Online Proceedings

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Molecular Basis for Human Disease

Session Moderator: Caroline Harwood, Microbiology
MGH 271
12:30 PM to 2:15 PM

* Note: Titles in order of presentation.

Utilizing Budding Yeast to Investigate Human Dwarfism
Mackenzie Erin (Mackenzie) Croy, Senior, Biology (Molecular, Cellular & Developmental)
Mentor: Bonita Brewer, Genome Sciences

Meier-Gorlin syndrome (MGS) is a rare form of proportionate dwarfism that has been linked to mutations in proteins that function during the initiation and elongation steps of DNA replication. Although mutations in DNA replication proteins have been shown to be causative for MGS, it is still not clear how defects during S-phase lead to the phenotypes observed in patients with this condition. Therefore, further research is necessary to better understand the molecular and cellular consequences of cells harboring MGS mutations. Tools for studying DNA replication in humans are limited, however, there are several well-established tools for studying this process in the budding yeast *S. cerevisiae*. Some individuals with MGS have been shown to have mutations in the DNA replication initiation factor Cdc45, which functions during both the initiation and elongation steps of DNA replication. Because a specific MGS mutation in CDC45 is in a residue that is conserved between humans and yeast, we have replaced the wild type yeast gene with an allele that contains the equivalent human mutation. Yeast with the MGS mutation exhibit increased sensitivity to elevated temperatures and a reduced ribosomal DNA (rDNA) copy number. In budding yeast, the rDNA locus contains ˜150 copies of a 9.1 kb repeat which encodes the template needed to make ribosomal RNA—the main structural component of ribosomes. Additionally, each repeat contains an origin of replication. We hypothesize that this loss of rDNA repeats is a consequence of impaired replication initiation at the rDNA locus and will test this using 2-D gel electrophoresis. We anticipate to expand our study outside of the rDNA locus and examine how replication might be perturbed at other locations across the genome. Ultimately, we hope studying MGS in yeast will elucidate our understanding of the pathogenesis of this condition in humans.

Understanding Inflammation Cascades in Juvenile Idiopathic Arthritis
Samuel Dollard (Sam) Dodson, Senior, Biochemistry
Mentor: Anne Stevens, Pediatrics
Mentor: Sriharsha Grevich

Juvenile Idiopathic Arthritis (JIA) is the most common rheumatologic disorder found in children, affecting millions worldwide. It has no known cause, prevention, or cure, and can lead to debilitating destruction of the joints. In adult Rheumatoid Arthritis (RA), previous research suggests a connection between RA and oral bacteria that cause inflammatory diseases. Specifically, *Porphyromonas gingivalis*, found in patients with periodontitis, expresses an enzyme that modifies the amino acid arginine to citrulline, which the immune system recognizes as foreign, reacting to produce anti–citrullinated protein antibodies (ACPAs), which attack otherwise healthy proteins in the body. Therefore, we hypothesize that in JIA, the unique bacterial colonies of the mouth induce a chronic ACPA production that leads to arthritis. We use enzyme-linked immunosorbent assays to assay patient plasma for ACPAs with 6 peptides. We have tested plasma from 79 patients with JIA, 56 children recruited from dental clinic, and 29 healthy children for antibodies to citrullinated filaggrin, a known autoantigen in RA. Compared to healthy controls, JIA patients had a significant increase in anti-citrullinated filaggrin antibodies (relative levels 0.88 vs. 0.45, p=0.001 by t-test). Anti-filaggrin antibodies were elevated in patients with oligoarticular JIA, (mean 0.62, p=0.0012) and extended-oligoarticular JIA, (mean 0.88, p=0.007), but not polyarticular JIA (mean 0.49, p=0.47). In addition, there is a higher prevalence of bleeding on probing (BOP, an indicator of gingival inflammation) in JIA, with an average BOP score of more than two times that of children from dental clinic (p=0.008). Our next step is to see if there are any specific microbes associated with higher BOP scores and ACPA levels. If we find that oral pathogens are associated with inflammatory disease, prevention can be designed to eliminate plaque-associated pathogens through var-

ious dental hygiene practices.

Microfluidic Flow Chamber Array to Study Viridans Group Streptococci

Christian Joseph (Christian) Matthews, Senior, Bioengineering

Mary Gates Scholar, NASA Space Grant Scholar, UW Honors Program

Mentor: Wendy Thomas, Bioengineering

Bacterial endocarditis is a life-threatening disease that occurs when bacteria bind to the inner surface of cardiovascular tissue. One group of bacteria in particular, the viridans group Streptococci, cause a high percentage of cases of endocarditis. It is known that adhesion is mediated by specialized proteins on the surface of bacteria capable of binding to specific glycans expressed outside of host cells, and that binding is also dependent on shear stress from flow through allosteric regulation of the binding site. However, the exact mechanisms and resulting rates of binding are not sufficiently understood. If adhesion of the viridans group Streptococci can be experimentally tested and characterized, it could lead to the development of anti-adhesive therapies to treat or prevent diseases such as endocarditis. Current methods of testing bacterial adhesion utilize a parallel plate flow chamber to test the binding of one species of bacteria to a single glycan under one flow rate. Significant progress could be made through the construction of a microfluidic flow chamber compatible with a glycan array capable of testing bacterial adhesion to various glycans under a range of shear stresses in a single run. I have designed such a device using CAD and COMSOL fluid modeling software, and will construct it through the process of 3D stereolithography and laser-cutting. Additionally, I have conducted experiments to identify conditions that minimize non-specific adhesion, maximizing specificity for the device. Bacteria were fluorescently tagged, run through the flow chamber and across the glycan array, and then adhesion was analyzed using a microarray scanner. Data gathered from this device will help collaborators develop treatments to treat and prevent this type bacterial endocarditis. The flow chamber is also now accessible to others in the field to be used to study other clinically relevant types of bacterial adhesion.

KSHV Modulates the Expression of Genes Involved in Peroxisome Biogenesis

Mira Naidoo, Senior, Microbiology

Levinson Emerging Scholar, Mary Gates Scholar, UW Honors Program

Mentor: Michael Lagunoff, Microbiology

Kaposi's sarcoma-associated herpesvirus (KSHV) is the causative agent of Kaposi's Sarcoma (KS), a cancer of endothelial cell origin that is the most common malignancy among AIDS patients worldwide. Previous research in our lab has established that the number of peroxisomes is increased during latent KSHV infection. Peroxisomes are multifunctional cellular organelles involved in a variety of metabolic pathways important to KSHV pathogenesis. My project is to evaluate the cellular mechanism by which KSHV induces peroxisome biogenesis, thereby elucidating one of the key pathways involved in KSHV latency. I hypothesize that KSHV increases the transcription of specific regulatory genes responsible for peroxisome biogenesis. I evaluated gene expression of a known transcription factor, peroxisome proliferator-activated receptor alpha (PPARA), that has been implicated in peroxisome biogenesis during mock and KSHV latent infection of endothelial cells. I used real-time PCR to quantify gene expression of PPARA, in addition to other genes involved in peroxisome formation and function. My data demonstrates that KSHV infection upregulates PPARA and peroxisome-associated genes, suggesting that PPARA may be a key regulator of the expression of peroxisome biogenesis. To further establish the role of PPARA in peroxisome biogenesis, I am currently working on silencing PPARA expression using small interfering RNA (siRNA). I will determine if the knockdown of PPARA prevents KSHV upregulation of peroxisome-associated genes. In the absence of PPARA, I expect that expression of peroxisome-associated genes will be downregulated, suggesting that PPARA regulates them at the transcriptional level. These results will establish a key mechanism in KSHV pathogenesis, and potentially contribute to the development of novel therapeutic avenues for KS treatment.

Low-level Mutation Detection of Megalencephaly with ddPCR

Andrew Piacitelli, Senior, Neurobiology

Mentor: Ghayda Mirzaa, Human Genetics

While many brain malformations at birth are caused by *de novo* variants– genetic mutations that arise early during fetal development– in some cases deleterious mutations are inherited from a parent unaffected by the mutation. If the parent harbors the mutation in a small amount of cells, it is possible for them to lack a disease phenotype despite being able to pass on the mutant gene to their offspring. This genetic variability in somatic cells is called mosaicism. Mosaicism is challenging to detect with traditional methods of sequencing, as it is difficult to determine if a small read number of mutant DNA is an error in sequencing or if it is a biologically real low-level sequence variation. In order to confidently identify low-frequency mosaic mutations, we utilize deep-coverage next-generation sequencing methods with very low thresholds for mutant DNA detection. Digital-droplet PCR, or ddPCR, is a promising new sequencing technique ideal for detecting low-level mosaicism. As this is a recently developed technology, we first optimized the protocol to determine the level of attainable coverage in mosaic samples and compare this with other high-depth methods. When this was

successful, we tested samples from families of children affected by serious brain malformations. We selected families with healthy parents and multiple affected children, as this is a strong indication that one of the parents carries a mosaic mutation. We also tested dysplastic brain samples from affected children who underwent epilepsy surgery to quantify the level of mutant cells. Overall, we were able to optimize ddPCR to detect a mutant allele frequency of 0.2%, which is substantially better than other high-depth methods. This work provides a promising new avenue into the detection of low-frequency mosaicism, which can be used to present clinically-relevant information for the diagnosis and genetic consultation of children affected by various genetic conditions.