resulted in a downregulation of GAPDH, an enzyme involved in glycolysis and upregulation of GLUL, an enzyme that is involved in glutamine metabolism.

**Conclusion:** These changes indicate a shift away from glucose metabolism and into alternative metabolisms. This shift in metabolism aids in the survival of the tumor by making more glucose available to the tumor cells, and preventing this shift will result in a reduced ability for TAMs to carry out their protumor functions and decreased tumor proliferation. Future studies may examine other genes involved in this metabolic shift as well as the factors causing the upregulation of VSIG4.
Seattle Children’s Hospital is one of many sites participating in this nationwide study that originated at Northwestern University. The purpose of this study is to better understand the social impact of chronic skin diseases on children. We want to see if there was a stigma associated with skin conditions and if the impact is severe enough to lessen their quality of life. We are also looking for any correlation between how age, gender, race, and socioeconomic background change how the skin condition impacts the patient. With this information, we can come up with ways to minimize the effect. Possible solutions include intervening early, and treating conditions with the intent of healing both body and mind. We administered the study through a series of surveys that the patient and their parents took. Questions include how the patients feel about having a visible skin condition, how they perceive themselves because of their condition, and how other people react to and act towards the patient because of their condition. There is also a depression and anxiety screening to see if there is a correlation between having a chronic skin disorder and having a mental disorder. By having both the child and the parent take the survey, we will be able to identify differences and similarities between how the parents perceive the skin condition impacting their child’s life and how the children feel the skin condition impacts them. Because the study has only just begun, we do not have enough data to draw conclusions. However, I predict that the more visible the skin condition, the greater impact it will have on a child’s life. I believe that because the children who take the study are between ages eight to seventeen and typically in school which means that they see other children every day and there is more room for them to be embarrassed by their condition. I also suspect that children who are minorities, have only one parent, or have a lower socioeconomic status are more predisposed to showing depressive symptoms because they do not have access to the support that other children may receive. I hope that in the future this study will be used as a stepping stone towards eradicating the stigma and negative impacts of childhood skin conditions.
Identification of Synergistic and Antagonistic Antibiotic Combinations for Treatment of Mycobacterium Abscessus

Mycobacterium abscessus (Mabsc) infections occur in more than 5% of patients with cystic fibrosis (CF), increasing over the last decade. Antibiotic treatment for those with the infection can take many months. In countless cases, antibiotics are not successful at eradicating the organism, requiring long-term maintenance therapy. Prolonged treatment raises the risk of increased resistance within the patient. Currently, there is not an effective and evidence-based drug regimen for treating Mabsc infections in patients with CF. The conventional recommended treatment consists of 3-4 drugs, which together have an eradication rate as low as 20% in Mabsc subspecies abscessus. The goals of this project were two-fold: to establish an efficient method for set-up and analysis of checkerboard interaction assays for Mabsc, and then to collect preliminary data to train the INDIGO-MABSC, an in silico model for predicting synergistic and antagonistic antibiotic interactions to treat M. abscessus infections. Using broth microdilution, we observed the interactions between two different drugs and the combination’s efficiency in inhibiting the growth of Mabsc type strain ATCC19977. Checkerboard assays were set up in two different media, Cation-Adjusted Mueller Hinton Broth (CAMHB) and Middlebrook 7H9, to determine whether the type of media would alter the minimum inhibitory concentrations (MICs) and/or any observed interactions. CAMHB plates were incubated at 30°C and 7H9 plates were incubated at 37°C, without supplemental CO2. Several methods for reading assay plates were also tested to identify a reproducible and reliable data collection method, including: visual reading for inhibition of growth, measurement of OD600 by microplate reader, use of resazurin sodium as an indicator both read by eye for color change and by fluorescence. Drug interactions were determined by calculating the sum fractional inhibitory concentration (FIC) of each combination using the visual boundary between growth/no growth. Preliminary results show putative synergistic interactions between drug pairs Cefoxitin-Rifampin, Imipenem-Cefoxitin, Imipenem-Clarithromycin, and Imipenem-Linezolid. Based on our observations, the type of media does not greatly affect the MIC of individual drugs with the exception of Clarithromycin, nor does it appear to alter whether interactions between antibiotics are synergistic or antagonistic. Future directions include using 7H9 as the main media, since Mabsc grows more quickly in it compared to CAMHB. Furthermore, for quantitative measurement of growth inhibition, OD600 measured without lids provides reliable data. These results will allow us to efficiently collect antibiotic interaction data going forward for training the INDIGO-MABSC model.
Calcium Imaging Shows Neuron Activity and Contradicts the Notion that Five Minute Simulations with Glutamate, NMDA, and DHPG Induce Excitotoxicity

Calcium ion concentrations play a critical role in neuron function. Intracellular calcium signals are responsible for a wide range of processes such as triggering neurotransmitter release and inducing activity-dependent changes in synaptic plasticity. This makes calcium imaging particularly useful in our research, which explores how various genes linked to autism spectrum disorder (ASD) disrupt activity-dependent changes in protein interaction networks (PIN). Previously, to gain insight into the dynamics of glutamatergic signaling, our lab utilized quantitative multiplex co-immunoprecipitation (QMI) to measure activity-dependent changes in an autism-linked PIN by stimulating neurons with glutamate, NMDA, and DHPG.

Here, I utilized calcium imaging while stimulating neurons for five minutes with 100 μM glutamate, NMDA, DHPG, and with aCSF as control, as previously reported (Lautz et al., 2018). During stimulations, I measured intracellular calcium using genetically-encoded calcium indicators (GECIs) to confirm stimulations produced strong calcium flux, and did not induce excitotoxicity. Furthermore, we hoped to correlate intracellular calcium flux with the immediate changes that occur in our targeted PIN.

In calcium imaging of glutamate and NMDA stimulations, we used GCaMP6 expressed in VGlut-CRE+ neurons in culture. But when stimulating with DHPG, we used ER-GCaMP6-150, an ER GECI.

Our results showed that during stimulation with glutamate or NMDA, intracellular calcium concentrations rise, and peak at approximately 1 minute. After removal of stimuli, intracellular calcium concentrations decline and eventually reach baseline. After half an hour, I would then re-stimulate with KCl to increase spontaneous firing. Intracellular calcium concentrations again increased after stimulation KCl, demonstrating that the neurons were still alive and our stimulations did not induce excitotoxicity.

However, we were unable to show changes in intracellular calcium when stimulating with DHPG as a result of peak calcium concentrations not being great enough with DHPG stimulation.

“It’s Like 1998 Again”: Why are Parents Still Refusing and Delaying Vaccines?

Background: The vast majority of empirical studies regarding why parents refuse or delay childhood vaccines are greater than five years old and consistently document vaccine safety as a primary reason. There is a need to update this literature given recent outbreaks of vaccine-preventable diseases (VPDs) and an increase in parental vaccine refusal.

Objective: In this study, we seek to identify provider perspectives on why parents are currently refusing or delaying childhood vaccines.

Design/Methods: We conducted four focus groups and four semi-structured interviews of pediatric providers (n=33) in two states, Washington and Colorado. This study was part of a larger study to design and test the effect of a provider vaccine communication strategy for childhood vaccine uptake. In these focus groups and semi-structured interviews, we specifically asked providers about their recent experiences regarding why parents have refused or delayed vaccines for their child. The focus groups and semi-structured interviews were recorded and transcribed. We conducted a thematic analysis of these transcripts with inductive coding to identify themes related to parental refusal or delay of childhood vaccines. We then assessed the frequency of identified themes by counts.

Results: Five major themes (each with several subthemes - see Table 1) relating to parental refusal or delay of childhood vaccines were identified. (1) Vaccine safety (concerns regarding the potential physical harm that the composition and administration frequency of vaccines may impose on a child), (2) decision-making process (reasons related to how a parent identifies a decision and refers to information to solidify decision to refuse or delay), (3) low perceived risk of contracting VPD (reasons regarding the perceived chances child will contract or spread disease), (4) lack of trust (parent is untrustworthy of provider and/or government), and (5) religious objection (refuses or delays vaccinating child due to religious or spiritual concerns regarding vaccines and its contents). Vaccine safety was referred to 45 times by providers, making it the most prevalent theme. Decision-making process was the second most prevalent theme with 14 references. Low perceived risk and lack of trust were each cited 9 times. Religious objection was referred to the least (n=6).

Conclusion: The reasons expressed by providers regarding why parents are refusing or delaying vaccines for their child are similar to reasons documented in existing literature. These results reveal a need to continue research to develop and test strategies that address parental vaccine concerns and improve uptake.
Food Insecurity in Seattle Children’s Hospital Emergency Department

Background: Food insecurity (FI) is the limited or uncertain availability of nutritionally adequate or safe foods. Children in food-insecure households may experience adverse health outcomes due to inadequate quantity and/or quality of food. FI is associated with poorer overall health and increased hospitalizations. FI in families seeking emergency care may be high; in one urban ED study population it was 21%. Past research suggests family members may be more comfortable reporting FI during ED visits than a primary care visit.

Objective: To understand the food needs of patients visiting Seattle Children’s Hospital (SCH) ED and to examine associations between patient demographics and FI status in the SCH ED population in order to inform future efforts and interventions.

Methods: A cross-sectional, quality improvement-pilot was conducted. During a convenience sample of screening hours spanning weekday and weekend mornings, afternoons, and evenings, SCH ED families were screened for FI using a validated 2-question Hunger Vital Sign Screening Tool. Siblings, repeat visits, critically ill patients, minor-age patients without a guardian, and families that providers asked not to disturb were excluded. Families answered two FI screening questions verbally or in writing, based on preference. Those who screened positive received information about food-related resources in the community and resources specific to SCH.

Results: Of 513 patients seen during screening hours, 439 families were screened, and 18% screened positive for FI. On average the screening tool required 5 minutes (range 3-10 minutes) to complete. FI varied by preferred language of care: 54/374 (14%) of English-speakers, 15/29 (52%) of Spanish-speakers, 7/10 (50%) of those speaking African/Middle Eastern languages, 0/12 of those speaking Asian languages, and 1/10 (10%) other non-English. Insurance was also associated with FI: 9/209 (4%) with commercial insurance, 65/207 (31%) with Medicaid and 3/22 (14%) with other. FI was similar between patients discharged (18%) versus admitted (15%). To minimize missing FI families, results suggest the SCH ED should target a combination of Medicaid, uninsured, and patients in families with Limited English Proficiency (LEP). This could result in screening approximately 51% of ED patients and possibly missing 13% of patients in food insecure households.

Conclusion: FI was common in ED patients in this urban, pediatric ED in the Pacific Northwest, similar to what has been reported in the northeastern United States. At SCH ED, LEP and public or no insurance status were associated with FI. Screening ED patients for FI could provide important information relevant to providing optimal emergency health care.

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Impact of Diabetes Technology Use on Glycemic Control in Adolescents and Young Adults with Type 1 Diabetes

**Background:** Less than a quarter of adolescents and young adults (AYAs) with type 1 diabetes (T1D) are meeting recommended targets for glycemic control. To successfully manage their diabetes, AYAs with T1D face complex and demanding diabetes self-management tasks such as needing to check blood glucose levels several times a day and administer multiple daily insulin injections. The use of diabetes technology such as continuous glucose monitors (CGM), insulin pumps, and hybrid closed loop (HCL) systems may help support diabetes management and improve glycemic control in AYAs with T1D.

**Objective:** To examine differences in glycemic control between AYAs with T1D using diabetes technology compared to those who do not.

**Methods:** Eligible participants included AYAs with T1D who received diabetes care in the Seattle Children’s AYA Diabetes Transition Clinic designed to assist AYA patients transition to adult diabetes care in 2017-2019. CGM use was defined into three categories: 1) Not prescribed CGM; 2) Prescribed CGM, not actively using; 3) Prescribed CGM, actively using. Insulin delivery methods included multiple daily injections (MDI), insulin pumps, and hybrid closed loop (HCL) systems. Glycemic control was assessed based on a HbA1c measurement at participant’s first AYA clinic visit. ANOVA tests were used to examine differences in mean HbA1c between participants who use diabetes technology and those who do not.

**Results:** Participants included 179 AYAs with T1D (mean age 20 ± 2.3 years, mean diabetes duration 10 ± 4.7 years, 54% male, 75% non-Hispanic white, and 68% private insurance). There was a difference among the three CGM groups in terms of HbA1c levels with active CGM users having the lowest mean HbA1c (Table 1; F=8.3, p<0.01). There was also a difference in HbA1c levels among insulin delivery method groups with HCL system users having the lowest mean HbA1c (Table 1; F=6.2, p<0.01).

**Conclusion:** Our results demonstrate that the active use of diabetes technology can help support improved glycemic control in AYAs with T1D. Given that AYAs struggle meeting glycemic targets and have a generally favorable perception of technology, diabetes providers should consider incorporation of diabetes technology to improve engagement and adherence to diabetes self-management.
The Effects of Sleep Deprivation on Epilepsy in a Mouse Model of Megalencephaly

Background: Megalencephaly (MEG) is a developmental disorder of brain overgrowth associated with intellectual disability and epilepsy. Sleep disturbances are associated with poor seizure control. Poor sleep can lead to behavioral and attentional problems which can ultimately contribute to social and intellectual difficulties in life. In clinics, epilepsy is diagnosed with Electroencephalogram (EEG) which can detect myoclonic and generalized tonic-clonic seizures as well as other abnormal brain activity such as interictal spikes. Interictal spikes are hallmarks of the epileptic brain and they help to confirm the diagnosis of epilepsy and to characterize generalized vs. partial epilepsies.

Objective: Our research aimed to determine the effect of sleep deprivation on epilepsy in a mouse model of MEG.

Design/Methods: The Kalume lab in collaboration with the Dobyns and Millen labs generated the Pik3ca mouse model of MEG which recapitulates the human features of MEG. PIK3CA is a gene in the mTOR signaling pathway that is well known to be involved in abnormal brain malformation.

In this experiment, there were two groups of mice, sham (n=3) and sleep deprivation (SD) (n=3), that were implanted with EEG and electromyography (EMG) electrodes using techniques developed in the lab. The EEG electrodes were placed bilaterally over frontal and posterior cortices. The EMG electrodes were placed underneath neck muscles and a reference electrode at the midline cerebellum.

Both groups were placed in an enclosed chamber for 5 hours for either SD or sham treatment. The chamber contained curtains controlled by LabChart software and PowerLab which moved randomly in different patterns and speeds. In the SD group, mice were kept awake by gentle touch during curtains movement in the cage. However, in the sham group, the curtain did not move and mice were allowed to sleep. Then, the mice were moved to a recording chamber for 3 hours and video EEG/EMG activity was recorded. Raw data were processed through filters to extract EEG signals and sleep and epileptic events were characterized.

Results: We found when exposed to SD, MEG mice experienced more interictal spikes than when kept in control (sham) condition. Interictal spikes were defined as high amplitude, sharply contoured EEG waveforms, and no association with movement on video. Thus, this finding indicates that SD increases the number of epileptic EEG events in MEG mice.

Conclusion: This study demonstrates sleep can influence the expression of epilepsy, poor sleep can worsen or exacerbate the epilepsy phenotype in MEG.
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