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Injured proximal tubule cells are increased in kidney transplant rejection and are spatially located in distinct niches containing mixed immune cells†

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OBJECTIVES/GOALS: Kidney transplant rejection is a leading cause of graft failure. Diagnosis is dependent on allograft biopsy that 1: focuses on immune infiltrate and 2: does not inform treatment or recovery potential. Using single-cell transcriptomic technologies, we assess rejection's impact on kidney cells and begin to define outcome-affecting interactions. METHODS/STUDY POPULATION: Single-cell RNA sequencing was performed on 25 kidney biopsies from 15 separate patients who had undergone acute cellular rejection. Cell typing was performed using canonical genes, and differential gene expression profiling was performed in R with Seurat. The proportion of cell types present at different time points and grades of rejection was determined. We focused first on renal parenchymal cell types and their gene expression changes with rejection grade and treatment of rejection. The spatial localization of these cell states was determined with spatial transcriptomics using the Xenium Prime 5K pan-human tissue panel on 11 kidney biopsy sections. After annotation, spatial-informed niches were identified to investigate cellular changes associated with injury. RESULTS/ANTICIPATED RESULTS: From the single-cell data, we were able to identify several renal parenchymal cell states. Focusing on the proximal tubule (PT) cells as the most numerous cell type in the renal cortex, we identified healthy, injured, and damaged cell states based on expression of tubular injury markers, genes associated with epithelial to mesenchymal transition, and expression of interferon-response genes. The proportion of PT cells in an injured state increased with rejection severity and decreased with rejection treatment. Utilizing the spatial transcriptomic data, we were able to identify both healthy and injured PT states and define their localization with immune cell types and histologic injury patterns. We found varied transcriptomic niches and that niches with injured PT cells were enriched with immune cells. DISCUSSION/SIGNIFICANCE OF IMPACT: PT cells exhibit patterns of injury in response to rejection and when injured cells exist in spatial niches containing immune cells that are contributing or responding to injury. Understanding these interactions will further define the injury process caused by rejection and guide development of personalized rejection treatment.

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Salivary DNA methylation signatures as potential biomarkers for chronic painful temporomandibular disorders: A pilot study

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OBJECTIVES/GOALS: To evaluate the feasibility of developing salivary DNA methylation-based biomarkers for objective diagnosis and stratification of Temporomandibular Disorders (TMD), addressing the critical need for quantitative diagnostic tools to complement current subjective assessments and clinical examinations. METHODS/STUDY POPULATION: This cross-sectional pilot study enrolled 13 TMD patients and 4 pain-free controls from the University of Minnesota TMD Orofacial Pain Clinic. Participants underwent comprehensive DC/TMD diagnostic evaluation and completed validated instruments measuring pain intensity (VAS), psychological status (PHQ-9, PCS), and functional impairment (GCPS). Saliva samples were analyzed using Illumina EPIC BeadChip arrays interrogating ~850,000 CpG sites. Bioinformatic analyses included principal component analysis, identification of differentially methylated sites, and development of composite Methylation Profile Scores for patient stratification RESULTS/ANTICIPATED RESULTS: Principal component analysis showed clear clustering separation between TMD patients and controls. Methylation profile scores demonstrated clear stratification between controls, high-impact, and low-impact TMD subgroups. High-impact TMD participants (n=6) showed significantly elevated clinical severity scores compared to low-impact TMD (n=7): pain intensity (52.0±11.3 vs 25.0±13.8), depression (20.5±7.3 vs 8.4±4.9), and catastrophizing (12.6±8.1 vs 6.4±6.5). Promoter-level analysis identified candidate genes associated with TMD severity phenotypes, supporting the potential for epigenetic biomarkers to enable objective patient stratification and guide precision treatment selection. DISCUSSION/SIGNIFICANCE OF IMPACT: Epigenetic biomarkers capturing TMD heterogeneity could transform clinical practice by enabling early-risk stratification, preventing progression to high-impact chronic pain through personalized interventions, and reducing the substantial healthcare burden of this prevalent orofacial pain condition.

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Safety, feasibility, child, and parent perceptions following biofeedback and breathing practices in children with attention deficit hyperactivity disorder: The healing minds study

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OBJECTIVES/GOALS: Attention deficit hyperactivity disorder (ADHD) affects 5.9% of youth worldwide and is associated with mental health disorders. In adults, HeartMath biofeedback improves ADHD, emotional regulation, and anxiety. Breathing practices promote similar outcomes. Our goal was to examine the synergistic effect of these therapies in children with ADHD. METHODS/STUDY POPULATION: A 10-week, 2-arm randomized controlled clinical trial was conducted in a rural health care clinic to examine

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