Poster 122

Long-read cfDNA sequencing detects hypofragmentation phenotype with distinct motifs

J. van den Berg, D.C.L. Vessies, E. Post, K. Roohollahi, D.J.S. Makarawung, F. van Asch
RenovaroCube, Amsterdam, The Netherlands

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Introduction

- Cell-free circulating tumour DNA (ctDNA) is a minimally invasive biomarker
- cfDNA fragmentomics is an emerging research field

- Short fragments (<400bp) have been heavily investigated
- Longer fragments "invisible" with Illumina sequencing
- Hypofragmented phenotype was reported earlier using long-read sequencing [1-3]

Methods and cohort

- PCR-free Oxford Nanopore Technologies (ONT) sequencing
- Count reads in exponentially increasing bin sizes up to 10 kbp
- Cohort:
  - ➢ 25 healthy
  - ➢ 25 lung cancer patients
    - 11 stage I
    - 5 stage II
    - 6 stage III
    - 3 stage IV

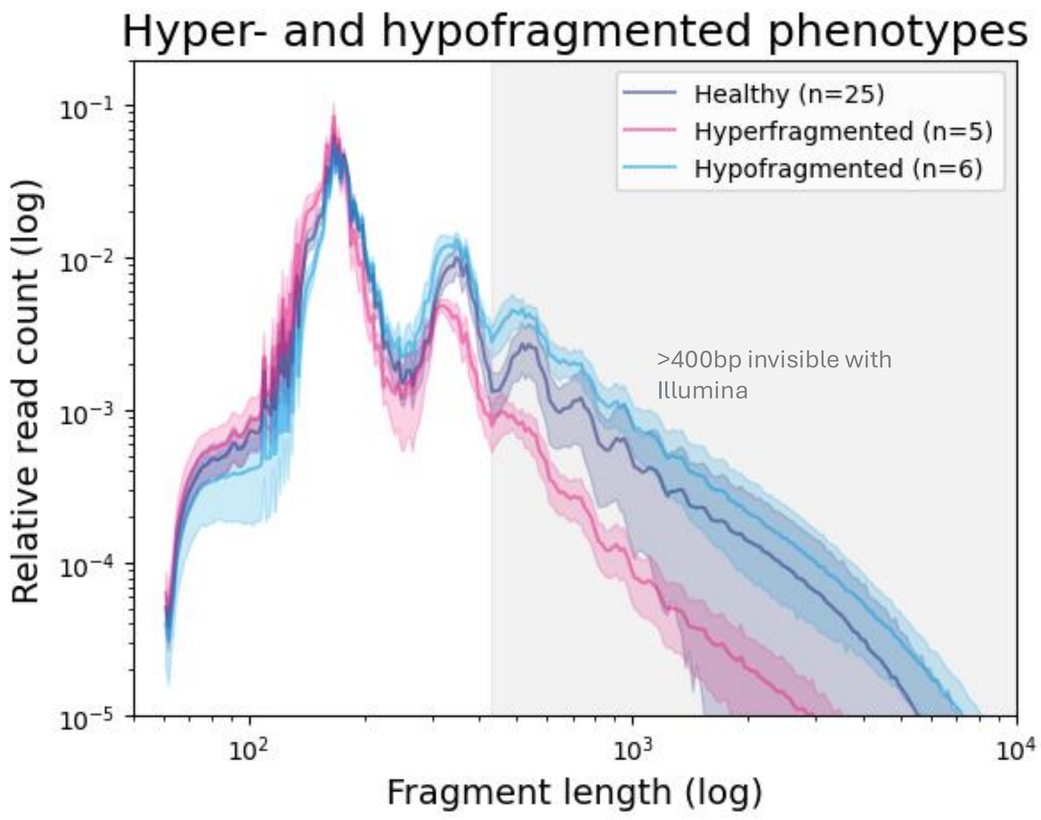

Figure 1. *Distribution of cfDNA fragment sizes in healthy patients, and a subset of lung cancer patients identified as having either hyperfragmentation or hypofragmentation phenotype.*

Cross-breakpoint motif analysis

- Count occurrences of each 2bp motif around the 5' fragment breakpoint
- Compare **hyperfragmented (n=5)** to **hypofragmented (n=6)** motifs across fragment lengths

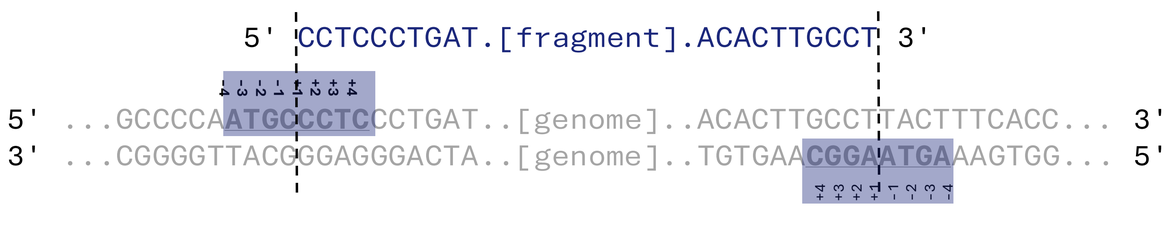

Figure 2. *Schematic representation of 5' cross-breakpoint motifs.*

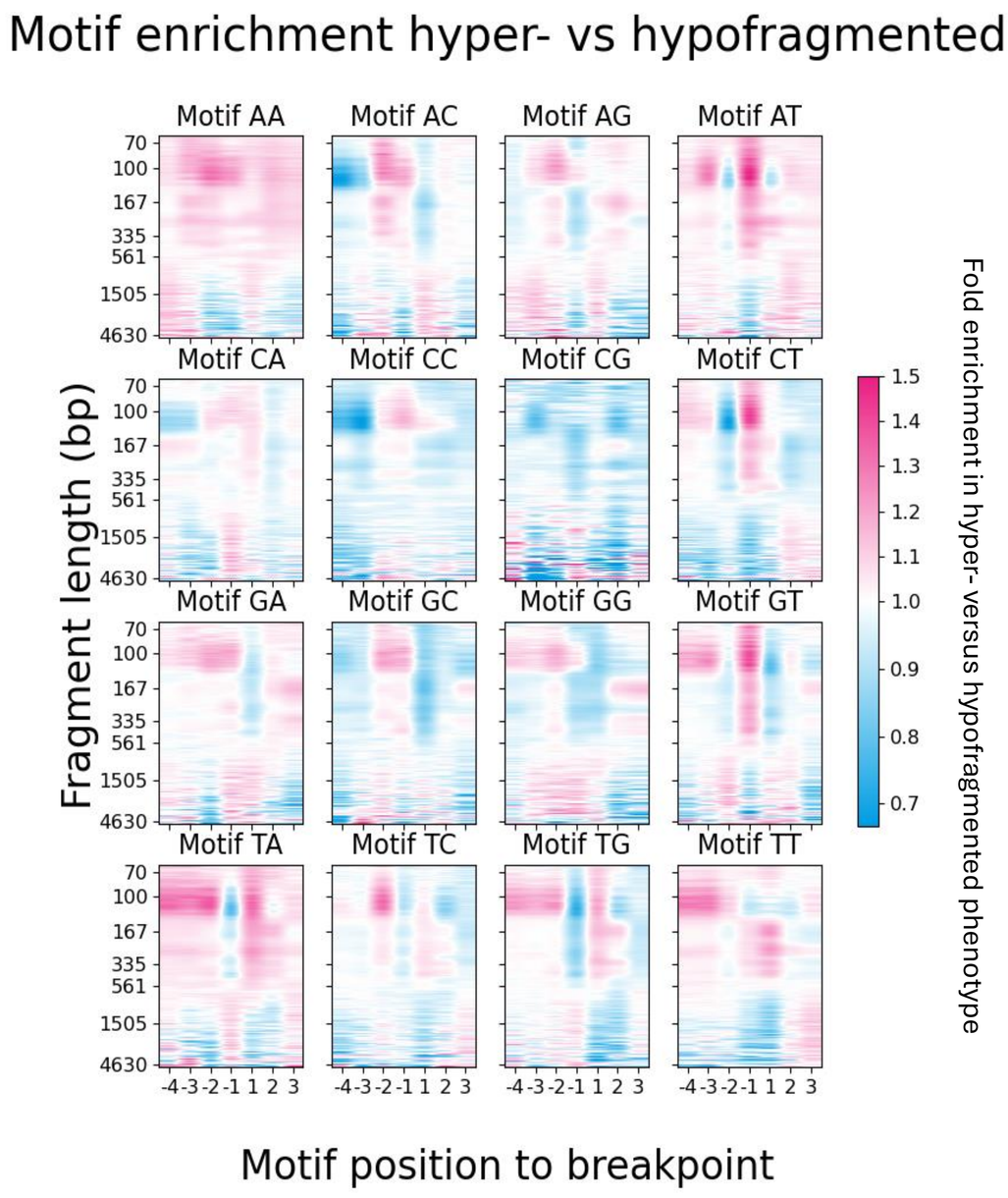

Figure 3. *Differential motif enrichment in hyper-fragmented versus hypo-fragmented samples.*

Cancer vs healthy classification

- 100x random subsample 200,000 reads per patient
- Count 5' end nucleotides across fragment lengths
- Support vector machine (SVM) model
- Leave-one-patient-out cross validation

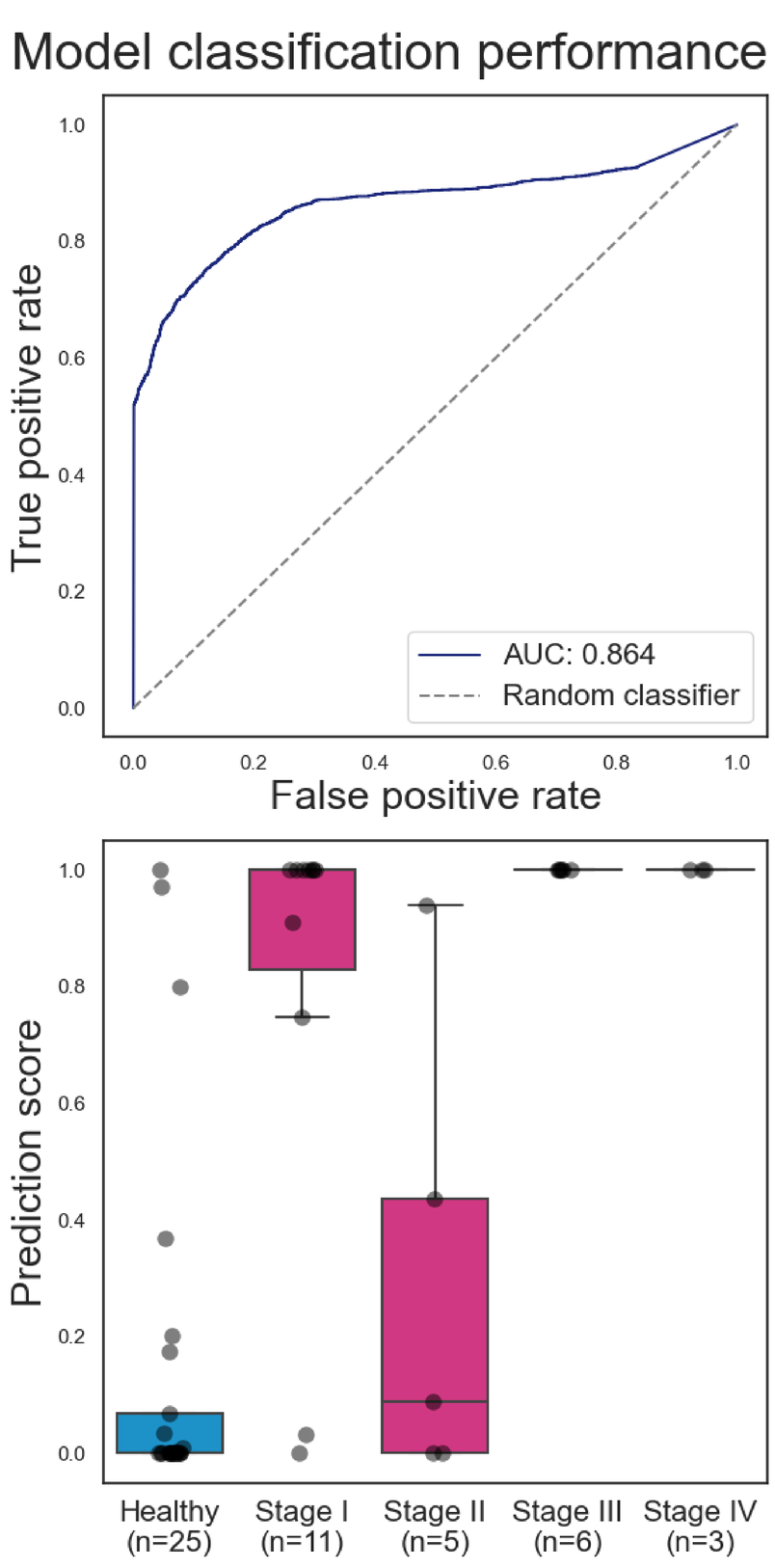

Figure 4. *Cancer prediction based on motif enrichment across fragment lengths.*

Conclusions

- Hyperfragmented vs hypofragmented phenotype confirmed
- End motifs are different between:
  - ➢ Short reads vs long reads
  - ➢ Hyperfragmented vs hypofragmented
- Preliminary model reaches AUC 0.864 in cross validation
- Improved model performance with more data

References:

1. Lois Choy LY, Peng W, Jiang P, Cheng SH, Yu SCY, Shang H, et al. Single-Molecule Sequencing Enables Long Cell-Free DNA Detection and Direct Methylation Analysis for Cancer Patients. Clin Chem. 2022;68(9).
2. Linthorst J, Nivard M, Sistermans EA, Linthorst J, Nivard M, Sistermans EA. GWAS shows the genetics behind cell-free DNA and highlights the importance of p.Arg206Cys in DNASE1L3 for non-invasive testing. Cell Rep. 2024 Oct 22;43(10):114799.
3. Berman BP, Erdman SA, Turatsinze JV, Cayford J, Kelly TK. Long-read sequencing reveals aberrant fragmentation patterns and origins of circulating DNA in cancer. bioRxiv [Internet]. 2024 Jan 1;2024.05.02.592182.

Corresponding authors:
jasper@renovarocube.com, daan@renovarocube.com, khashayar@renovarocube.com
Reach out to us!