

Prevalence of *BRCA1* Promoter Methylation in Cohorts of Individuals With and Without Cancer Assessed by Circulating Cell-Free DNA

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ABSTRACT

PURPOSE *BRCA1* promoter methylation (*BRCA1*meth) affects 20%–27% of triple-negative breast cancer (TNBC) and ovarian cancer (OvCa) and has therapeutic predictive value in both cancer types. *BRCA1*meth is established during early embryonic development as a constitutional event, and its detection in the peripheral blood cells of unaffected women associates with increased risk for breast cancer and OvCa. Thus, facile and sensitive assessment of *BRCA1*meth is an important component for both risk assessment and the therapeutic management of these cancer types.

METHODS We used the GRAIL circulating cell-free DNA (cfDNA)-based targeted methylation platform to quantify *BRCA1*meth in cfDNA (cf*BRCA1*meth) from plasma samples of individuals with and without cancer.

RESULTS Detection of cf*BRCA1*meth was accurate and sensitive, with an empirical LoD₉₅ of 0.0081. cf*BRCA1*meth was found in 4.1% (113/2,790) of individuals without cancer, and it was significantly more prevalent in females compared with males (4.8% v 2.9%, $P = .016$), suggesting that the dynamics of *BRCA1* promoter methylation and its consequences for cancer development may differ by sex. In a pan-cancer cohort comprising 2,849 patients and representing 15 tissue types, cf*BRCA1*meth was enriched only in TNBC (15.2%, $P = .001$) and OvCa (13.9%, $P = .014$). Importantly, in these cf*BRCA1*meth-positive cases, the fraction of cf*BRCA1*meth was correlated with a methylation-based estimate of tumor burden.

CONCLUSION Our findings demonstrate that *BRCA1*meth can be robustly detected using cfDNA assessment both as a constitutional event in noncancer samples and as a metric of *BRCA1*meth tumor burden in individuals with *BRCA1*meth cancers. Therefore, the GRAIL assay provides a single quantitative platform that can be used to explore the role of *BRCA1*meth in cancer risk and therapeutic response.

ACCOMPANYING CONTENT

 [Data Sharing Statement](#)

 [Data Supplement](#)

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BACKGROUND

BRCA1 deficiency induced by *BRCA1* promoter methylation (*BRCA1*meth) is found in the tumor tissue of 20%–27% of patients with ovarian cancer (OvCa) and triple-negative breast cancer (TNBC)^{1–4} and is considered causative in the development of these cancer types.⁵ Although *BRCA1*meth tumors share similar genomic signatures with *BRCA1*-mutant tumors,^{2,6} our previous work demonstrated that *BRCA1*meth associates with worse treatment response. This reduced sensitivity arises because *BRCA1*meth is frequently lost after chemotherapy exposure, leading to *BRCA1* re-expression, restoration of homologous recombination proficiency, and acquired treatment resistance.²

Recently, *BRCA1*meth was observed constitutionally in the peripheral blood cells (PBCs) of 5%–9% of women

without cancer.^{5,7} This is generally recognized as a primary epimutation event, where an epigenetic change occurs in the absence of a DNA mutation, as opposed to a secondary epimutation, where an epigenetic change is triggered by a cis- or trans-acting genetic mutation.⁸ Constitutional *BRCA1*meth typically affects a single allele and has a mosaic presentation, affecting a small fraction of somatic cells: the median *BRCA1*meth allelic fraction ranges between 0.01 and 0.05 (on a 0–1 scale), rarely reaching up to 0.3.^{5,7,9–12} Since *BRCA1*meth is detected in cord blood samples and in adult tissues from all germinal layers, and because of the intra-individual consistency of the affected allele, it is proposed that constitutional *BRCA1*meth occurs as a single primary epimutation event during early embryonic development and that it is maintained into adulthood, with affected individuals carrying at least a 2.5- and 1.8-fold increased risk of incident TNBC and OvCa, respectively.^{5,11,13} A recent

CONTEXT

Key Objective

Can *BRCA1* promoter methylation (*BRCA1*meth) be detected in cell-free DNA as a molecular biomarker in cancer?

Knowledge Generated

Our method sensitively detects cell-free *BRCA1*meth (cf*BRCA1*meth), with an empirical LoD_{95} of 0.0081. In individuals without cancer, cf*BRCA1*meth occurs in 4.8% of women and 2.9% of men ($P = .016$). cf*BRCA1*meth prevalence significantly increases to 15.2% and 13.9% in women with triple-negative breast cancer and ovarian cancer, respectively, and its abundance correlates with epigenetic measures of tumor burden.

Relevance

This study provides a single quantitative platform that allows to explore the role of *BRCA1*meth in cancer risk and therapeutic response.

epidemiologic study described two unique cases of familial breast cancer and OvCa, where *BRCA1*meth occurred as a secondary epimutation, due to an inherited DNA mutation in the 5' untranslated region (5'UTR) of the *BRCA1* gene.¹⁴ The pedigrees of the two families examined showed a clear inheritance pattern with the 5'UTR mutation and its associated *BRCA1*meth status segregating with cancer.¹⁴ Although these cases are extremely rare, they support a driving role for constitutional *BRCA1*meth in tumorigenesis.

Given the potential impact of *BRCA1*meth on cancer risk assessment and prediction of therapeutic outcomes, a facile and quantitative detection of *BRCA1*meth that does not require tissue biopsy is likely to be an important biomarker in clinical cancer medicine.

The GRAIL circulating cell-free DNA (cfDNA)-based targeted methylation platform is a robust, biopsy-free, scalable assay derived from a previously validated next-generation sequencing (NGS) platform.¹⁵⁻¹⁷ Herein, we show that this assay can be used for sensitive and quantitative cell-free *BRCA1*meth (cf*BRCA1*meth) detection in large cohorts of individuals with and without cancer, with potential utility both as a predictive biomarker and as a cancer risk assessment tool in precision oncology.

METHODS

CCGA Study Cohort

The Circulating Cell-Free Genome Atlas (CCGA, ClinicalTrials.gov identifier: [NCT02889978](https://clinicaltrials.gov/ct2/show/study/NCT02889978)) is a previously reported multicenter, observational, longitudinal, case-control study.¹⁶ Participants with cancer had a confirmed diagnosis of invasive cancer, whereas noncancer participants had no previous or current cancer diagnosis and no acute illness at enrollment. In total, 5,639 cfDNA samples from CCGA Substudy 2 and 3¹⁶ were

analyzed for cf*BRCA1*meth, representing 2,849 cancer and 2,790 noncancer individuals.

TCGA Study Cohort

Methylation beta-values from the Illumina Infinium HumanMethylation27 and HumanMethylation450 arrays were downloaded from the UCSC Xena Browser in January 2024. Only tumor types found in the CCGA cohort were included in the analysis, accounting for 7,789 unique samples across multiple tumor types. For each tumor sample, the average beta value of the probes mapping to the *BRCA1* promoter region (chr17:41277134-41277486; GRCh37) was computed and the threshold of 0.2 was applied to identify *BRCA1*meth cancers.

Determining *BRCA1*meth in Circulating cfDNA (cf*BRCA1*meth)

An existing targeted methylation assay that was originally developed to detect cancer methylation signatures for multicancer early detection (MCED) was used.¹⁷ Although the MCED assay captures over a million CpG sites genome-wide, this analysis focuses only on sequencing reads overlapping the *BRCA1* promoter region (chr17:41277134-41277486; GRCh37) and spanning at least four CpG sites (ie, informative reads). Informative reads were required to have at least two PCR duplicates to ensure robustness; however, they were then deduplicated so that each read represents a unique molecule of cfDNA in the sample. Across the cohort, the number of informative fragments per sample ranged from 21 to 237 (median = 92). A fragment was considered methylated if at least four of its CpGs were methylated. Fragments contained a median of 11 CpGs (range, 4-20) per sample with similar distributions for methylated and nonmethylated samples. Fragments tended to be either predominantly methylated (>75% CpGs are methylated) or predominantly unmethylated (<25%). The threshold of 4 methylated CpG

sites was chosen such that the theoretical probability of erroneously calling a sample methylated due to sequencing or other technical error is <0.0001 . A sample was considered methylated (ie, *cfBRCA1meth*) if at least one informative fragment was methylated (Data Supplement, Fig S1). For each sample, the *cfBRCA1meth* fraction was computed as the ratio of the number of methylated fragments over the number of informative fragments (scale: 0–1). To avoid confusion, we use percentages when discussing the prevalence of *cfBRCA1meth* within groups of samples (scale: 0%–100%, Data Supplement, Fig S1).

Empirical Limit of Detection Analysis

To assess the limit of *cfBRCA1meth* detection, seven serial dilutions of commercially available material with defined global methylation levels were analyzed as proxies for expected *BRCA1meth* fractions. At contrived methylation levels of 0, 0.1, 0.9, and 1.0, samples were prepared by mixing biochemically methylated or unmethylated, sheared, and size-selected genomic DNA samples from HCT116 DKO cell lines, with 7–8 replicates per level. For lower contrived methylation levels (0.003–0.0243), SeraSeq ctDNA reference material (SeraCare) was spiked into a background noncancer sample, with 19–26 replicates per level. In all mixtures, the reported contrived methylation reflects only the spiked-in material and excludes background methylation. Linearity was assessed for samples with contrived methylation levels >0.001 . Sensitivity was evaluated using the empirical limit of detection 95 (LoD_{95}), defined as the minimum *cfBRCA1meth* fraction (estimated from the contrived methylation level) at which 95% of replicates are classified as positive.

Statistical Analyses

All plotting and statistical analyses were performed in R version 4.3.2. Where prevalence was measured within a group (eg, on the basis of cancer type, sex, etc), the prevalence and a 95% exact binomial confidence interval were reported (using the *Hmisc* function of the *binconf* R package, *method* = “exact”). For comparisons across groups, Fisher’s exact test for count data was applied if at least one of the cell counts was ≤ 5 or if the total count was $\leq 1,000$. Otherwise, the chi-square test was used.

Ethics Approval and Consent to Participate

This study used data from the CCGA study,¹⁷ a prospective, multicenter, observational study sponsored by GRAIL, LLC. The CCGA study is registered at ClinicalTrials.gov under the identifier [NCT02889978](#). All participants were required to provide written informed consent. The study was approved by the institutional review board or an independent ethics committee at each participating trial site, and conducted in accordance with the International Conference on Harmonisation for Good Clinical Practice guidelines and the Declaration of Helsinki.

RESULTS

cfBRCA1meth Can Be Detected in a Sensitive and Quantitative Manner

In this study, we used data from a blood-based cfDNA assay originally designed as a targeted bisulfite sequencing approach to detect differences in methylation patterns between cancer and noncancer individuals.¹⁷ Although the original assay queries over a million CpGs, we focused on the assessment of the *BRCA1* promoter locus, as described in Methods and in the Data Supplement (Fig S1).

To determine the linearity of *cfBRCA1meth* fraction and our limit of *cfBRCA1meth* detection, we assessed a dilution series of biochemically methylated contrived samples with expected *cfBRCA1meth* fractions ranging from 0.0003 to 1 (where 1 = 100% methylated). The assay showed a strong linear correlation between observed and expected *cfBRCA1meth* fractions across the full range tested ($R = 0.97$, $P < .001$, Fig 1A). The empirical limit of detection 95 (LoD_{95}),¹⁸ determined from the dilution series, corresponded to a *cfBRCA1meth* allelic fraction of 0.0081 (Fig 1B), indicating that samples with fractions ≥ 0.0081 would be classified as positive in 95% of replicates. Thus, our approach is both sensitive and accurate in detecting and quantifying *BRCA1meth* in circulating cfDNA.

Constitutional *cfBRCA1meth* Is Detected in Individuals Without Cancer

We analyzed *cfBRCA1meth* status in 2,790 individuals without cancer, originally collected as part of the CCGA, a previously reported multicenter, observational, longitudinal, case-control study.¹⁶ In this noncancer cohort, 4.1% (113/2,790) of individuals harbored evidence of *cfBRCA1meth* (Table 1 and Data Supplement, Table S1), a percentage comparable to previous assessments of constitutional *BRCA1meth* detected by ultradeep targeted bisulfite sequencing of PBCs from adults.^{5,11} The *cfBRCA1meth* fractions in *cfBRCA1meth*-positive samples ranged from 0.004 to 0.32 (median = 0.015, Data Supplement, Fig S2A), with most *cfBRCA1meth*-positive individuals (92/113, 81%) having a *cfBRCA1meth* fraction <0.05 (Data Supplement, Fig S2A). Again, the fractional range of *cfBRCA1meth* is comparable to what found in previous surveys using PBC DNA,⁵ confirming that constitutional *BRCA1meth* can be sensitively and accurately detected using cfDNA. Interestingly, we observed a significantly lower incidence of *cfBRCA1meth* in males compared with females (31/1,080 [2.9%] *cfBRCA1meth*-positive cases in males v 82/1,710 [4.8%] in females, $P = .016$, Table 1), although positive cases from both sexes shared similar distributions of *cfBRCA1meth* fractions (Data Supplement, Fig S2A). A recent study of DNA obtained from the umbilical cord blood of newborns and from PBCs of their parents also showed a significant sex bias for constitutional *BRCA1meth*, with 9% of newborn girls but only 4.5% newborn boys, and 8% of mothers versus 3% of fathers showing evidence of constitutional *BRCA1meth*.⁷ Intriguingly, this

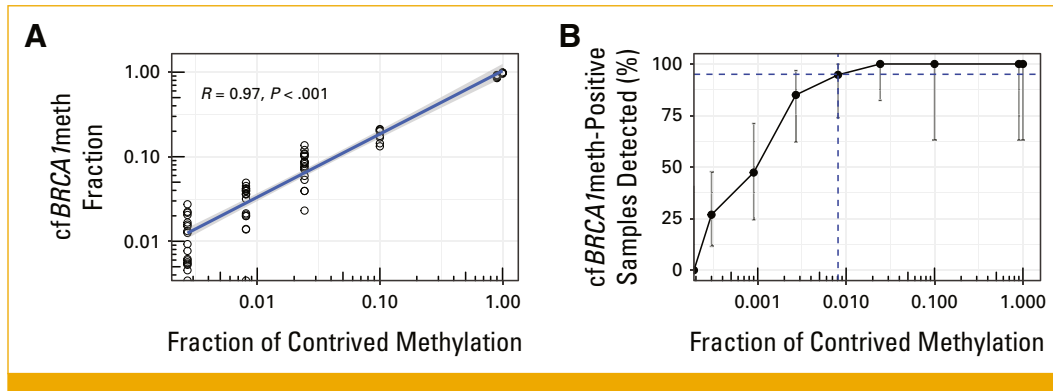


FIG 1. Detection and quantification of *cfBRCA1meth* in samples with known fraction of contrived genome-wide methylation. (A) A scatter plot of fraction of contrived methylation (x-axis) against the estimated per-sample fraction of *BRCA1* methylated fragments (y-axis). A linear regression line is shown in blue to visualize linearity of quantitative estimation. Pearson correlation (R) and the associated P value are shown alongside the regression equation. (B) Percentage of samples detected as having *cfBRCA1meth* (y-axis), with error bars representing the 95% binomial CI. 95% detection is marked with a horizontal blue dashed line, and the corresponding LoD_{95} is indicated with a vertical blue dashed line. *cfBRCA1meth*, cell-free *BRCA1meth*.

study also suggests a potential age bias in the rates of constitutional *BRCA1meth*. We therefore explored the possibility that *cfBRCA1meth* may be more prevalent in younger adults. However, we did not observe any significant age-related change in the rate of *cfBRCA1meth* within our

TABLE 1. Percentage of *cfBRCA1meth* Across Demographic Characteristics in the Noncancer Cohort

Characteristic	CCGA Cohort: Noncancer (n = 2,790)	
	<i>cfBRCA1meth</i> , No. (%)	P
All	113/2,790 (4.1)	—
Sex		
Female	82/1,710 (4.8)	.016
Male	31/1,080 (2.9)	
Age, years		
20-39	15/387 (3.9)	NS
40-49	22/602 (3.7)	
50-59	34/697 (4.9)	
60-69	28/745 (3.8)	
70-85	14/359 (3.9)	
Race/ethnicity		
White, non-Hispanic	80/2,186 (3.7)	NS
Hispanic	13/242 (5.4)	
Black, non-Hispanic	9/229 (3.9)	
Asian, Native Hawaiian, or Pacific Islander	5/62 (8.1)	
Other/missing	5/59 (8.5)	
Smoking status		
Never-smoker	67/1,580 (4.3)	NS
Ever-smoker	44/1,187 (3.7)	

Abbreviations: CCGA, Circulating Cell-Free Genome Atlas; NS, not significant.

noncancer cohort that includes individuals ranging from age 19 to 86 years (median = 57 years, Table 1). This was true even when the analysis was restricted to each sex (Data Supplement, Table S2). Moreover, we did not detect any correlation between age and *cfBRCA1meth* fraction (Data Supplement, Fig S2B). Finally, we did not find any associations between *cfBRCA1meth* prevalence and additional demographic features, including self-reported race/ethnicity and smoking history (Table 1).

cfBRCA1meth Is Enriched in Triple-Negative Breast and Ovarian Cancers

We then quantified *cfBRCA1meth* in 2,849 patients with cancer from the CCGA cohort (Data Supplement, Table S1). We found that the prevalence of *cfBRCA1meth* was significantly higher in women with cancer compared with those without cancer (6.4% v 4.8%, $P = .052$, Fig 2), while the opposite trend was true for the male cohort (1.4% with cancer v 2.9% without cancer, $P = .014$, Fig 2). When we assessed each tumor type individually, *cfBRCA1meth* was found to be significantly enriched only in female patients with TNBC (17/112 [15.2%], $P < .001$, Fig 2) and OvCa (14/101 [13.9%], $P < .001$, Fig 2). Furthermore, upon histologic subclassification, we observed that *cfBRCA1meth* was specifically enriched in the high-grade serous subtype of OvCa (high-grade serous ovarian carcinoma: 6/41 [14.6%], $P = .015$), as well as in the indeterminate OvCa subset (5/26 [19.2%], $P = .008$, Data Supplement, Fig S3), for which there was insufficient pathologic data for subtype assignment. No other cancer types showed a significant enrichment of *cfBRCA1meth* patients over noncancer controls (Fig 2 and Data Supplement, Fig S3). Conversely, we noted lower *cfBRCA1meth* rates in prostate cancer (4/484 or 0.8%, Fig 2), which contributes to the reduced *cfBRCA1meth* rates in the male all-cancer cohort. The distribution of *cfBRCA1meth* prevalences across different tumor types in the CCGA cohort

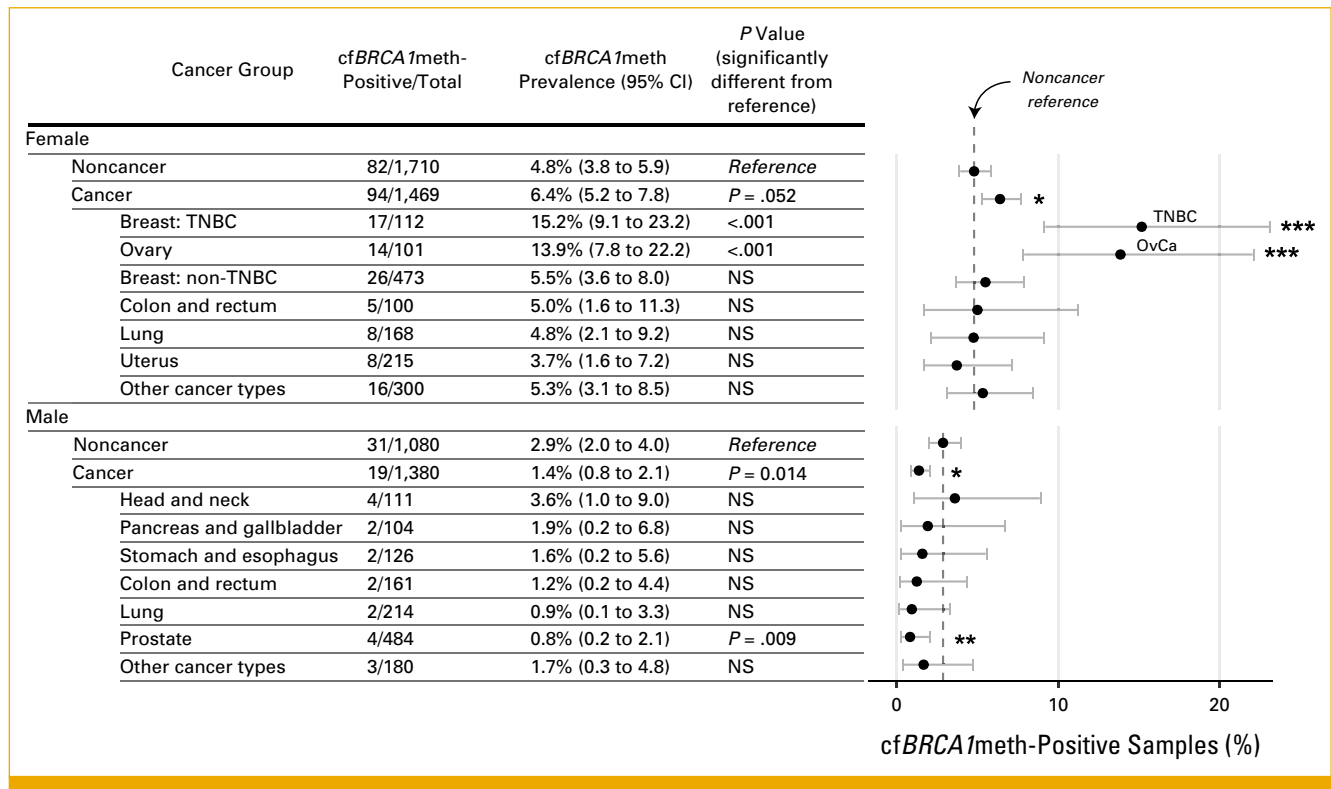


FIG 2. Prevalence of cfBRCA1meth in the CCGA cohort. The prevalence of cfBRCA1meth was computed for females and males across the entire noncancer and cancer cohorts and for each tumor type that comprised at least 100 samples. Tumor types with < 100 samples were combined as other cancer types. The percent of individuals with cfBRCA1meth (x-axis) is shown, with error bars denoting the 95% binomial CI. Prevalences for individual cancer groups were compared against the sex-matched reference group of noncancer individuals. A complete breakdown of patient number and cfBRCA1meth percentages across each individual tumor type analyzed is provided in the Data Supplement (Fig S3). * $P \leq .05$; ** $P \leq .01$; *** $P \leq .001$. CCGA, Circulating Cell-Free Genome Atlas; cfBRCA1meth, cell-free BRCA1meth; NS, not significant; OvCa, ovarian cancer; TNBC, triple-negative breast cancer.

is comparable to that of BRCA1meth assessed in The Cancer Genome Atlas (TCGA) data set directly from tumor tissue DNA samples, using Illumina methylation arrays (Data Supplement, Fig S4).

The median cfBRCA1meth fractions in patients with TNBC and OvCa were approximately 3-fold higher than those observed in individuals without cancer ($P < .001$, Fig 3A). This is expected, as unlike the more traditional analysis of PBC DNA, the cfBRCA1meth signal detected in patients with cancer captures both the potential presence of constitutional BRCA1meth and the BRCA1meth signal originating from BRCA1meth-driven cancer cells. To deconvolve these two components, we explored cfBRCA1meth levels as a function of the tumor methylation fraction (TMeF), a metric that aggregates the GRAIL genome-wide signal to provide an estimate of tumor burden in cfDNA, which generally correlates with tumor mass.¹⁹ The cfBRCA1meth fraction positively correlated with TMeF in both TNBC ($R = 0.68$, $P = .005$) and OvCa ($R = 0.65$, $P = .03$; Fig 3B), suggesting that, in these tumor types, the bulk of cfBRCA1meth originates from BRCA1meth cancer cells that release BRCA1meth DNA into the plasma. When all female cancers other than TNBC and OvCa were analyzed separately (ie, other cancers), there was no

significant association between cfBRCA1meth fraction and TMeF (Data Supplement, Fig S5). However, the median cfBRCA1meth fraction in the other-cancer female subset was 1.5-fold higher than that in the noncancer female subset ($P = .04$, Fig 3A), indicating that some women with cancers other than TNBC and OvCa either have higher constitutional cfBRCA1meth levels than noncancer individuals or have occasional tumors driven by BRCA1meth.

DISCUSSION

BRCA1meth is a highly consequential epigenetic state, which manifests constitutionally and associates with an increased risk of incident breast cancer and OvCa in otherwise healthy individuals.⁵ In patients with established tumors, BRCA1meth associates with poor therapeutic outcomes.² Accurate, reproducible, and quantitative tests for BRCA1meth therefore will have clinical utility in both cancer risk assessment and therapeutic guidance.

Although several methods have been developed to assess locus-specific methylation over the years, including methylation-specific PCR,²⁰ methylation-sensitive high-resolution melting,²¹ and clonal bisulfite Sanger sequencing,²² these

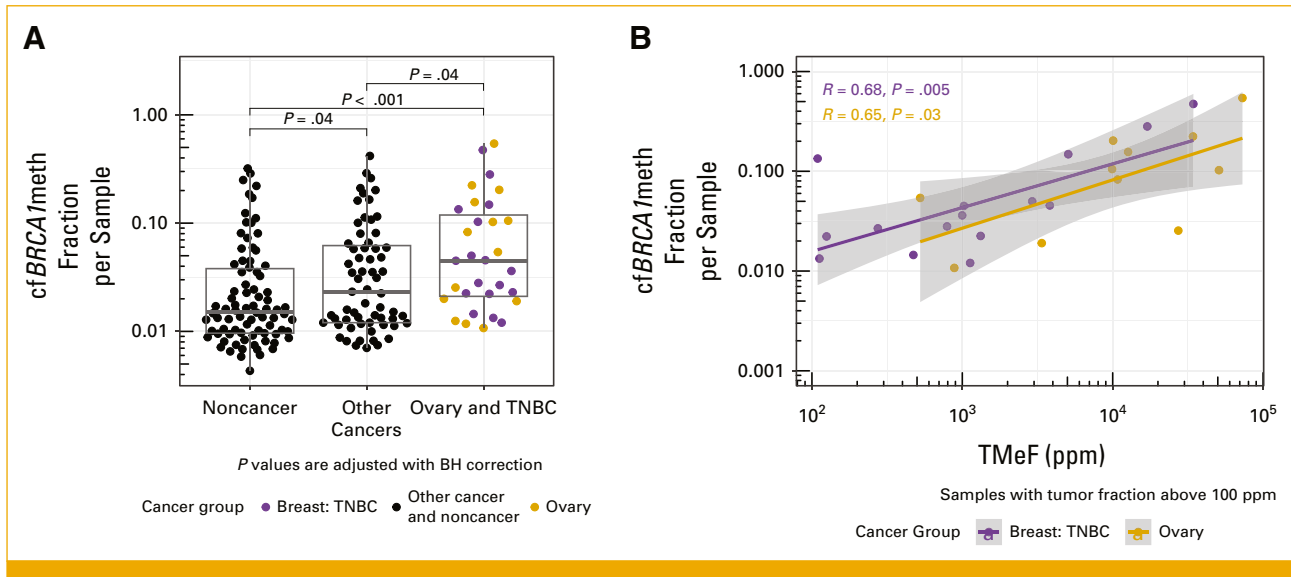


FIG 3. Distribution of *cfBRCA1meth* fraction and association with TMeF in female individuals with evidence of *cfBRCA1meth*. (A) Boxplots show the distribution of *cfBRCA1meth* fractions for OvCa and TNBC samples compared with other cancers and noncancer samples. Only *cfBRCA1meth*-positive samples from female donors are included in the analysis. (B) The correlation between TMeF (x-axis, log₁₀-transformed) and *cfBRCA1meth* fraction (y-axis, log₁₀-transformed) is shown for *cfBRCA1meth*-positive OvCa and TNBC samples. A linear regression line is shown for each cancer type, and the R value and the associated P value from a Pearson's correlation test are also indicated. *cfBRCA1meth*, cell-free *BRCA1meth*; OvCa, ovarian cancer; TMeF, tumor methylation fraction; TNBC, triple-negative breast cancer.

strategies are limited in their sensitivity and throughput and do not allow the quantification of low methylation frequencies, such as those associated with constitutional *BRCA1meth*. NGS methodologies address these limitations by allowing ultradeep coverage combined with CpG phasing, and generate highly sensitive and granular CpG-level information.²³ Our approach, based on a noninvasive cfDNA NGS assay, is agnostic to the cellular origin of the *cfBRCA1meth* signal, enabling the assessment of *BRCA1meth* across the entire body rather than within specific tissues. Furthermore, being built on a clinically validated targeted-methylation platform for comprehensive cancer-signal assessment, it allows interpretation of *BRCA1meth* signals alongside other metrics, such as a tumor burden estimate.¹⁹

Across 2,790 individuals without cancer, we detected *cfBRCA1meth* in 4.8% and 2.9% of females and males, respectively, with most patients having a *cfBRCA1meth* fraction <0.05 , in alignment with findings using *BRCA1meth* from PBC DNA.^{5,7} We acknowledge that with an LoD₉₅ of 0.0081, we may be missing a small proportion of individuals with even lower *cfBRCA1meth* frequencies down to 0.001, which has been established as the detection limit in other ultradeep NGS studies that directly sequence blood or buccal DNA.^{5,7} To overcome this potential limitation, future studies should directly compare the sensitivity of NGS approaches based on cfDNA versus PBC DNA from the same donors.

The intriguing observation of an almost two-fold increased prevalence of *cfBRCA1meth* in females over males confirms

the findings of a recent study in newborns and their parents,⁷ but with larger numbers. Although it has been speculated that constitutional *BRCA1meth* is more common in younger individuals, our study, comprising the largest cohort ever assessed across a wide age range (19–86 years), showed no association between age and *cfBRCA1meth* prevalence, nor *cfBRCA1meth* fraction, at least within this age range. Although our observations point to a consistent rate of *cfBRCA1meth* throughout an individual lifespan, we cannot exclude modest age-associated effects, which, ideally, should be investigated in longitudinal studies with repeated intraindividual samplings over the years.

We found that patients with *BRCA1meth*-linked cancers (TNBC and OvCa) have generally higher *cfBRCA1meth* fractions, suggesting that cfDNA can be used to accurately and quantitatively detect clinically meaningful *BRCA1meth* from cancer tissues, thus bypassing the need for invasive biopsies. Although a high fraction of *cfBRCA1meth* alone is not indicative of the presence of cancer, as we did observe sporadic instances of noncancer samples with *cfBRCA1meth* fractions in the range of 0.10–0.37 that are comparable to those observed in patients with *BRCA1meth*-linked cancers, the *cfBRCA1meth* fraction is a useful quantitative experimental metric, especially when coupled with cancer detection tools that quantify tumor burden.

Interestingly, we observed that women with other cancers (ie, neither TNBC nor OvCa) had a statistically higher *cfBRCA1meth* fraction than women without cancer. Similarly, concordant

tumor and blood *BRCA1*meth signals have been observed in a small percentage of estrogen receptor–positive breast cancers.⁷ Thus, female cancers that are not typically associated with loss of *BRCA1* can also show tumor *BRCA1*meth, possibly from a constitutional origin, which has critical implications for risk assessment and clinical outcomes. Indeed, as *BRCA1* disruptions appear to drive a small fraction of other malignancies such as biliary tract, gastric, lung, and pancreatic cancers,²⁴ assessing the frequency of *cfBRCA1*meth may identify a subset of these refractory cancers where *BRCA1* meth drives tumorigenesis, which may be used to guide treatment planning.

An unexpected observation from our study was the depletion of *cfBRCA1*meth cases among patients with prostate cancer, which aligned with no *BRCA1*meth cases detected across the 502 TCGA prostate cancer cases examined. This suggests that not only *BRCA1*meth is exceedingly rare in this tumor type—as are genetic mutations in the *BRCA1* gene (although not *BRCA2* mutations²⁵)—but that even constitutional *BRCA1*meth is underrepresented in patients with prostate cancer. Whether these patterns are reflective of a selection process at play in male patients during the development of

prostate cancer or whether it is the result of a developmental peculiarity remains to be elucidated.

In summary, we present a new approach for the detection of constitutional *BRCA1*meth in the cfDNA of individuals with and without cancer. We show that the prevalence and fraction of *cfBRCA1*meth align with previous NGS studies using PBC and tissue DNA, and provide novel insights into demographic and clinical associations. Future studies in larger prospective cohorts of noncancer individuals, including longitudinal sampling, will elucidate dynamic changes in *cfBRCA1*meth fraction as a function of normal aging and/or tumorigenesis, validating the predictive utility of *cfBRCA1*meth for cancer risk and therapeutic outcomes. As constitutional and somatic DNA methylation has been observed for several cancer-relevant genes other than *BRCA1* (eg, *MLH1*, *MLH2*, and *MGMT*²⁶), assays like the one presented here, which detect cfDNA methylation at extremely low frequencies and in association with tumor status and tumor burden, represent a powerful tool to explore the prevalence and dynamic evolution of pathogenic methylation signals in epidemiological studies.

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DATA SHARING STATEMENT

A data sharing statement provided by the authors is available with this article at DOI <https://doi.org/10.1200/PO-25-00854>.

Because of participant privacy considerations and data use restrictions, the raw individual-level data from the CCGA study are not publicly available. Access to these data is limited to GRAIL and its authorized collaborators under institutional agreements. Researchers interested in accessing the data may contact GRAIL directly to inquire about potential collaborations and data access procedures. Sample-level data supporting the findings of this study are provided within the Data Supplement of this publication.

AUTHOR CONTRIBUTIONS

Conception and design: Edison T. Liu, Tracy Nance, Francesca Menghi

Financial support: Edison T. Liu

Administrative support: Edison T. Liu

Collection and assembly of data: Margaret Antonio, Tracy Nance

Data analysis and interpretation: All authors

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Open Payments is a public database containing information reported by companies about payments made to US-licensed physicians ([Open Payments](http://OpenPayments.org)).

Edison T. Liu

Patents, Royalties, Other Intellectual Property: I am employed by The Jackson Laboratory, a nonprofit research institution focused on mammalian genetics. I have several patents and patent pending relating to my research in cancer genomics especially around *BRCA1* and their genomic consequences

Margaret Antonio

Employment: GRAIL

Stock and Other Ownership Interests: GRAIL

Patents, Royalties, Other Intellectual Property: Patent applications related to multicancer early detection, including International Patent Publication WO/2026/015665 (Inst)

Tracy Nance

Employment: GRAIL, Karius

Stock and Other Ownership Interests: GRAIL, Karius

No other potential conflicts of interest were reported.

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