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OBJECTIVES/GOALS: To identify population-specific and ancestry-informed genetic loci for blood pressure traits, characterize X chromosome associations, and evaluate evidence for evolutionary pressures influencing blood pressure risk across ancestries, which may be used downstream clinically for personalized medicine and drug target identification. METHODS/STUDY POPULATION: We conducted multi-ancestry and ancestry-stratified genome-wide association studies (GWAS) of systolic blood pressure (SBP), diastolic blood pressure (DBP), and pulse pressure (PP) across >2.5M individuals (40% non-European) from 16 cohorts and biobanks. Analyses were adjusted for age, age^2, sex, body mass index, and the first 10 principal components. Fixed-effects meta-analyses were followed by SuSiEx cross-population fine-mapping to identify ancestry-specific loci. We evaluated genetic differentiation using the fixation index (FST) and allele frequency directionality using the sign test to infer potential evolutionary pressures. RESULTS/ANTICIPATED RESULTS: Multi-ancestry meta-analyses identified 2,406 significant independent loci for SBP (1,594 novel), 2,358 DBP (1,509 novel), and 2,312 PP (1,432 novel). Chromosome X analyses revealed 8 SBP, 80 DBP, and 8 PP loci. SuSiEx fine-mapping identified ancestry-specific signals, including a pulse pressure SNP near CTSL (Asian-specific) and a DBP SNP in CACNA1D (African-specific). Sign tests indicated enrichment of DBP risk alleles when comparing African and Asian populations and when comparing Admixed American and Asian populations ($p < 0.01$), supported by elevated FST values for variants with larger effect sizes. These findings suggest that ancestry-specific selection may influence blood pressure regulation. DISCUSSION/SIGNIFICANCE OF IMPACT: This is the largest and most ancestrally diverse blood pressure genetics study to date and one of few to include X chromosome analyses, which enabled discovery of thousands of novel and population-specific loci and revealed evolutionary and biological insights that may advance precision medicine for hypertension.

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Improving T-cell immunity using interleukin-7 therapy

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OBJECTIVES/GOALS: Our goal is to improve health outcomes for sepsis survivors experiencing immune-paralysis. We propose a functional diagnostic tool to identify at-risk patients and a novel therapeutic called interleukin-7 to boost the patient's T-cell-mediated immunity against infection. METHODS/STUDY POPULATION: This multi-center research study recruits healthy adult volunteers and adult septic patients in the intensive care unit to consent to giving blood draws. Blood draws containing immune cells are assessed for functionality through our diagnostic tool. Interleukin-7, a therapeutic candidate to improve immune cell activity, is assessed by flow cytometry, ELISA, and bulk RNA sequencing. RESULTS/ANTICIPATED RESULTS: Using IL-7 during T-cell stimulation in the ELISpot assay increased the number of T-cells producing protective effector protein IFNgamma. Statistical analysis was performed using Brown-Forsythe ANOVA test with Dunnett's T3 multiple comparisons test with a P-value<0.001. Flow cytometry confirms IL-7 treatment increased the frequency of T-cells to produce IFNgamma and preferentially impacts memory T-cells in sepsis patients. Bulk mRNA sequencing demonstrated increased differentially expressed mRNA transcripts FDR<.001 related to cell survival

https://doi.org/10.1017/cts.2026.10562 Published online by Cambridge University Press

and T-cell-mediated effector molecules with IL-7-treated cells. IL-7 improved effector protein production was confirmed by a multiplex assay. DISCUSSION/SIGNIFICANCE OF IMPACT: There are no diagnostic tools to identify immunocompromised patients or therapies to reverse the immune suppression. Our ELISpot diagnostic tool identifies at-risk immune-impaired patients. Furthermore, our results demonstrate interleukin-7 as a potential therapeutic to improve immune activity.

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Metallothionein 2A loss shifts mitochondrial function and metabolism in high-grade serous ovarian cancer*

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OBJECTIVES/GOALS: Metallothioneins (MT) are cysteine-rich proteins that bind metals and regulate oxidative stress. MT2A, the most expressed MT gene, is lost in ~70% of High Grade Serous Ovarian Cancer cases. We hypothesize that MT2A loss disrupts mitochondrial function and metabolism, promoting tumor progression. METHODS/STUDY POPULATION: To explore this, MT2A was knocked down (KD) in CAOV3 HGSOC cells via lentiviral shRNA. KD was confirmed transcriptionally via RT-qPCR and functionally using a nuclei-counting assay, which assesses sensitivity to cadmium chloride ($CdCl_2$), a cytotoxic heavy metal. Mitochondrial membrane potential (MMP) was assessed via flow cytometry, and oxygen consumption rate (OCR) was measured with Resipher. Clonogenic potential was evaluated by soft-agar colony formation assays. RESULTS/ANTICIPATED RESULTS: MT2A KD cells showed a significant decrease in nuclei count in response to $CdCl_2$. MMP was reduced in MT2A KD cells, indicating compromised mitochondrial integrity. OCR was significantly decreased in MT2A KD cells, suggesting impaired oxidative phosphorylation. These phenotypes are characteristic features of the Warburg effect, which is a hallmark of aggressive cancers. Soft agar assays revealed that MT2A loss significantly enhanced the cell's ability to multiply adhesion free from a single cell, a phenotype associated with aggressive oncogenic transformation. DISCUSSION/SIGNIFICANCE OF IMPACT: In summary, MT2A loss disrupts mitochondrial dynamics and oxidative metabolism while promoting tumor aggressiveness. These findings support our hypothesis that MT2A plays a key role in regulating mitochondrial homeostasis and may influence metabolic adaptation in HGSOC.

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Molecular dynamics driving phenotypic divergence among KRAS mutants in pancreatic tumorigenesis

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OBJECTIVES/GOALS: To investigate how distinct KRAS mutations (G12D, G12V, and G12R) drive distinct molecular and phenotypic programs in pancreatic tumorigenesis, linking early lineage and signaling differences to clinical patterns such as stage at diagnosis, nodal status, and survival to ultimately inform allele-specific therapies. METHODS/STUDY POPULATION: We analyzed clinical cohorts (MSK-IMPACT n=1,360; COMPASS n=100) and conducted lineage-tracing studies in mice carrying KRAS-G12D, KRAS-G12V, or KRAS-G12R alleles under inflammatory, genetic, and pharmacologic perturbations. Spatial transcriptomic and proteogenomic profiling of resected PDAC tissues characterized EMT and immune signaling states. Functional studies assessed EGFR–PI3K/AKT–RAC1/VAV1 signaling, including RAC1 inhibition and constitutive AKT activation, to reveal allele-specific susceptibilities. RESULTS/ANTICIPATED RESULTS: KRAS-G12D drove robust lineage plasticity, enhancer remodeling, and early neoplastic progression. KRAS-G12R/V showed limited EMT and increased inflammatory signatures, with impaired EGFR–RAC1/VAV1 activation restricting lineage reversion. Constitutive AKT activation rescued the tumor-initiating defect of KRAS-G12R *in vivo*, pinpointing a downstream signaling bottleneck, which could be exploited therapeutically. Spatial and transcriptomic profiling of resected human PDAC revealed that KRAS-G12R tumors were more often early-stage, node-negative, and linked to improved survival relative to KRAS-G12D. DISCUSSION/SIGNIFICANCE OF IMPACT: Integrating patient and mouse data reveals a mechanistic hierarchy among KRAS alleles that governs lineage fate, signaling reliance, and clinical course, pointing to opportunities for allele-tailored therapeutic strategies in pancreatic cancer.

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Engineering a precision organoid model to uncover matrix-driven tumorigenesis

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OBJECTIVES/GOALS: Develop a precision hydrogel-organoid model to determine how extracellular matrix (ECM) biomechanics drive the malignant transformation of oral epithelial dysplasia (OED) to oral squamous cell carcinoma (OSCC) and identify mechanoresponsive pathways. METHODS/STUDY POPULATION: Patient-derived oral epithelial dysplasia (OED) and oral squamous

https://doi.org/10.1017/cts.2026.10562 Published online by Cambridge University Press