

were significantly associated with hypokalemia. In multivariate analysis, age (OR 0.961, 95% CI [0.936, 0.985], $p = 0.00167$) remained significant, and creatinine clearance (OR 0.986, 95% CI [0.974, 0.999], $p = 0.0386$) became significant. BMI, weight, systolic and diastolic blood pressure, sex, and urine protein concentration were not significant. **DISCUSSION/SIGNIFICANCE OF IMPACT:** In the multivariate analysis, age and creatinine clearance was statistically significant; however, their effect sizes were small and unlikely to be clinically meaningful. The underlying reasons for the high prevalence of hypokalemia in this population remain unclear, warranting further investigation to identify contributing factors.

106

Unraveling the genetic architecture of aging through comparative genomics

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OBJECTIVES/GOALS: To identify the genomic mechanisms underlying cross-species regulation of longevity among mammals and birds and to characterize the impact of those conserved pathways on human aging. More broadly, this study aims to develop a novel evolutionary approach to understand the genetics of complex traits. **METHODS/STUDY POPULATION:** High-quality genome sequences for 194 bird species and 295 mammal species with reliable longevity and body size data were obtained from publicly available resources. The data include coding sequence alignments of 16,863 mammalian and 14,565 avian one-to-one orthologous genes. Gene-wise relative evolutionary rates (RERConverge) and maximum likelihood phylogenetics (PAML) were computed to assess for evidence of purifying selection and positive selection for longevity. As part of ongoing analysis, human orthologs of selected results will be examined in the UK Biobank for validation. **RESULTS/ANTICIPATED RESULTS:** Preliminarily, we have found a signal of concordant positive selection between two classes of vertebrates separated by hundreds of millions of years. Several genes show signals of positive selection in long-lived species of both birds and mammals. Ongoing work focuses on elucidating the relationship between relative evolutionary rates and positive selection, the overlap in selection signature between long-lived animals with large body sizes and those who are exceptionally long lived for their body size, and further elaborating on convergence between mammals and birds. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Currently, most scientific knowledge about aging is from experiments on short-lived model organisms. By systematically studying the genomes of long-lived mammals and birds, we aim to develop a new method for studying complex traits and uncover novel insights into the mechanisms of longevity.

108

The Resist! Project: Use of a mixed-methods approach to identify substance use resistance factors

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OBJECTIVES/GOALS: The aim of the study is to identify resistance factors for substance use (i.e., factors that explicitly help to avoid or reduce drug use). Identification of resistance factors could inform strategies that seek to reduce the prevalence of substance use and related disorders. **METHODS/STUDY POPULATION:** Adult twins aged 30–70 years were recruited from the Mid-Atlantic Twin Registry. A mixed-method approach, group concept mapping, was used to identify factors influencing participants to resist using substances. Approximately 155 participants produced 97 statements reflecting substance use resistance factors. Hierarchical cluster analysis and multidimensional scaling assessed how participants sorted and rated statements for their lifetime and current importance. Factor analysis was used to reduce data dimensionality. Reliability analyses were conducted to identify a subset of statements anticipated to consistently represent each cluster. Results were shared with participants to assess accuracy with their experiences. **RESULTS/ANTICIPATED RESULTS:** Participants sorted 97 statements into 9 thematic clusters: (1) Controlling Personal, Negative Consequences; (2) Concern About Health and Well-being; (3) Lack of Desire; (4) Outside Influences; (5) Social Norms and My Reputation; (6) Career and Legal Impacts; (7) Avoiding Harm to Family and Relationships; (8) Preserving Family Relationships; and (9) Family and Friends Impact on Me. Participants consistently identified health concerns as an important substance use resistance factor. The statements will be further reduced to represent a smaller subset for future use as a scale to measure exposure to resistance factors. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Health concerns related to substance use were identified as an important resistance factor. This has been supported by research on smoking cessation and implemented in smoking prevention campaigns. Therefore, prioritizing health-related outcomes in prevention may be important to reduce substance use prevalence.

109

Retrospective analysis of sociodemographic and geographic risk factors for presenting keratitis severity in a South Indian population

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OBJECTIVES/GOALS: Infectious keratitis is the leading cause of corneal blindness worldwide, causing two million cases of monocular blindness per year. Of these cases, developing countries are disproportionately affected, in part due to sociodemographic disparities. Our study examined risk factors for severe keratitis presentation in a South Indian population. **METHODS/STUDY POPULATION:** 156 patients aged ≥ 16 years with clinically diagnosed infectious keratitis presenting to Aravind Eye Hospital in Pondicherry, India, from January 1, 2023 to July 31, 2024, were retrospectively reviewed. Univariate logistic regression was used to evaluate associations between specific potential risk factors (including age, sex, awareness of keratitis, travel distance to hospital, education level,

missed work wages, and ability to afford care) and keratitis clinical severity (defined using thresholds of poor visual acuity, size of stromal infiltrate measured on slit lamp examination, occurrence of perforation, and need for corneal transplant surgery). Individual risk factors found to be significant were incorporated into a multivariable logistic regression model. **RESULTS/ANTICIPATED RESULTS:** We anticipate that the results of this study will identify multiple risk factors for more severe keratitis presentations among patients at baseline. We expect these factors to include increased travel distance from the patient's home to the base hospital, delays between time of diagnosis and initiation of treatment, treatment nonadherence, lower educational levels, lack of familiarity with keratitis, treatment and transportation costs, increased time off work, and missed work wages, among others. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This study elucidates barriers to early keratitis diagnosis in a low-resource setting. Study findings can inform strategies to improve keratitis prevention using decentralized care approaches such as community eye screenings and expanding outreach via vision centers. Such strategies can improve timely access to care for vulnerable populations.

110

Computational methods to monitor treatment response and toxicity in immune-checkpoint-inhibitor treated metastatic melanoma using methylated cell-free DNA

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OBJECTIVES/GOALS: Immune checkpoint inhibitors (IO) have dramatically improved survival outcomes in patients with metastatic melanoma. Still, many patients do not respond these treatments, and others may experience harmful adverse events (irAEs). Thus, there is an unmet need for biomarkers for real-time monitoring and management of patients exposed to IO therapies. **METHODS/STUDY POPULATION:** Serial serum samples were collected from patients with BRAFV600-mutant metastatic melanoma treated with ipilimumab/nivolumab (IO, n = 14) or dabrafenib/trametinib (TT, n = 10). Methylated cell-free DNA (cfDNA) was isolated and sequenced using enzymatic methyl-seq. We develop a robust computational pipeline to identify the top 250 cell-type specific regions of differential methylation (DMRs) across 24 cell-types. Using these differentially methylated regions, a deconvolution tool was developed to determine the abundance of cell type-specific cfDNA in patient serum, and changes in abundance were tracked over treatment time-course to assess response to treatment and identify signals of adverse events. **RESULTS/ANTICIPATED RESULTS:** We demonstrated improved precision in DMR detection evidenced by a higher area under the receiver operator characteristic curve (AUROC) of 0.85 on average. Pathway and functional annotation analysis revealed melanocyte-specific methylation marker regions regulated genes related to melanocyte development and differentiation, including MITF, SOX9/10, and FOXD3. We show these regions are conserved through the transformation to malignant melanoma, indicating melanocyte cfDNA abundance can be used as a marker for tumor burden. We characterize the dynamics of melanocyte-derived cfDNA over the course of treatment in responders and

nonresponders to both IO and TT. We observe that changes in concentrations of cfDNA from other cell types correlate with clinically observed irAE-mediated damage to normal tissue. **DISCUSSION/SIGNIFICANCE OF IMPACT:** We demonstrated the utility of decoding the origins of cfDNA fragments obtained from serial liquid biopsy samples. Using cell-specific methylation marks, we identified a signature from the primary melanoma to assess response to treatment, while also obtaining a signal from other tissues throughout the body to monitor immune related adverse events.

116

Acute inorganic arsenic exposure and antibiotic perturbation of the murine gut microbiome induce interindividual susceptibility to a sepsis-like disease

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OBJECTIVES/GOALS: We investigate how the gut microbiome protects against arsenic toxicity, showing antibiotic perturbation increases toxicity and causes interindividual susceptibility to a sepsis-like disease state in mice. Here, we aim to understand how baseline microbiomes from various mouse vendors impact these outcomes and characterize the observed disease. **METHODS/STUDY POPULATION:** We developed a novel mouse model where mice are exposed to an antibiotic (cefoperazone) for 2 days, followed by co-exposure to the antibiotic and 100 ppm arsenate. So far, we have evaluated C57BL/6N mice from MSU's in-house colony, Taconic Biosciences (TAC), and Jackson Labs (JAX), along with C57BL/6J mice from JAX. To determine if the baseline microbiome drives inter-vivarium differences, we established in-house breeding colonies of TAC- and JAX-origin mice at MSU. This allowed us to assess whether, when housed under identical conditions, these mice still show differences in mortality based on their original microbiomes. To characterize the arsenic-induced sepsis-like disease, we performed blood biochemistry assays to quantify the white blood cell populations, and sepsis biomarkers used in clinical settings. **RESULTS/ANTICIPATED RESULTS:** We observed differences in survival rates between genetically identical mice from MSU (45%), TAC (30%), and JAX (2.5%) in our model. From this, we characterized the baseline composition of the gut microbiomes of these mice and found they were significantly different from each other. We are still awaiting results from our in-house TAC and JAX experiments but expect them to have similar gut microbiome compositions to those directly purchased from TAC and JAX and respond similarly. In our blood biochemistry analysis, we found sick mice presented with low WBC counts and notable biomarkers indicative of liver, heart, and kidney distress. We also anticipate that 16S sequencing results of cecal contents will further support findings by providing evidence of a bacterial infection in the ceca of sick mice. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Collectively, our work demonstrates that antibiotic perturbation of the gut microbiome induces an inter-individual and inter-vivarium susceptibility to an arsenic-induced sepsis-like disease state. This work highlights the importance of considering antibiotic use in the risk assessment of arsenic to better protect the health of those exposed.