Urine Proteomics Reveals Ancestry-Driven Heterogeneity in Patients with Localized Prostate Cancer

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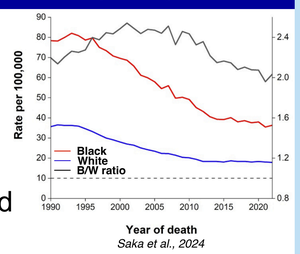
Prostate cancer clinical disparity

- **Men of African Ancestry** are disproportionately affected in prostate cancer (PCa).
- Incidence: 1.67 times more.
- Mortality: 2.05 times more.
- Clinical disparity is confounded by socio-economic factors.
- Underlying biological differences showed contribution to disease heterogeneity.
- We aim to **identify the influences of ancestry on the urinary proteome of patients with localized PCa**.



Urine proteomics prostate cancer cohort

Our group has collected **post-DRE** (digital rectal exam) **urine** samples from **329 patients** with localized PCa. The diverse cohort contains self-identified Black (N =194) and White (N = 135) patients that span the **entire risk spectrum**.

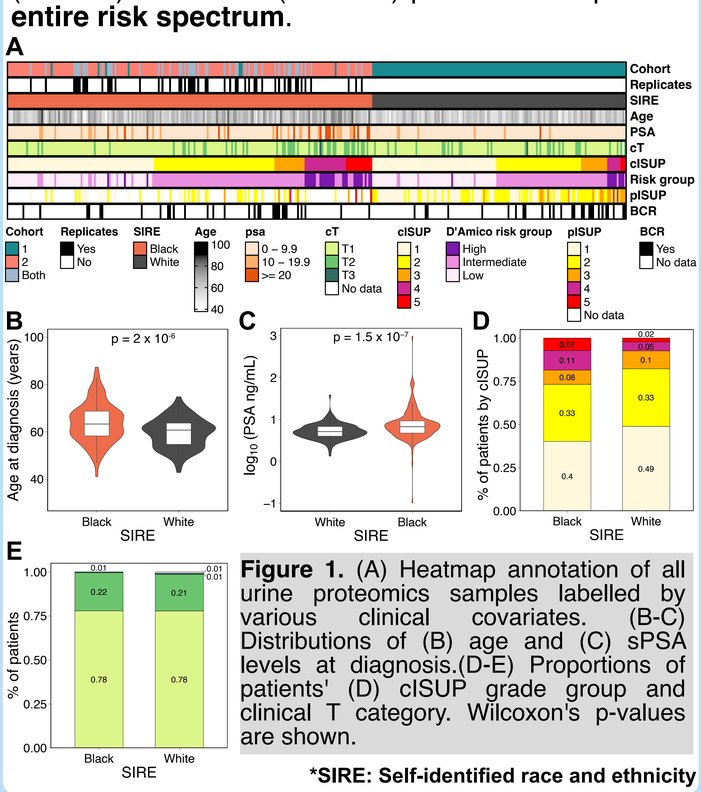
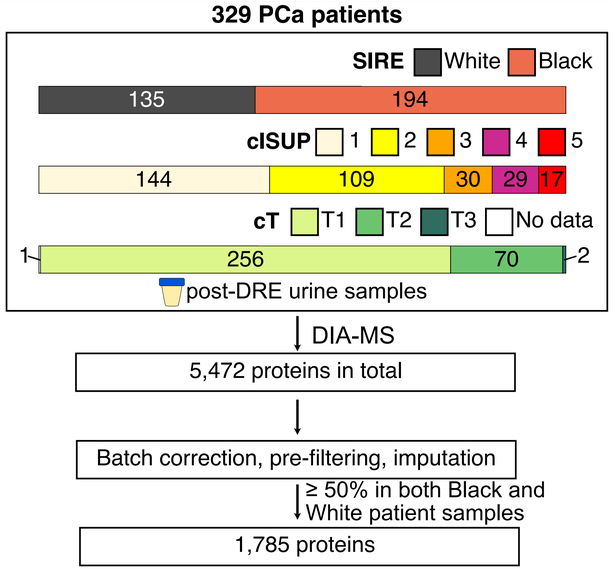


Figure 1. (A) Heatmap annotation of all urine proteomics samples labelled by various clinical covariates. (B-C) Distributions of (B) age and (C) sPSA levels at diagnosis.(D-E) Proportions of patients' (D) cISUP grade group and clinical T category. Wilcoxon's p-values are shown.

***SIRE: Self-identified race and ethnicity**

Urine proteomics workflow



All post-DRE urine samples were processed using the MStern Blotting protocol (Berger *et al.*, 2015) followed by LC-MS/MS analysis using DIA-MS. Following a stringent cutoff of protein detection rate of at least 50% in both self-identified Black and White patients, 1,785 proteins were used for analyses.

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The authors declare no competing financial interests.

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Ancestry-driven heterogeneity in the urinary proteome

Adjusting for other clinical variables (e.g. age, cISUP, sPSA), we observed that **110 proteins** are significantly associated with SIRE, with top-ranking proteins showing **independent SIRE-driven effects across tumour grade group** (cISUP GG). Notably, immune-related proteins are elevated in Black patients, while prostate-derived proteins (e.g. MSMB, TGM4) are elevated in White patients.

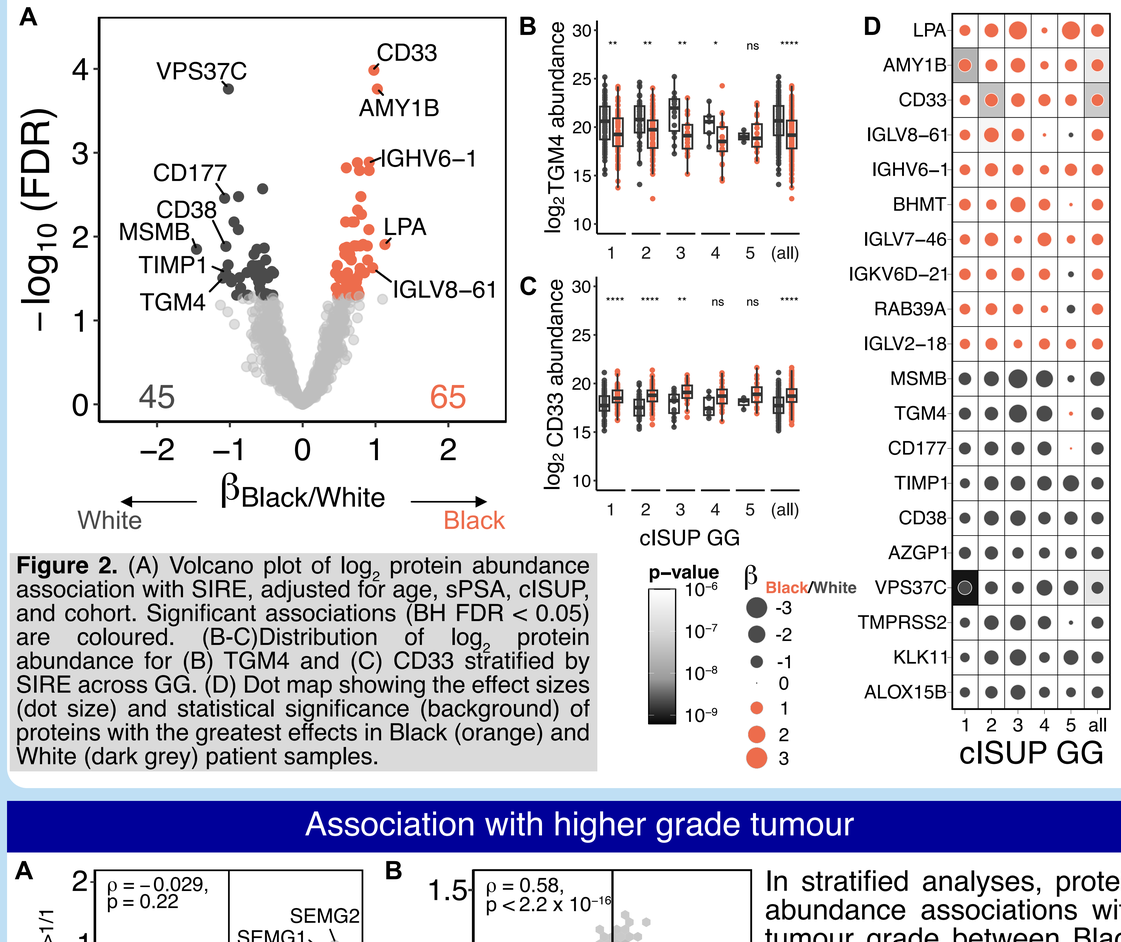
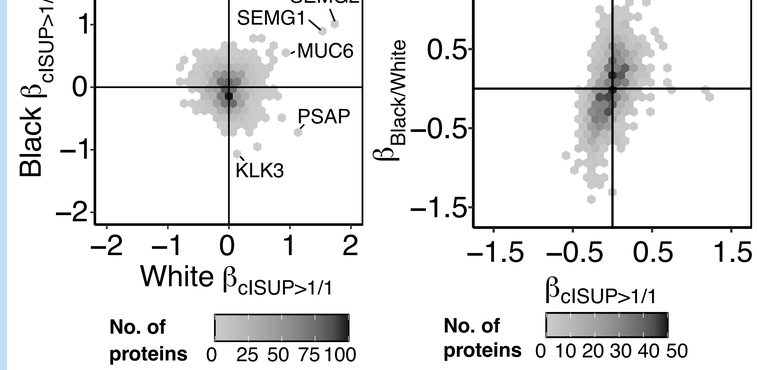


Figure 2. (A) Volcano plot of $\log_2$ protein abundance association with SIRE, adjusted for age, sPSA, cISUP, and cohort. Significant associations (BH FDR < 0.05) are coloured. (B-C)Distribution of $\log_2$ protein abundance for (B) TGM4 and (C) CD33 stratified by SIRE across GG. (D) Dot map showing the effect sizes (dot size) and statistical significance (background) of proteins with the greatest effects in Black (orange) and White (dark grey) patient samples.

Association with higher grade tumour



In stratified analyses, protein abundance associations with tumour grade between Black and White patients were not correlated. We also found that proteins elevated in higher grade tumours are correlated with those elevated in Black patients, suggesting that the **urinary proteome of Black patients may resemble that of clinically significant tumours**.

Figure 3. (A) Spearman's correlations of $\log_2$ protein abundance associations with (A) disease stage (cISUP >1 vs 1) between Black and White patients from stratified multivariable analyses, (B) SIRE and disease stage not stratified.

Verification of immune responses in TCGA-PRAD transcriptomics

Using genetically inferred ancestry transcriptomics data from TCGA-PRAD, **ancestry also had a major effect** in the tumours of localized PCa patients. Comparing between the 2 datasets, **immune dysregulation and depleted androgen response** are consistently detected.

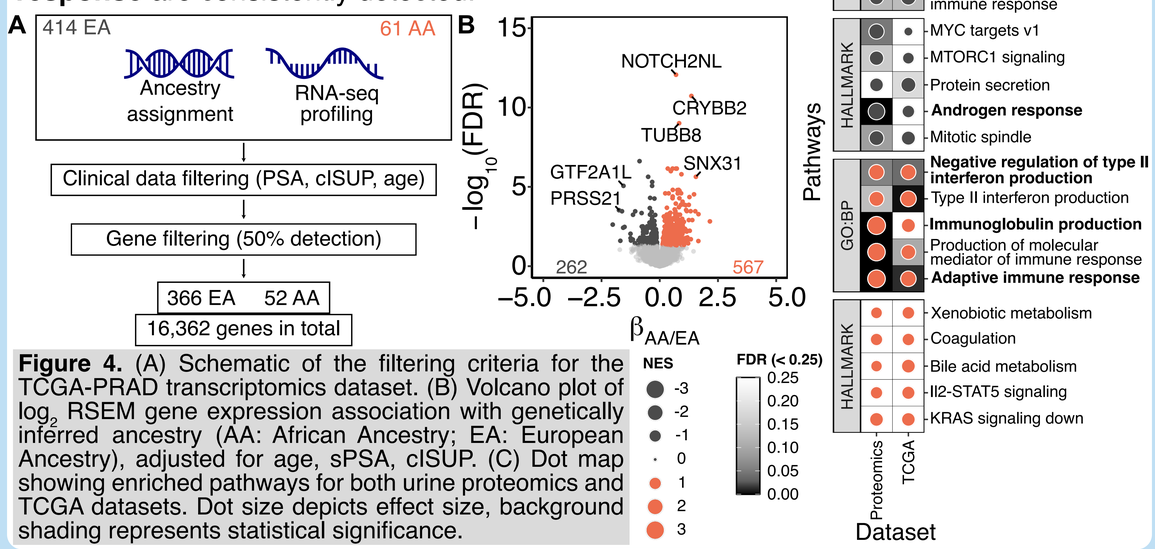


Figure 4. (A) Schematic of the filtering criteria for the TCGA-PRAD transcriptomics dataset. (B) Volcano plot of $\log_2$ RSEM gene expression association with genetically inferred ancestry (AA: African Ancestry; EA: European Ancestry), adjusted for age, sPSA, cISUP. (C) Dot map showing enriched pathways for both urine proteomics and TCGA datasets. Dot size depicts effect size, background shading represents statistical significance.

Subset cohort for prognosis analysis

To evaluate **whether protein prognosis for biochemical recurrence (BCR) varies by SIRE**, we assessed **protein interaction effects** with BCR-free survival. For this analysis, we subset our analysis to patients belonged to the **intermediate risk group** that represented a clinically balanced population. **Overall BCR-free survival was not associated with clinical covariates** (e.g. age, SIRE, processing cohort) in this subset.

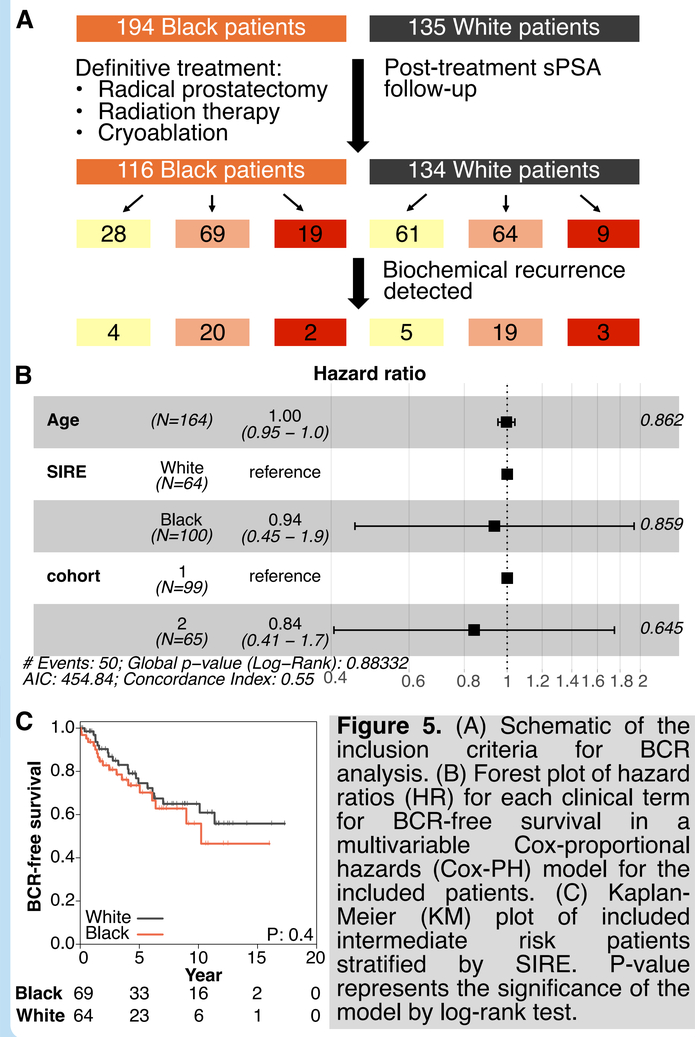
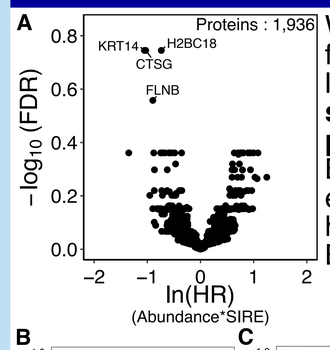


Figure 5. (A) Schematic of the inclusion criteria for BCR analysis. (B) Forest plot of hazard ratios (HR) for each clinical term for BCR-free survival in a multivariable Cox-proportional hazards (Cox-PH) model for the included patients. (C) Kaplan-Meier (KM) plot of included intermediate risk patients stratified by SIRE. P-value represents the significance of the model by log-rank test.

Urinary protein prognosis differs by SIRE



While statistical significance for interaction effect was limited, **several proteins showed differences in the prognostic trends** between Black and White patients. For example, CTSG abundance had varying effects between Black and White patients.

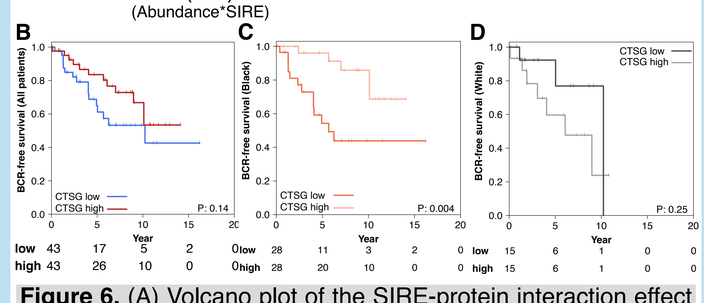


Figure 6. (A) Volcano plot of the SIRE-protein interaction effect associated with BCR. (B-D) KM plot stratified by urinary CTSG abundance in (B) all patients, (C) within Black patients, (D) within White patients. P-values represent the significance of the model by log-rank test.

Conclusions

- First study that acquired a large ethnicity-driven prostate cancer urine proteomics cohort.
- Ancestry-driven differences revealed immune dysregulation.
- Identifying ancestry-adjusted biomarkers hence may benefit personalized clinical-decision making.