Quantification of Breast Cancer 1 gene (*BRCA1*) promoter methylation in individuals with and without cancer using cell-free DNA

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BACKGROUND

BRCA1 promoter hypermethylation (*BRCA1*meth), a driver of homologous recombination deficiency (HRD), is observed in up to 25% of triple negative breast cancers (TNBCs) and ovarian carcinomas (OvCas).

*BRCA1*meth is associated with worse outcome compared to *BRCA1* mutations and, when found in individuals without cancer (i.e., constitutive *BRCA1*meth), it is associated with increased risk of breast and ovarian cancer.

Current methodologies to determine the presence of *BRCA1*meth in a cancer sample require tissue biopsy and have not been adopted for standard clinical assessment.

The GRAIL cell-free DNA (cfDNA) targeted methylation platform is a robust, biopsy-free, scalable assay that was developed to distinguish cancer methylation patterns across different cancer signal origins. Here, we describe the GRAIL platform's ability to detect and quantify *BRCA1*meth.

METHODS

As part of the CCGA study (NCT02889978, *Liu et al., PMID:33506766*), circulating cfDNA was isolated from the plasma of 2,790 individuals without cancer and of 2,958 patients with cancer prior to any treatment.

The DNA samples were processed on GRAIL's targeted methylation platform and a focused analysis was performed on sequencing reads overlapping the *BRCA1* core promoter region (*chr17: 41277134-41277486*; GRCh37), to determine the presence and extent (i.e., allele frequency) of cell-free *BRCA1* promoter methylation (cf*BRCA1*meth).

Sequencing fragments with >=4 methylated CpG sites were considered methylated, and samples with >=1 methylated fragments were considered positive for cf*BRCA1*meth. The methylation frequency for cf*BRCA1*meth samples was calculated as the fraction of methylated fragments over the total number of sequencing fragments mapping to the *BRCA1* core promoter region.

CONCLUSIONS

This work shows that the GRAIL targeted-methylation platform, as a single blood-based assay, can sensitively detect and quantify *BRCA1*meth from cfDNA, which may inform cancer susceptibility in non-cancer bearing individuals and may also have potential treatment selection implications for cancer patients.

RESULTS

(1) Our quantification of cf*BRCA1*meth fraction is linear with respect to expected sample-wide cf*BRCA1*meth fraction in biochemically methylated contrived samples (Fig. 1A). Our limit of cf*BRCA1*meth detection (LoD95) in these samples is 0.0081 cf*BRCA1*meth fraction (i.e., true detection probability of > 0.95 for samples with expected cfBRCA1meth fraction >= 0.0081, Fig. 1B).

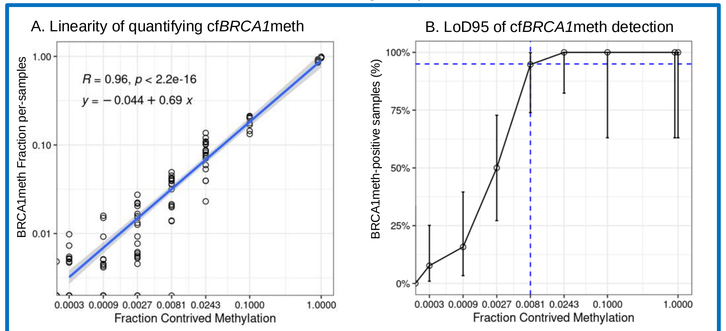

Fig. 1. Detection and quantification of cf*BRCA1*meth in contrived samples.

(2) cf*BRCA1*meth is detected in 113/2,790 individuals without cancer (4%; 95% CI: 3.4-4.8%), and it is more prevalent in females vs. males (Fig. 2, blue highlights, p = 0.0158).

(3) cf*BRCA1*meth is more prevalent in females vs. males (Fig. 2, blue highlights, p = 0.0158) and it is significantly enriched in patients with TNBC and OvCa but not other types of cancers (Fig. 2), reflecting the known cancer-type specific prevalence of *BRCA1*meth (*Menghi et al. PMID:35857626*).

(4) In TNBC and OvCa, the median cf*BRCA1*meth fraction is ~3-fold higher than that found in individuals without cancer (Fig. 3A), and it significantly correlates with the methylation-based estimate of tumor burden (Fig. 3B).

(5) Overall higher cf*BRCA1*meth frequencies were also observed in individuals with cancers other than TNBC/OvCa (Fig. 3A, other cancers), with a few rare cases showing cf*BRCA1*meth as high as 0.21; suggesting that BRCA1 deficiency may be implicated in these rare cases, making them candidates for targeted treatments.

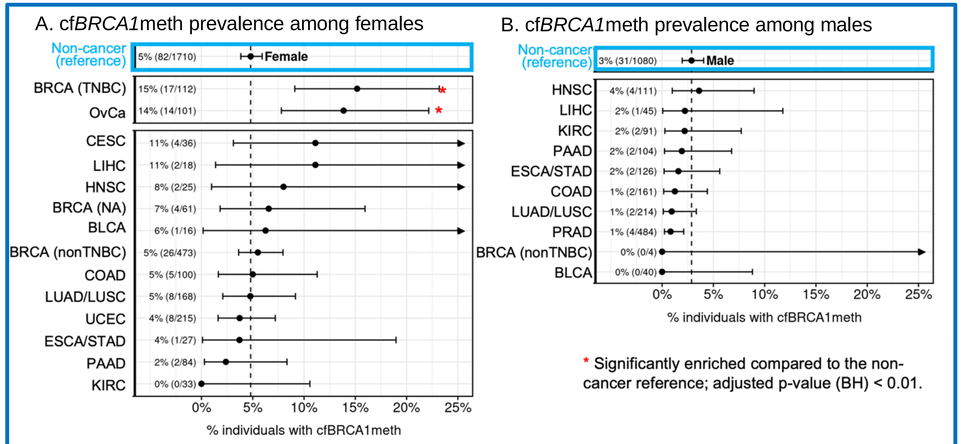

Fig. 2. Prevalence of cf*BRCA1*meth as a function of gender, cancer status, and cancer type. Cancer type abbreviations shown are based on The Cancer Genome Atlas (TCGA).

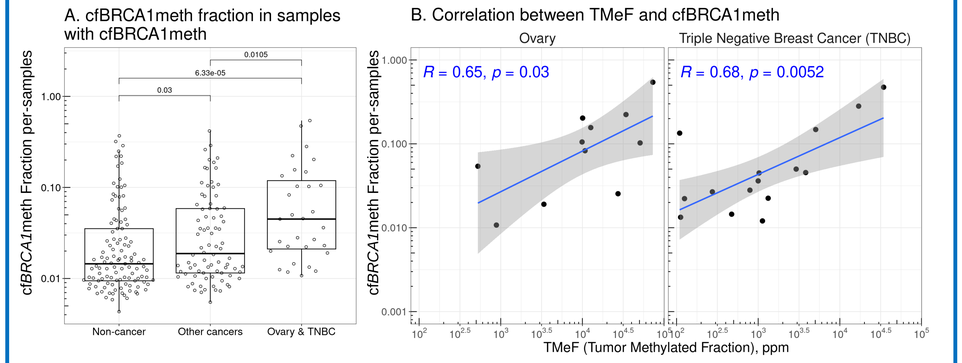

Fig. 3. cf*BRCA1*meth frequencies as a function of cancer status and tumor fraction. (**A**) cf*BRCA1*meth fractions across non-cancer and cancer samples. P value by Student's t-test (BH-corrected). (**B**) In OvCa and TNBC, cf*BRCA1*meth fraction is correlated with tumor methylated fraction (TMeF), a previously described metric that aggregates panel-wide signals to provide an estimate of tumor fraction (*Melton et al., PMID: 38201510*). In both (A) and (B), only samples that are cf*BRCA1*meth-positive are depicted.

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