Frame-Specific Depletion of the TRBV23-1 Pseudogene in Human TCR Repertoires

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Introduction

- V(D)J recombination generates TCR diversity, but most rearrangements are non-productive
- Non-productive V(D)J rearrangements are observed in sequencing when a successful rearrangement on the homologous allele rescues cell survival
- Non-productive and pseudogene rearrangements are usually considered biologically inert
- Emerging evidence suggests pseudogenes may influence immune regulation
- **Objective:** Test whether TCR pseudogene rearrangements show non-random, frame-specific patterns across deep sequenced repertoires in large human cohorts

Methodology

Datasets:

- 6,000 DNA-based TCRβ repertoires from 9 cohorts
- Independent RNA-seq cohort for validation
- 1,986,248,402 sequences examined in total.

Frame classification:

- F0 (in-frame), F1 and F2 (out-of-frame)
- F0 subdivided into PTC-free (F0NT) and PTC-containing (F0T)

Statistical analysis:

- Quantitative model for expected in-frame/out-of-frame ratios
- Multinomial null model with chi-squared testing to identify outliers

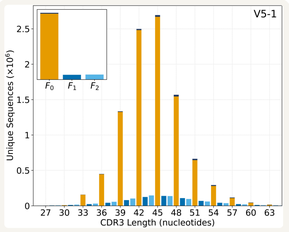
Validation:

- RNA-seq analysis of frame-specific transcript abundance

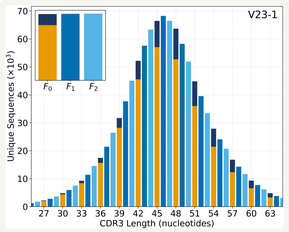
Preprint & Website

Results

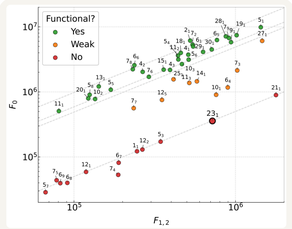
Frame usage distinguishes functional V genes from pseudogenes



Functional V genes show in-frame enrichment

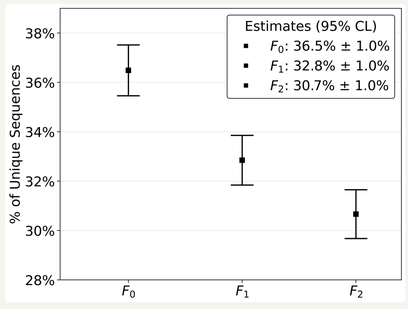


Pseudogenes show no frame dependence



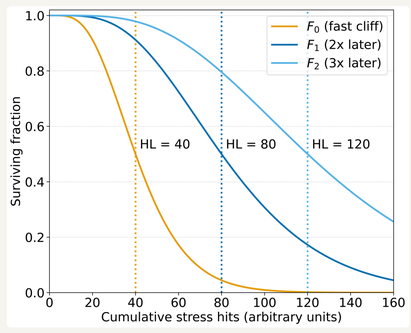
In/Out ratios separate genes into categories

RNA-seq results



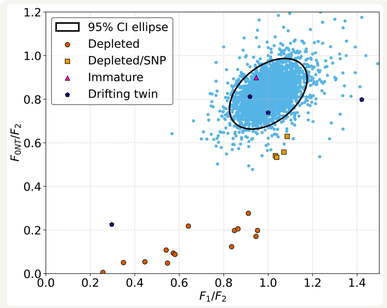
F0 > F1 > F2 expression hierarchy in an independent RNA-seq cohort

Cumulative damage model

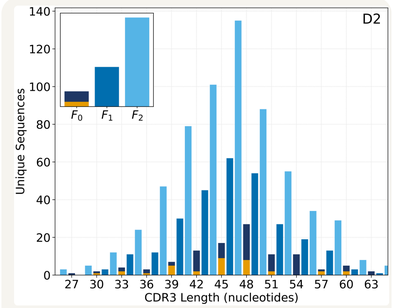


Frame-dependent attrition can generate sawtooth depletion

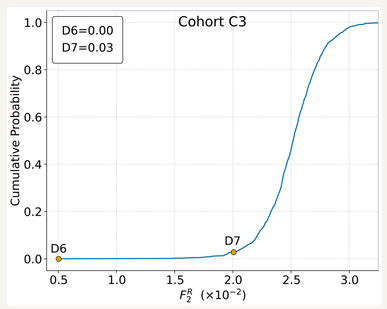
Rare pseudogene-specific frame-ordered CDR3 deviations



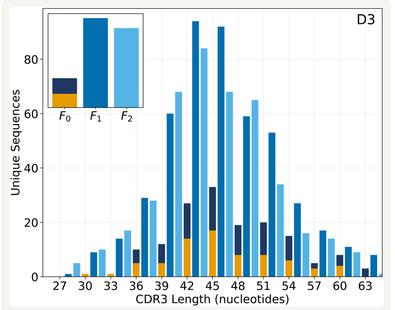
Four distinct outlier classes identified



Modulo-3 depletion sawtooth in TRBV23-1 outlier



Cohort benchmarking confirms TRBV23-1 depletion



Outlier with similar behaviour in TRBV5-3

Conclusions

- TRBV23-1 exhibits robust, frame-dependent depletion in human TCR repertoires
- Patterns are inconsistent with technical artifacts, recombination bias, or rare alleles
- DNA–RNA concordance implicates post-transcriptional or immune-mediated mechanisms
- Findings challenge the assumption that TCR pseudogenes are biologically inert
- Results motivate experimental testing of pseudogene-derived peptide presentation and immune regulation

Acknowledgments

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- RNA-seq data from Mikelov et al.
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- Tigg J, Bektashi-Brown A. Frame-specific depletion of the TRBV23-1 pseudogene in human TCR repertoires. bioRxiv, 2025