95233

Analysis of 5'UTR Variation in Rare Disease Patients Reveals Variants of Potential Disease Relevance

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ABSTRACT IMPACT: This work sheds diagnostic insight on patients with idiopathic rare disease and has the potential to further their care and treatment as a result. OBJECTIVES/GOALS: Correct diagnosis is imperative to treating patients with idiopathic, suspected genetic conditions, yet sequencing approaches leave up to 70% of these patients undiagnosed. We sought to improve diagnosis rates for a cohort patients referred for sequencing by characterizing deleterious variants within the 5'UTR. METHODS/STUDY POPULATION: We retrospectively analyzed whole exome sequencing (WES) data from 472 unsolved rare disease patients within the Mayo Clinic Center for Individualized Medicine to identify variants within the 5'UTR that affect the presence of upstream open reading frames (uORFs). uORFs are short regions (typically 30bp - 600bp) that typically influence downstream gene translation by sequestering ribosomes. We specifically searched for variants with the potential to disrupt existing uORFs or introduce new uORFs within the 5'UTR, and developed a pipeline to annotate these variants with information including GnomAD allele frequency and gene loss of function intolerance (pLI) score. To aid in variant interpretation, we applied two deep learning tools to predict variant impacts on transcript ribosome load (TITER and FramePool). RESULTS/ANTICIPATED RESULTS: Our pipeline identified a median of 21 variants per patient that were predicted to have a deleterious impact on the translational efficiency of protein coding transcripts, primarily by introducing new start codons within the 5'UTR or by altering the Kozak consensus of existing start codons. A median of 10 of these variants occur upstream of haploinsufficient genes with an existing disease association. We also identified a subset of variants that are predicted to introduce translationally active N-terminal extensions to protein coding transcripts, with the potential to disrupt protein localization and processing. DISCUSSION/SIGNIFICANCE OF FINDINGS: This work demonstrates that analysis of 5'UTR variants can be incorporated into existing WES pipelines, and identifies a group of variants with potential significance to patient disease. Further experimental evidence is necessary to ascertain the pathogenicity of these variants.

Clinical Trial

17230

Agreement between point-of-care intestinal ultrasound (POCUS) and magnetic resonance enterography for assessment of the terminal ileum through sigmoid colon in pediatric patients with inflammatory bowel diseases: A diagnostic cross-sectional study

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ABSTRACT IMPACT: Preliminary results will inform the formal evaluation of the reliability of point-of-care ultrasound (POCUS) done by the gastroenterologist compared to standard of care methods such as MR-Enterography. OBJECTIVES/GOALS: Evaluation of mucosal healing is standard for pediatric patients with inflammatory bowel disease (IBD). Point-of-care ultrasound is a non-invasive, cost-efficient tool for assessing intestinal inflammation. We aim to evaluate the agreement between POCUS and typical cross-sectional imaging, such as MR-Enterography (MRE). METHODS/STUDY POPULATION: In this cross-sectional study, we recruited consecutive patients newly diagnosed with IBD, presenting to the specialty outpatient clinic or hospitalized in a pediatric tertiary care center between August to November 2020. They underwent POCUS performed by a single gastroenterologist, in addition to MRE. The sonographer was blinded to MRE results. Bowel wall thickness (BWT) was measured across different bowel segments and recorded twice in longitudinal view and twice in axial view. An average segmental BWT of the four measurements of more than 3 mm was considered inflamed. Agreement between sections of the bowel measured as inflamed were compared to inflamed bowel segments seen by MRE, using Cohen's kappa. RESULTS/ANTICIPATED RESULTS: Eight of 12 patients completed both MRE and POCUS.A total of 40 bowel segments were assessed, namely the terminal ileum, ascending, transverse, descending and sigmoid colon. There were 4 girls with a median age of 15 years (IQR 14.25-16 years), and 6 patients were diagnosed with Crohn's disease. Median PCDAI was 32.5 (IQR 30.6-40), and median PUCAI was 75 (72.5-77.5). Agreement between MRE and point-of-care ultrasound was substantial to perfect for the terminal ileum ******(Îº= 0.75, 95%CI 0.31-1), transverse colon (Îº= 1, 95%CI 1-1) and sigmoid colon (Îº= 1, 95%CI 1-1). The agreement was poor for the ascending (Îº= 0, 95%CI 0-0) and moderate for the descending colon. (Îº= 0.6, 95%CI -0.07-1) DISCUSSION/SIGNIFICANCE OF FINDINGS: In pediatric patients with IBD, we found a high agreement between POCUS and MRE for imaging of the terminal ileum, transverse and sigmoid colon, areas commonly involved in IBD. This reinforces adult data, outlining the potential of POCUS as an evaluation tool of disease activity in clinical practice.

Data Science/Biostatistics/Informatics

12621

Targeted Chemical-Genetic Screen Platform for Identifying Drug Modes-of-Action

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ABSTRACT IMPACT: The key to advancing precision medicine is to deepen our understanding of drug modes-of-action (MOA). This project aims to develop a novel method for predicting MOA of potential drug compounds, providing an experimental and computational platform for more efficient drug discovery. OBJECTIVES/GOALS: To develop (1) a targeted CRISPR-Cas9 chemical-genetic screen approach, and (2) a computational method to predict drug mode-of-action from chemical-genetic interaction profiles. METHODS/STUDY POPULATION: Screening drugs against a gene deletion library can identify knockouts that modulate drug

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sensitivity. These chemical-genetic interaction (CGI) screens can be performed in human cell lines using a pooled lentiviral CRISPR-Cas9 approach to assess drug sensitivity/resistance of single-gene knockouts across the human genome. A targeted, rather than genome-wide, library can enable scaling these screens across many drugs. CGI profiles can be derived from phenotypic screen readouts. These profiles are analogous to genetic interaction (GI) profiles, which represent sensitivity/resistance of gene knockouts to a second gene knockout rather than a drug. To computationally predict a drug's genetic target, we leverage the property that a drug's CGI profile will be similar to its target's GI profile. RESULTS/ANTICIPATED RESULTS: Five proof-of-principle screens will be conducted with compounds that have existing genome-wide profiles and well-characterized MOA. I will generate CGI profiles for these five compounds and identify genes that are drug-sensitizers or drug-suppressors. I will then evaluate whether targeted library screens can recapitulate the CGIs found in genome-wide screens. Finally, I will develop a computational tool to integrate these CGI profiles with GI profiles (derived from another project) to predict gene-level and bioprocess-level drug targets. These predictions (from both targeted and genome-wide profiles) will be benchmarked against a drug-target and drug-bioprocess standard. DISCUSSION/SIGNIFICANCE OF FINDINGS: This work will develop a scalable, targeted chemical-genetic screen approach to discovering how putative therapeutics work. The targeted screen workflow provides a method for higher-throughput drug screening. The computational pipeline provides a powerful tool for exploring the MOA of uncharacterized drugs or repurposing FDA-approved drugs.

38081

A Whole Blood Signature of Neutrophil Expression and Adaptive Immune Downregulation Characterizes Sepsis Mortality

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ABSTRACT IMPACT: This work identifies host immune perturbations in sepsis mortality that suggests targets for a precision medicine paradigm in the host response to infection. OBJECTIVES/GOALS: To compare the early whole blood transcriptome during sepsis between 30-day survivors and non-survivors in the Intensive Care Unit (ICU), and to evaluate for pathway enrichment that might explain sepsis lethality. METHODS/STUDY POPULATION: We enrolled 162 sepsis patients in the first 24 hours of ICU admission, particularly targeting individuals requiring vasopressors. Peripheral whole blood was collected in PAXgene vacutainers. Isolated RNA was analyzed with Affymetrix Human Genome ST 2.0 microarray. Differential gene expression was performed with Bioconductor/R/limma using log2 fold-change +/-0.6 as a threshold for differential expression, and a Benjamini-Hochberg adjusted p-value <0.05 to declare significance. Functional gene enrichment was performed using the Gene Ontology (GO) database with PANTHER overrepresentation test (Fisher's Exact) on all transcripts with adjusted p-value <0.05. Pathways analysis was performed with the Reactome Project using the raw fold change and significance data to identify dysregulated pathways. RESULTS/ANTICIPATED RESULTS: There were 58 non-survivors (36% mortality). We identified 39 genes as differentially expressed between sepsis non-survivors and survivors; 31 were upregulated in non-survivors and 8 had reduced expression. Several of the most overexpressed transcripts are neutrophil-specific, including LCN, MPO, OLF4M4, DEFA3, and DEFA4. A functional gene overrepresentation test further supports this finding, as the most enriched gene ontologies were neutrophil-mediated killing, neutrophil cytotoxicity, neutrophil extravasation, and respiratory burst, all demonstrating higher than 10-fold enrichment and FDR < 0.02. Pathway analysis of the peripheral blood transcriptome was notable for immune response derangement, specifically down-regulation of both innate and adaptive immune pathways (FDR < 0.00001). DISCUSSION/SIGNIFICANCE OF FINDINGS: We identified increased expression of neutrophil-related genes in sepsis non-survivors, replicating candidates previously identified in pediatric sepsis mortality and ARDS. These immune perturbations in sepsis mortality may represent key targets for eventually employing precision medicine strategies in sepsis.

54443

Pharmacogenomic Profiling of East and West African Populations

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ABSTRACT IMPACT: Genomic variation likely plays a role in differences in rates of adverse reactions and efficacy for African populations, and this research will add to the understudied field of pharmacogenomics in African populations. OBJECTIVES/GOALS: We aim to characterize the frequency of variants in clinically relevant genes in East and West African populations to assess the prevalence of potential drug-gene interactions. METHODS/STUDY POPULATION: Our pilot study will consist of 100 Somali patients enrolled at Mayo Clinic Rochester and 100 Ghanaian patients recruited at Teaching Hospitals in Ghana. Germline DNA will be extracted from pre-existing blood samples. Sequencing will be performed using Admera Health's PGxOne Plus test, interrogating a panel of 62 genes. Variants will be reported along with the predicted response for a list of drugs. Differences between frequencies of variants in East and West African populations will be analyzed. We will look for correlations with reported adverse reaction rates. We will then compare our findings with allele frequency data from publicly available data bases. Additionally, we will analyze the flanking regions of the panel for novel variants, using machine learning to predict gene-drug interactions. RESULTS/ANTICIPATED RESULTS: African populations are known to have more genetic diversity than any other population. Additionally, only African-Americans, African-Caribbeans from Barbados, Esan and Yoruba Nigerians, Gambians, Kenyans, and Sierra Leoneans are accounted for within the publicly available data bases most often used for variant studies. Thus, it is anticipated that we will find differences in the variant allele frequencies of our populations compared to European allele frequencies, and differences in frequencies between the East and West African populations. In the 200 base pair flanking regions that are sequenced in the assay along with the known variant regions, we may find novel previously unreported variants. DISCUSSION/

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