Genetics in Medicine Open (2025) 3, 101899

Genetics in Medicine OPEN
An Official Journal of the ACMG
www.journals.elsevier.com/genetics-in-medicine-open

ARTICLE

Genome sequencing enhances the diagnostic yield and expands the genetic landscape of male breast cancer

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ARTICLE INFO

Article history:
Received 9 February 2024
Received in revised form
23 October 2024
Accepted 25 October 2024
Available online 6 November 2024

Keywords:
Genetic testing
Genome sequencing
Male breast cancer
Mutational landscape
Polygenic risk score

ABSTRACT

Purpose: To understand the broader genetic landscape of male breast cancer (MBC), focusing on the utility of genome sequencing (GS) beyond *BRCA1/2* (HGNC: 1100, 1101) variants.

Methods: Twenty-four patients with MBC underwent a multistep genetic analysis. Initial screening targeted *BRCA1/2* variants followed by GS to identify pathogenic/likely pathogenic germline variants through a 3-tiered classification. Polygenic risk score analysis was further incorporated using a model for female breast cancer with 2666 noncancer controls. Exome sequencing was used to transition from germline to somatic investigations, assessing second-hit variant and mutational signatures.

Results: The GS analysis unveiled previously unrecognized pathogenic/likely pathogenic germline variants in *BARD1*, *ATR*, *BRIP1*, and *CHEK2* (HGNC: 952, 882, 20473, 16627) among 21 *BRCA1/2*-negative patients with MBC, elevating the diagnostic yield from 12.5% to 33.0% in all MBC. Elevated average polygenic risk score was noted compared with controls, with a significant correlation to early-onset MBC when combined with high-penetrance germline pathogenic variants ($P = 1.10 \times 10^{-4}$). Exome sequencing analysis further identified significant somatic oncogenic drivers and revealed a dominant mutational signature SBS3 across *BRCA1/2*-negative samples, reinforcing the contribution of omologous recombination deficiency underlying the MBC development.

Conclusion: Our findings extended the MBC genetic spectrum beyond BRCA1/2 and highlighted the intricate interplay of monogenic and polygenic predispositions, presenting a comprehensive MBC genomic profile.

The Article Publishing Charge (APC) for this article was paid by Jiaqi Liu.

Wen Wen, Sen Zhao, and Yiwen Jiang contributed equally to this study.

Given his role as Editor, Nan Wu had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to the Editor-in-Chief, Robert D. Steiner, MD.

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doi: https://doi.org/10.1016/j.gimo.2024.101899

Introduction

Cancer is a pivotal public health adversary and a leading global cause of death.[1] Despite rare cancers constituting approximately 22% to 27% of all cancers and accounting for 25% of cancer-related deaths,[2,3] their genetic underpinnings, which are crucial for evolving precision medicine strategies, remain largely unknown.[4-6]

Male breast cancer (MBC) exemplifies the complexities of rare cancers. Representing less than 1% of all breast cancers, it exhibits an incidence rate of 0.4 per 100,0000 man years.[7] A notable 20% of patients with MBC have a family history of breast cancer, which increases the lifetime risk by almost 3-fold, suggesting a significant genetic contribution to MBC predisposition.[8,9] Consequently, MBC ranks highest in the postdiagnosis germline genetic testing rate among all cancer types.[10] However, known pathogenic (P) variants, mostly found in *BRCA1/2*, *ATM*, and *PALB2* (HGNC: 1100, 1101,795, 26144), are reported in only about 16% of patients with MBC.[11,12]

Although traditional genomic diagnostic tools focus on panel sequencing of cancer predisposition genes, this may miss significant genetic information.[13] Exome sequencing (ES) can dramatically expand the detection range of genes,[14] and genome sequencing (GS) dives deeper, capturing noncoding region variants, structural variations (SVs), copy-number variations (CNVs), and even polygenic risk scores (PRS), thereby revealing the genetic etiology of rare cancers more comprehensively.[15,16] Despite the remarkable success of ES and GS in diagnosing congenital defects and neonatal diseases,[17-19] their implications for rare cancers are yet to be fully realized. The significant portion of unexplained genetic etiologies in rare cancers, such as MBC, accentuates the need for thorough research, setting the stage for utilizing GS in decoding rare cancers.[20,21]

In this study, we performed *BRCA1/2* panel genetic testing in 24 Chinese patients with MBC, deep GS for *BRCA1/2*-negative patients with MBC, comprehensive analysis at monogenetic and polygenetic levels, and further ES in tumor tissue to extend the landscape of germline and somatic variants in Chinese patients with MBC and improved the genetic diagnosis.

Materials and Methods

Study participants

We recruited male patients with breast cancer and individuals without any cancer at the time of recruitment as the noncancer controls from multiple centers between 2017 and 2019. None of them had a previous cancer history. The histological examination confirmed all breast cancer diagnoses. Biopsy specimens were obtained from primary breast tumors during surgical excision. The phenotypic information was collected and evaluated according to the well-defined criteria (Supplemental Method). The study was approved by the Institutional Review Board of the hospitals, and written informed consent was signed by all participants.

DNA sequencing

According to the National Comprehensive Cancer Network guidelines for genetic assessment (https://www.nccn.org),[22] initial *BRCA* testing should be performed for breast cancer, followed by advanced next-generation sequencing analysis using GRCh38 if the *BRCA* result is negative. The protein-coding regions of *BRCA1/2* were analyzed using targeted gene panels in all participants, following an established procedure.[23] Genomic DNA of *BRCA1/2*-negative patients obtained from peripheral blood and tumor samples were subjected to GS and ES, respectively (Supplemental Method). The choice of tumor samples for subsequent analyses depended on the availability of tissue.

Variant calling and filtering

Germline variant calling and filtering were performed using the Peking Union Medical College pipeline.[24-26] Somatic variant calling was detected using Sentieon, by comparing the sequencing data obtained from tumor and matched blood samples. Multiple filter procedures were applied at both variant and sample levels (Supplemental Method). The authenticity of putative variants was confirmed through manual inspection using the Integrative Genomic Viewer.[27]

Variant interpretation

The methodologies for the interpretation of small variants and SVs were adapted from the guidelines set forth by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology[28,29] (Supplemental Method). Additionally, noncoding variants in the promoter flanking regions were evaluated for established hereditary breast cancer-related genes. The promoter regions were obtained from the Eukaryotic Promoter Database[30] (EPD-new, accessed on 2022/10/13; Supplemental Table 1).

Variant classification

A 3-tiered terminology system was developed to curate and categorize the candidate variants based on the existing genetic annotations and inferred clinical relevance. P/likely P (P/LP) variants in genes explicitly implicated in hereditary breast cancer were assigned to tier 1. The tier 2 encompassed variants indicative of a potential predisposition to breast cancer but lacking a conclusive P/LP classification, identified within other established hereditary cancer susceptibility genes. The tier-3 included variants of uncertain

significance (VUS) intersecting with these known hereditary cancer-related genes or other genes involved in tumorigenesis. The list of hereditary cancer-related genes comprises 78 genes exhibiting monogenic inheritance patterns, sourced from the Online Mendelian Inheritance in Man database[31] and literature (Supplemental Table 2).

PRS analysis

Given a shared genetic basis between MBC and ER-positive female breast cancer (FBC),[32] a published 313 variant PRS model (PRS313) for FBC was implemented,[33] comprising 305 SNPs that have been significantly associated with overall FBC with a P value of less than 10^{-5}, 6 additional SNPs that were associated with ER-positive FBC with a P value of less than 10^{-6} but not with overall breast cancer at $P < 10^{-5}$, and 2 rare variants ([*BRCA2*] NM_000059.3:c.9976A>T p.[Lys3326Ter], [*CHEK2*] NM_007194.4:c.470T>C p.[Ile157Thr] [HGNC: 16627]). The coefficients of the optimal model for ER-positive breast cancers were used to calculate the PRS for all MBC samples and noncancer controls (Supplemental Table 3).

The PRS calculation formula is as follows:

$$PRS = \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k \cdots + \beta_n x_n$$

β_k: effect size of a given allele

x_k: allele count of the individual, 0 for non-heterozygote,1 for heterozygote, 2 for homozygote

Statistical analysis

All statistical analyses were performed in R (version 3.6.3).[34] A 1-sided P value of less than05 was considered statistically significant for testing the hypothesis that the mean of one sample exceeds that of the other. Given that this comparison was performed as a single test, the risk of type 1 error due to multiple testing was not a concern for this specific analysis, thereby eliminating the need for multiple-testing correction.

Mutational signature analysis

The mutational signatures of somatic single-nucleotide variants were analyzed using the R/Bioconductor package MutationalPatterns[35] and the known signatures was obtained from Catalogue of Somatic Mutations in Cancer database (v3.3)[36] (Supplemental Method).

Results

Participant characteristics

We enrolled 24 patients with MBC in this study, with a median age of 57.5 years (range: 34.0-76.0) at diagnosis. Among them, 37.5% (9/24) had a family cancer history, with 16.6% (4/24) reporting FBC or ovarian cancer (Table 1). A majority (70.8%; 17/24) presented at relatively early stages (0-II), including 8.3% (2/24) with ductal carcinoma in situ. Furthermore, 79.2% (19/24) were of the HR-positive/HER2-negative subtype, and 8.3% (2/24) were triple-negative. We enrolled 2666 individuals without any cancer who underwent GS as the noncancer controls in the analysis of the contribution of common variants to genetic susceptibility in patients with MBC.

GS Increases the detection rate of hereditary cancer predisposition in MBC

Initially, we identified 3 participants harboring P/LP variants using *BRCA1/2*-targeted panel testing initially (Figure 1A, Table 1). *BRCA1* P variants were found in 2 participants with a family history of breast cancer or ovarian cancer, and the *BRCA2* P variant was found in a participant without any family history of cancer. All 3 participants were with HR-positive/HER2-negative breast cancer.

Subsequently, GS was conducted for the remaining 21 *BRCA1/2*-negative participants. The mutational landscape revealed a broad spectrum of rare genetic variants distributed across these patients (Supplemental Figures 1 and 2). Careful interpretation of single-nucleotide variants, small insertions/deletions, CNVs, and SVs was performed using a 3-tier variant classification system (Figure 1B).

Our genome-wide analysis revealed that 23.8% (5/21) of these *BRCA1/2*-negative participants carried P/LP variants with an established clinical relevance (ie, tier 1), including missense variant, nonsense variant, splice altering variant, and CNV across 4 genes (*ATR*, *BARD1*, *BRIP1* [HGNC: 882, 952, 20473], and *CHEK2*) (Figure 2, Table 2). Cumulatively, P/LP variants were found in 33.3% (8/24) of the patients with MBC. Our result demonstrates that GS improved the diagnostic yield for MBCs compared with multigene panel testing,[37,38] thereby enhancing MBC diagnostic yield and underscoring the diagnostic capability of GS in rare cancers with obscure etiology.

In addition, we identified 18 VUS in 16 patients who were yet to be diagnosed with P/LP variants (Table 2). These VUS included likely gene-disruptive variants in known monogenic hereditary cancer susceptibility genes and SVs/CNVs intersecting genes implicated in tumorigenesis. The clinical significance of these variants is not yet fully understood and further investigation is required to determine their impact on breast cancer risk. Based on their projected influence, these variants were classified as tier 2 or tier 3.

Implications of homology recombination deficiency in MBC

Two distinct deleterious variants were identified in *BARD1* in MBC043 and MBC011 (Table 2). *BARD1* is a moderate-risk susceptibility gene in FBC,[39] but its involvement in

Table 1 Participant characteristics

Participant	Age of Onset	Family History	AJCC Stage	Histology	Grade	Molecular Subtype	Ki-67	*BRCA1/2* Status
MBC002	56	None	IIA	IDC	II	HR-pos/HER2-neg	40%	Negative
MBC003	75	None	IA	IDC	II	HR-pos/HER2-neg	10%	(*BRCA2*) NM_000059.3:c.7007G>A p.(Arg2336His) NC_000013.11:g.32316461G>A
MBC005	63	Ovarian cancer (mother)	IA	IDC	II	HR-pos/HER2-neg	30%	Negative
MBC006	67	None	IA	IDC	II	HR-pos/HER2-neg	30%	Negative
MBC007	67	None	IA	IDC	II	HR-pos/HER2-neg	20%	Negative
MBC011	68	Leukemia (mother)	IIIA	IDC	II	HR-pos/HER2-neg	25%	Negative
MBC016	63	None	0	DCIS	I	HR-pos/HER2-neg	8%	Negative
MBC017	53	None	IA	IDC	III	HR-pos/HER2-pos	30%	Negative
MBC022	53	None	IIA	IDC	II	HR-pos/HER2-Neg	40%	Negative
MBC023	58	None	IIIC	IDC	III	HR-pos/HER2-neg	40%	Negative
MBC024	76	Ovarian cancers (2 daughters)	IIIC	IDC	II	HR-pos/HER2-neg	25%	(*BRCA1*) NM_007294.3:c.5095C>T p.(Arg1699Trp) NC_000017.11:g.43045729C>T
MBC025	53	Breast cancer (mother)	IIA	IDC	II	HR-pos/HER2-neg	20%	Negative
MBC026	40	None	IIIA	IDC	III	HR-pos/HER2-pos	60%	Negative
MBC032	64	None	0	DCIS	NA	NA	NA	Negative
MBC039	34	None	IIA	Mucinous	I	HR-pos/HER2-neg	5%	Negative
MBC040	71	None	IIIC	IDC	III	HR-pos/HER2-neg	80%	Negative
MBC041	46	Lung cancer (father)	IA	IDC	I	HR-pos/HER2-neg	30%	Negative
MBC042	49	Lymphoma (uncle)	IV	IDC	III	TNBC	NA	Negative
MBC043	43	None	IIA	IDC	II	TNBC	30%	Negative
MBC044	57	None	IA	Mucinous	I	HR-pos/HER2-neg	NA	Negative
MBC045	54	None	IIA	IDC	I	HR-pos/HER2-neg	10%	Negative
MBC046	56	Gastric cancer (father)	IIIA	IDC	III	HR-pos/HER2-neg	60%	Negative
MBC047	55	Lung cancer (father)	IIB	IDC	II	HR-pos/HER2-neg	70%	Negative
MBC051	61	Breast cancer (sister)	IIB	IDC	II	HR-pos/HER2-neg	30%	(*BRCA1*) NM_007294.3:c.5401A>T p.(Lys1801Ter) NC_000017.11:g.43071077A>T

AJCC, American joint committee on cancer; *DCIS*, ductal carcinoma in situ; *HER2*, human epidermal growth factor receptor 2; *HR*, hormone receptor; *IDC*, invasive ductal carcinoma; *MBC*, male breast cancer; *neg*, negative; *pos*, positive; *TNBC*, triple-negative breast cancer.

MBC has remained unknown. The ultrarare (*BRCA1*) NM_007294.3:c.2155A>G p.(Thr719Ala) variant is located in the *BRCA1* C-terminal domain (gnomAD frequency 0.001%), critical for the recruitment and retention of the *BRCA1-BARD1* heterodimer to DNA damage site.[40] Functional analysis demonstrated the existence of homologous recombination deficiency (HRD) due to the variant.[41] Person MBC011 harbors the novel *BARD1* CNV with breakpoints in deep introns, resulting in a frameshift deletion of exon 6, beyond the detection capability of conventional techniques (Supplemental Figures 3 and 4). We further validated this deletion by real-time quantitative polymerase chain reaction (Supplemental Figure 5).

Additionally, an ultrarare missense variant ([*BRIP1*] NM_032043.3:c.1208G>A p.[Arg403Gln]; gnomAD frequency 0.003%) was identified in the *BRIP1* gene in person MBC025, documented in a Latin American female affected with breast cancer.[42] *BRIP1* functions in conjunction with *BRCA1* and the variant is predicted to be likely gene disruptive, providing evidence for the *BRCA1*-related HRD in developing MBC. Notably, the patient's mother, with a history of invasive breast cancer, harbored the same variant, whereas his unaffected daughter did not carry it (Supplemental Method, Supplemental Figure 6). This cosegregation suggests that this *BRIP1* variant is a potential explanation for the increased breast cancer susceptibility within the pedigree.

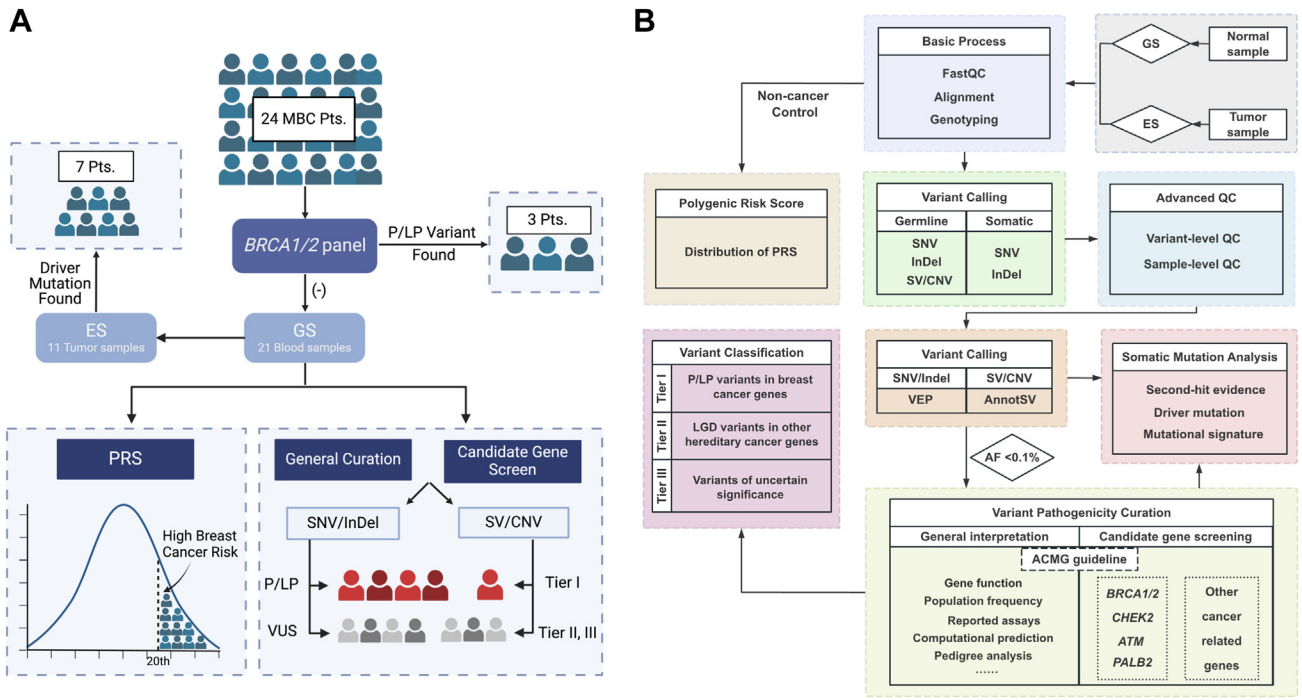

Figure 1 Schematic of the analytical workflow for elucidating genetic predisposition in male breast cancer. A. Overall workflow of the study, commencing with participant recruitment and culminating with the discernment of their diagnostic outcomes. It incorporates multiple stages of genetic predisposition evaluation, including identification of rare germline pathogenic variants, gathering somatic mutation evidence, and polygenic risk assessment. B. Rigorous data analysis pipeline used for the processing and interpretation of next-generation sequencing data. Each step is crucial in ensuring the accuracy and reliability of the derived genomic information. AF, allele frequency; LGD, likely gene-disruptive; P/LP, pathogenic/likely pathogenic; Pts, patients; QC, quality control; VUS, variant of uncertain significance.

Person MBC022 was found to harbor a nonsense variant ([*ATR*] NM_001184.4:c.5605C>T p.[Gln1869Ter]) in the middle of the *ATR* gene, working with *ATM* to initiate a complex network of DNA repair pathways, including homologous recombination.[43] Although variants in *ATM* are frequently associated with MBC, *ATR* variants are rarely observed and have only been reported in MBC tumor tissues.[37,44]

ATR activates *CHEK1* (HGNC: 1925) downstream, whereas *CHEK2* is activated downstream of *ATM*.[45,46] Another person, MBC045, was found to have a splicing variant in the intron 3 of *CHEK2* ([*CHEK2*] NM_145862.2:c.444+3A>C). This variant was predicted to be splice-disrupting by *SpliceAI*,[47] suggesting the high potential to alter messenger RNA and lead to effective loss of *CHEK2* function. Collectively, our results underscored the critical involvement of HRD in MBC development.

Identification of VUS and potential susceptibility loci in MBC

To capture potential genetic predisposition beyond routine curation, we conducted a more specific screening for rare variants in 78 hereditary cancer-related genes (Supplemental Method). Within this genomic spectrum, several VUS emerged in patients without a prior molecular diagnosis.

An experimental validated missense variant in *MSH2* (HGNC: 7325) ([*MSH2*] NM_000251.2:c.14C>A p.[Pro5Gln]),[48] a DNA mismatch repair gene with established association in Lynch syndrome,[49,50] was discovered in an early-onset patient aged 34 (MBC039). Histological examination revealed a mucinous carcinoma (Table 1), a rare subtype in breast cancer but common in Lynch-associated colorectal cancer.[51] Despite its inheritance from a healthy mother attenuating the penetrance (Supplemental Figure 7), the histological alignment suggests a possible role in MBC etiology, contrarily to previous studies negating the association of MSH2 with FBC risk.[52]

In another patient with triple-negative breast cancer, MBC042, with a family history of cancer (lymphoma in an uncle), we identified a novel missense variant in the *PTCH1* gene (HGNC: 9585) ([*PTCH1*] NM_000264.4:c.3346G>C p.[Val1116Leu]), a tumor suppressor gene associated with basal cell nevus syndrome. A more comprehensive pedigree cosegregation analysis is required to determine the pathogenicity of this *PTCH1* variant.

Other VUS with more ambiguous clinical significance were classified as tier 3, including VUS in cancer-associated genes and CNVs of uncertain significance (Table 2). Among these, *PALLD* (HGNC: 17068), previously implicated with familial pancreatic cancer,[53,54] emerged as a potential MBC susceptibility gene. Detected variants include 1 nonsense, 2 novel missense, and a recurrent microdeletion in *PALLD*

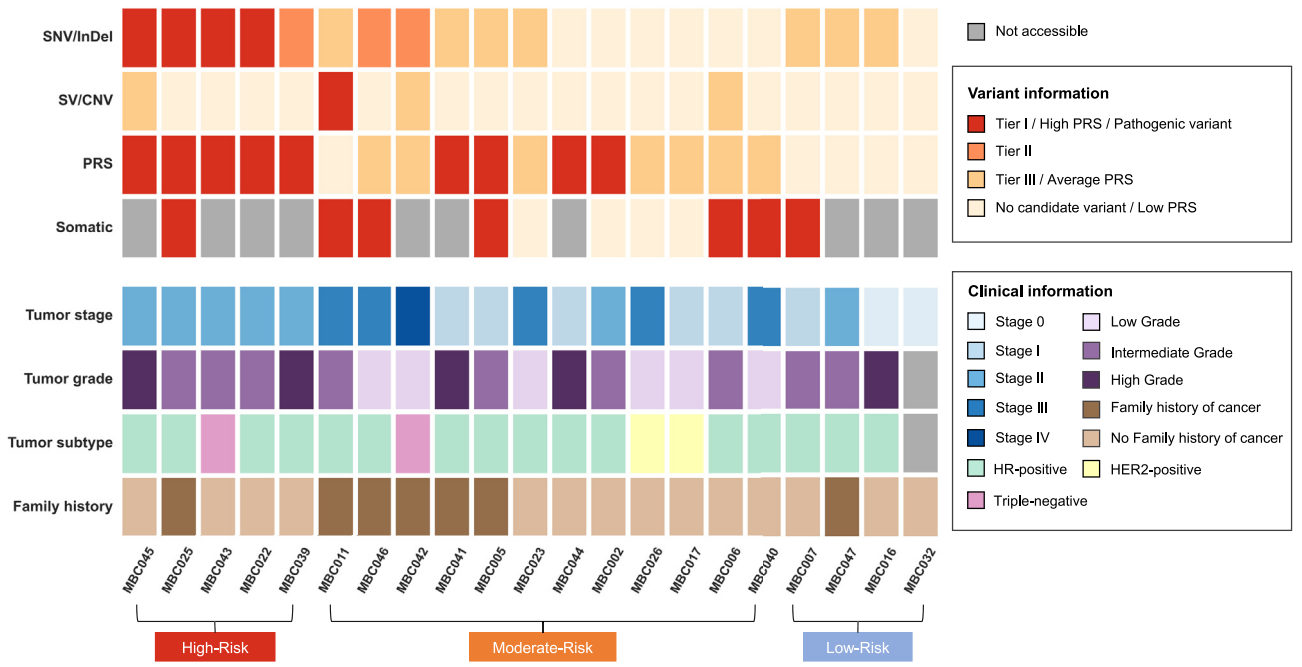

Figure 2 Integrated genomic and clinical landscape of patients with male breast cancer (MBC). This figure displays an intricate heatmap amalgamating genetic variant data and clinical features for individual MBC participants (represented by columns). Variant data include single-nucleotide variants, small insertions/duplications, copy-number variations, structural variations sorted into diagnostic tiers 1 to 3, polygenic risk scores, and somatic variants and are exhibited in a shared color gradient ranging from red to light yellow. These data are utilized to classify patients into 3 risk categories of MBC, reflecting different levels of genetic predisposition from high to low. Clinical parameters are concurrently illustrated, encompassing tumor stage, grade, subtype, and a documented family history of cancer. Gray cells designate samples for which data could not be obtained.

(Table 2), displaying a significant enrichment in our MBC cohort and indicating a likely association with MBC development.

Furthermore, we identified 2 novel CNVs in person MBC045, carrying a *CHEK2* splice variant classified as tier 1 (Table 2). Validated through sequencing methods, the presence of homologies surrounding these CNV breakpoints reflects the distinct mechanisms underpinning these nonrecurrent CNVs[55] (Supplemental Figure 8), supporting the genomic instability resulting from the impaired function of *CHEK2*. Specifically, the 19q13.3 deletion contains *BAX* (HGNC: 959), an apoptosis activator in *P53*-mediated apoptosis,[56] whereas the 17p13.3 duplication solely encompasses *YWHAE* (HGNC:12851). Both of *BAX* and *YWHAE* are reported to exhibit aberrant expression in breast cancer cells correlating with the respective copy number in our patient, impacting tumorigenesis and tumor growth.[57,58] Given that the pathogenicity of *CHEK2* variants is still controversial, the 2 CNVs serve as potential supplemental causal factors for person MBC045.

Contribution of common variants to genetic susceptibility in patients with MBC

To provide a comprehensive evaluation of the genetic predisposition, we implemented a classic PRS model for FBC to quantify the baseline breast cancer susceptibility in a polygenetic view. Our data suggest that MBC individuals exhibited a significantly greater average PRS than the control group ($P = .04$, 2-sample t test). This persisted even after gender-related confounding factors were controlled. (Figure 3A).

To investigate whether the monogenic factors influence PRS, we conducted subgroup analyses based on the carrying status of tier 1 or tier 2 variants. There were no significant differences in PRS values or age of onset between the 2 subgroups. However, we found that person MBC011, who harbored a *BARD1* microdeletion, exhibited an extremely low PRS of −0.45, indicating that the deletion may serve as a strong causal factor to override the protective effect of a low PRS. Excluding MBC011, we observed a statistically significant increase in PRS values and a decrease in the age of onset in molecular diagnosed patients compared with the undiagnosed ones ($P = 5.5 \times 10^{-5}$ and .03, 2-sample t test) (Figure 3B). No deviation in PRS distributions was observed when comparing germline P variant heterozygotes with nonheterozygotes among controls (Supplemental Figure 9, Supplemental Method).

Another subgroup analysis was performed further to investigate the interplay between monogenic and polygenic predispositions. MBC participants were stratified into 3 risk groups, each representing a distinct level of overall germline genetic susceptibility (Figure 2). Those with tier 1 or tier 2

Table 2 Variants in *BRCA1/2*-negative patients with MBC

Tier	Variant	Type	Gene	Patients	Inheritance
Tier 1	(*ATR*) NM_001184.4:c.5605C>T p.(Gln1869Ter) NC_000003.12:g.142495446C>T	stop gained	*ATR*	MBC022	NA
	(*BARD1*) NM_000465.4:c.2155A>G p.(Thr719Ala) NC_000002.12:g.215740789A>G	missense variant	*BARD1*	MBC043	NA
	(*BRIP1*) NM_032043.3:c.1208G>A p.(Arg403Gln); NC_000017.11:g.59877497G>A	missense variant	*BRIP1*	MBC025	Maternal
	(*CHEK2*) NM_145862.2:c.444+3A>C p.? NC_000022.11:g.29091855A>C	splice region variant	*CHEK2*	MBC045	NA
	(*BARD1*) NM_000465.4:c.1568+6443_1677+275del p.? NC_000002.12:g.214752175_214761042del	deletion	*BARD1*	MBC011	NA
Tier 2	(*MSH2*) NM_000251.3:c.14C>A p.(Pro5Gln) NC_000002.12:g.47630135C>A	missense variant	*MSH2*	MBC039	Maternal
	(*EXT2*) NM_207122.2:c.1495+2T>A p.? NC_000011.10:g.44715747T>A	splice donor variant	*EXT2*	MBC046	NA
	(*PTCH1*) NM_000264.5:c.3346G>C p.(Val1116Leu) NC_000009.12:g.98206734G>C	missense variant	*PTCH1*	MBC042	NA
Tier 3	(*NF1*) NM_000267.3:c.3469G>A p.(Val1157Ile) NC_000017.11:g.31232854G>A	missense variant	*NF1*	MBC023	NA
	(*MSH6*) NM_000179.3:c.1828A>G p.(Lys610Glu) NC_000002.12:g.47799811A>G	missense variant	*MSH6*	MBC005	NA
	(*PMS2*) NM_000535.7:c.2374G>A p.(Asp792Asn) NC_000007.14: g.5977659C>T	missense variant	*PMS2*	MBC007	NA
	(*PALLD)* NM_016081.4:c.160G>A p.(Glu54Lys) NC_000004.12:g.168511664G>A	missense variant	*PALLD*	MBC047	NA
	(*PALLD*) NM_016081.4:c.139C>T p.(Arg47Trp) NC_000004.12:g.168511643C>T	missense variant	*PALLD*	MBC016	NA
	(*PALLD*) NM_016081.4:c.3313C>T p.(Arg1105Ter) NC_000004.12:g.168925238C>T	stop gained	*PALLD*	MBC041	NA
	(*PTEN*) NM_000314.8:c.*75dup p.? NC_000010.11:g.87965547dup	3 prime UTR variant	*PTEN*	MBC011	NA
	(*TSC1*) NM_000368.5:c.937T>C p.(Ser313Pro) NC_000009.12:g.132911545A>G	missense variant	*TSC1*	MBC007	NA
	(*TSC2*) NM_000548.5:c.41A>T p.(Lys14Met) NC_000016.10:g.2048656A>T	missense variant	*TSC2*	MBC022	NA
	seq[GRCh38] dup(17)(p13.3p13.3) NC_000017.11:g.1332001_1358000dup	duplication	*YWHAE*	MBC045	NA
	seq[GRCh38] del(19)(q13.33) NC_000019.10:g.48942291_48988057del	deletion	19q13.3	MBC045	NA
	seq[GRCh38] inv(22)(q12.3) NC_000022.11:g.28736344_28736439inv	inversion	*CHEK2*	MBC007	NA
	seq[GRCh38] del(4)(q34.3) NC_000004.12:g.168698304_168700077del	deletion (intron)	*PALLD*	MBC006,MBC042	NA
	seq[GRCh38] dup(17)(q12q21.2) NC_000017.11:g.30757959_31963727dup	duplication	17q11.2	MBC006,MBC011	NA
	seq[GRCh38] del(2)(p25.3) NC_000002.12:g.10442173_10443229del	deletion	*ODC1*	MBC006	NA

DEL, deletion; *DUP*, duplication; *INV*, inversion; *UTR*, untranslated region; *MBC*, male breast cancer; *NA*, unknown inheritance.

variants and a high PRS (exceeding the top 20th percentile of the in-house population PRS distribution) were categorized as a high-genetic-risk group, representing a strong overall genetic predisposition. Conversely, patients with low PRS scores (below the bottom 20th percentile of the population PRS distribution) and no deleterious germline variants were assigned to the low-genetic-risk group, whereas those falling outside these criteria were assigned to the moderate-genetic-risk group.

Participants in the high-genetic-risk group had a significantly earlier age of onset compared with those in the low-genetic-risk and moderate-genetic-risk groups ($P = .003$ and .01, respectively; 2-sample t test) (Figure 3C), underscoring the importance of both a polygenic background and rare monogenic variant in determining the comprehensive genetic risk profile of MBC. Moreover, patients in the low-genetic-risk group predominantly exhibited tumors at early stages, majorly ductal carcinoma in situ/stage 0 or Ia

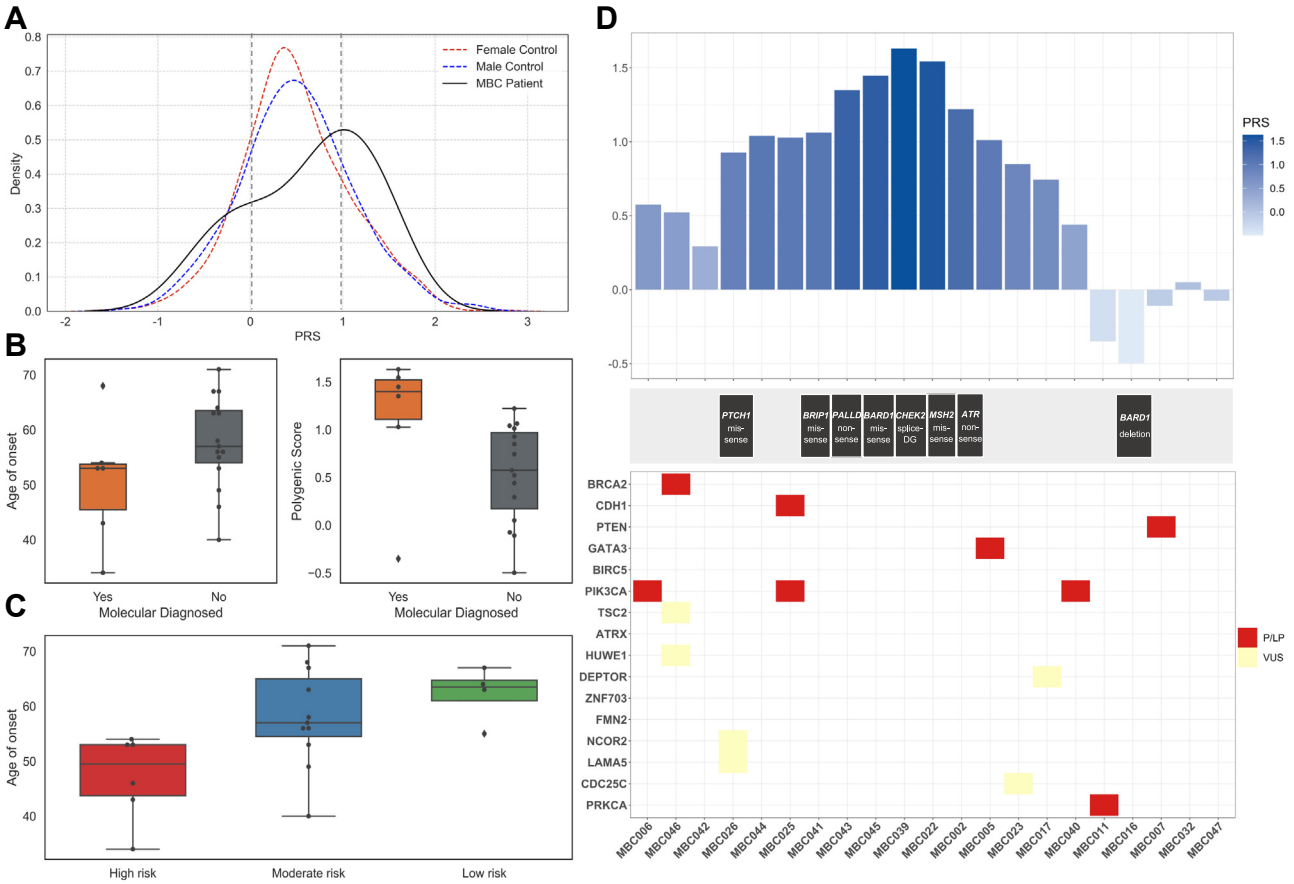

Figure 3 Mutation profiling and polygenic risk score (PRS) analysis. A. A visualization comparing PRS distribution among MBC participants with sex-segregated controls: red for females and blue for males. The 2 vertical dashed lines indicate the top and bottom 20th percentile of the PRS control distribution, with values of 0.0289 and 0.970, respectively. B and C. Boxplots of subgroup comparison. (B) shows the different distribution of onset age (left) and polygenic score (right) distributions between 2 subgroups: "molecular diagnosed" (orange) and " molecular undiagnosed" (gray) patients. (C) shows age distribution across different genetic risk groups, illustrated by different colored boxplots. Individual data points are shown in gray. D. A combined representation of PRS (top histogram), germline molecular diagnosis (middle panel), somatic mutation status (bottom heatmap). Sample IDs are shared on the *x*-axis to facilitate the concurrent interpretation of PRS and mutational patterns within each individual. MBC, male breast cancer.

(Figure 2, Table 1), indicating that genetic predisposition correlates not only with tumorigenesis but also tumor behavior, grading, and prognosis. Taken together, our results highlighted the intricate relationship between monogenic and polygenic predispositions in determining the genetic risk profile of MBC.

Somatic mutational profile enhance understanding of MBC tumorigenesis and personalized therapy

To elaborate on the germline findings, we conducted somatic mutational analysis on 11 patients with available cancer tissue samples following germline variant curation (Supplemental Method). We initially ascertain tumor-specific biallelic inactivation via loss of heterozygosity or second-hit somatic mutations in patients with putative germline variants to investigate the effect model of P variants in tumorigenesis. However, we did not identify any definitive instances of somatic biallelic inactivation.

Subsequently, a comprehensive screening of 238 oncogenes and tumor suppressor genes in breast cancer revealed driver mutations in well-established genes, including *CDH1*, *GATA3*, *PTEN, PIK3CA* (HGNC: 1748, 4172, 9588, 8975), and *BRCA2*, providing important implications for personalized therapies for patients (Supplemental Tables 4 and 5). Notably, a majority of these patients exhibited no deleterious germline variant or an extremely high PRS (Figure 3D). Remarkably, 3 patients were found to harbor P variants at the same position in *PIK3CA* ([*PIK3CA*] NM_006218.4:c.3140A>G p.[His1047Arg] and c.3140A>T p.[His1047Leu]), supporting its mutational hotspot relevance in *BRCA1/2*-negative MBC.[59,60]

To probe into the specific mutational patterns and underlying etiologies in MBC, we performed mutational signature analysis (Supplemental Method). A striking degree of homogeneity in mutational signature composition was observed in our MBC samples (Figure 4A and B). Compared with known signatures, SBS89 signature emerged as the most dominant contributor in the mutational signature spectrum

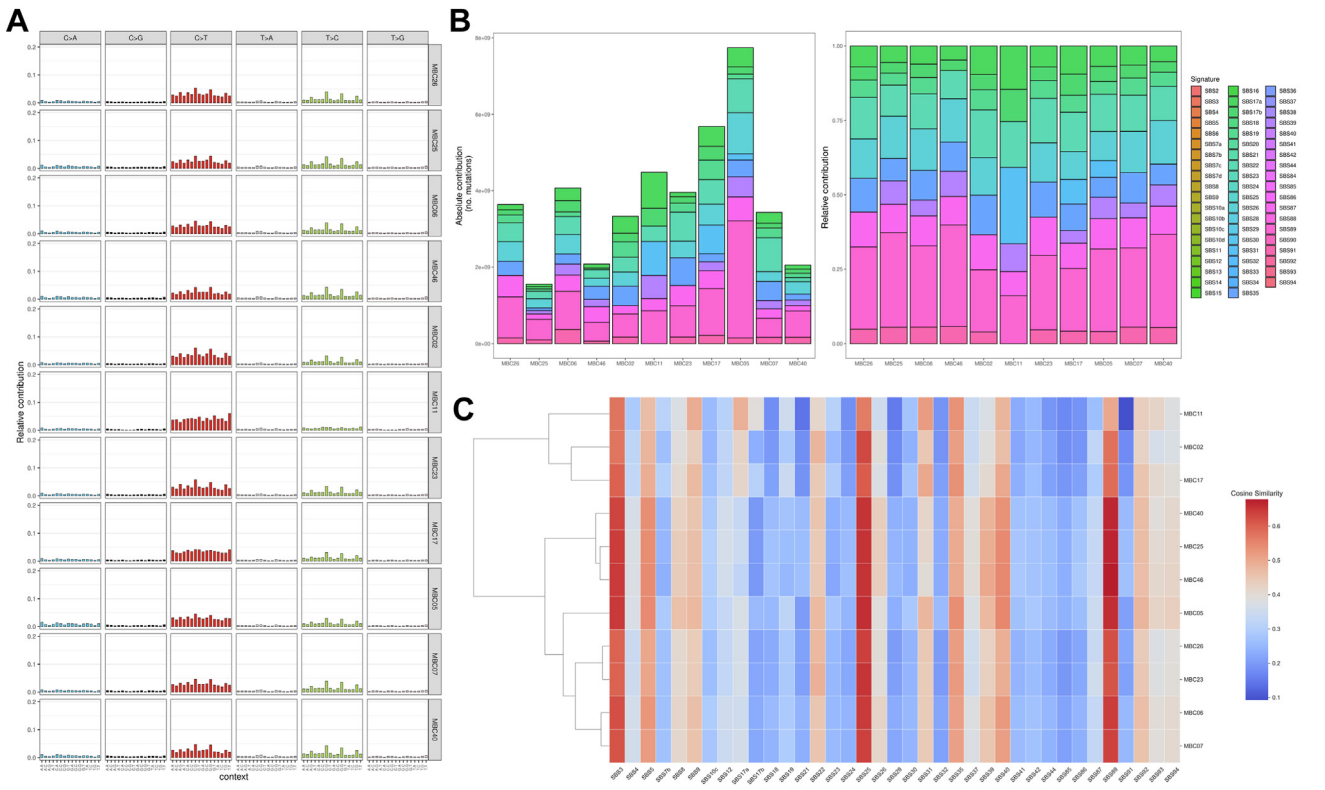

Figure 4 Mutational signature analysis of patients with male breast cancer. A. Display of the 96 SNV mutational signature profile, classified based on the conventional mutation type categorization. B. Bar graphs demonstrate the absolute and relative contributions of each mutational signature, compared with established Catalogue of Somatic Mutations in Cancer signatures (v3.3). C. The heatmap displays a correlation matrix between individual mutational profiles derived from each MBC participant and the known Catalogue of Somatic Mutations in Cancer signatures. Color gradation corresponds to the degree of cosine similarity, with warmer color indicating higher similarity. The participants are hierarchically clustered based on the similarity of their mutational signatures, revealing groups with similar mutational processes. MBC, male breast cancer.

across all samples, second to SBS25. Despite the limited understanding of its etiology, the SBS89 signature has been demonstrated to be most active during the first decade of life in normal colorectal epithelial cells.[61] Compared with the signature landscape of FBC,[62] the SBS17 and SBS26 were consistent, but rare in FBC samples (<5%) and common in our MBC samples (>90%). (Figure 4B, Supplemental Table 6). Moreover, the SBS3 signature, a well-documented biological footprint and efficient indicator of HRD,[62,63] exhibited striking similarities in the mutational patterns across most cases (Figure 4C), reinforcing the significance of HRD in MBC tumorigenesis, suggesting the utility of poly (adenosine diphosphate-ribose) polymerase inhibitors[64] and potential great benefit from platinum-based therapy.[65]

Discussion

The rarity and heterogeneity of MBC confines our understanding of its biological and genetic basis. To elucidate this under-explored genetic architecture, our study leverages the comprehensive genomic information offered by GS.

Initially, the *BRCA1/2*-target testing achieved a 12.5% diagnostic yield, addressing the clinical importance of utilizing target panels for efficient and cost-effective detection of P variants in MBC. Subsequent analyses using GS and adopting a 3-tier classification system, expanded the understanding of the genetic landscape of MBC beyond the conventional *BRCA1/2* spectrum, resulting in an aggregate diagnosis yield of 33.0%. The identified P/LP variants, alongside variants of potential clinical significance, including 30.4% SVs/CNVs, 8.7% splice site variants, and 4.3% noncoding variants, affirming the unparalleled detection proficiency of GS. Identified susceptibility genes for MBC, such as *ATR*, *BARD1*, *BRIP1*, and *CHEK2*, predominantly participate in the homologous recombination pathway, underlying the HRD-related tumorigenesis mechanism in *BRCA1/2*-negative MBC.

Furthermore, our investigation into PRS highlights the interplay between monogenic and polygenic susceptibility. The higher average PRS found in patients with MBC than the noncancer controls, inferred a significant contribution from polygenic common variants to MBC risk, supplementing the effects of rare monogenic variants. The strong association with high PRS and germline P variant in MBC further indicated the interaction and relevance between the monogenic and polygenic impact. Notably, patients harboring both a high PRS and a high-penetrance germline

P variant demonstrated a significantly earlier age of onset compared with the patients without either factor, demonstrating an overall genetic risk model comprising monogenic and polygenic predispositions. A similar phenomenon has been observed in FBC with P *BRCA1/2* variants,[66,67] our study corroborates and extends it to *BRCA1/2*-negative patients with MBC.

Incorporating genetic analysis of tumor tissue can offer important insights into the pathogenicity of germline variants.[19,68] Yet, this has not been explored in the diagnostic evaluation of rare cancers. To bridge this gap, we further performed somatic mutational analyses. For the majority, whose genetic predisposition remains unattributed to a germline P variant, high PRS, or familial history, a P somatic driver mutation was discovered, potentially instigated by aging. The *PIK3CA* hotspot emerged as the most common driver mutation, suggesting implications of target therapies for these patients with MBC. Mutational signatures in these patients manifested a dominant contribution of SBS89, a signature specific to early cellular age, suggesting that its high activity in later stages of life may potentially linked to age-related cancer development. Moreover, the prevalent SBS3 signature in our *BRCA1/2*-negative patients reinforced the hypothesis of its independence from germline *BRCA1/2* P variants[69,70] and emphasized the role of HRD in MBC development again.

Our analysis workflow can be applied to other rare cancers to decode their hereditary predisposition, particularly when clinical testing criteria are absent. However, there are some limitations. First, owing to low incidence of MBC, our sample size was limited. Second, following guidelines, we did not perform GS on *BRCA1/2*-positive patients, resulting in a gap in the PRS distribution for these individuals. The complex interaction between monogenic and polygenic predispositions in patients with MBC warrants further systematic investigation. Third, material constraints from tumor tissue permitted only ES analysis, rather than the more comprehensive GS, RNA sequencing or single-cell GS to further explore somatic mutations, which leads to an inconclusive result regarding our negative second-hit evidence. Lastly, due to the advanced onset ages of the patients with MBC (around 60 years old), trio (parental) validation was largely unavailable for our sample. Future research should account for these limitations and explore approaches to supplement or verify data in which trio validations or GS are unattainable.

Conclusion

Our investigation has underscored the effectiveness and promising potential of GS in elucidating the complex genetic landscape of MBC beyond *BRCA1/2*. The findings highlight the clinical utility of multidimensional analysis in enhancing diagnostic yield and risk assessment and informing personalized treatment strategies of rare cancers, laying a foundation for advanced research and precision medicine in MBC.

Data Availability

The supplemental data supporting this study's findings are available in the supplement. Deidentified participant data and analytic code are available upon request to Dr Jiaqi Liu (j.liu@cicams.ac.cn).

Acknowledgments

This study is part of the Genetic investigation of Inherited and Familial Tumor Syndrome study (GIFTS, the Chinese Clinical Trial Registry number ChiCTR1900024050). The authors thank all the individuals, families, and physicians involved in the study for their participation. The authors also thank Mr Haoxiang Xu for providing valuable assistance in the implementation and analysis of the Polygenic Risk Score model, contributing to the statistical approach and interpretation of the genetic data.

Funding

This research was funded in part by National Natural Science Foundation of China (82272938 to Jiaqi Liu), Beijing Nova Program (20220484059 to Jiaqi Liu), the CAMS Innovation Fund for Medical Sciences (2021-I2M-1-014 to Jiaqi Liu), the Beijing Hope Run Special Fund (LC2020B05 to Jiaqi Liu), and the Beijing Science and Technology Innovation Foundation for University or College students (2023zglc06013 to Yiwen Jiang).

Author Contributions

Conceptualization: J. Liu, W.W., S.Z.; Data Curation: W.W., S.Z., J. Liu; Formal Analysis: W.W., C.O.; Investigation: W.W., Y.J., C.G., Z.J., J. Li; Project Administration: N.W., X.W.; Supervision: J. Liu, N.W.; Funding Acquisition: J. Liu, Y.J.; Methodology: W.W., S.Z.; Software: Y.H., H.X., P.P.; Validation: Y.J., T.S., L.C.; Visualization: W.W., Y.J.; Writing-original draft: W.W., S.Z., Y.J.; Writing-review and editing: J. Liu, N.W., X.W.

Ethics Declaration

This study was reviewed and approved by the ethics committees at the Cancer Hospital of the Chinese Academy of Medical Sciences. Written informed consent was obtained from each participant.

Conflict of Interest

All authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gimo.2024.101899) contains supplemental material, which is available to authorized users.

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References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. 2023;73(1):17-48. http://doi.org/10.3322/caac.21763
2. DeSantis CE, Kramer JL, Jemal A. The burden of rare cancers in the United States. *CA Cancer J Clin*. 2017;67(4):261-272. http://doi.org/10.3322/caac.21400
3. Gatta G, Capocaccia R, Botta L, et al. Burden and centralised treatment in Europe of rare tumours: results of RARECAREnet-a population-based study. *Lancet Oncol*. 2017;18(8):1022-1039. http://doi.org/10.1016/S1470-2045(17)30445-X
4. Simon R, Roychowdhury S. Implementing personalized cancer genomics in clinical trials. *Nat Rev Drug Discov*. 2013;12(5):358-369. http://doi.org/10.1038/nrd3979
5. Tan AC, Huang PH. Translational genomics for rare cancers: challenges and opportunity. *Semin Cancer Biol*. 2020;61:iii-iv. http://doi.org/10.1016/j.semcancer.2020.02.014
6. Jahn A, Rump A, Widmann TJ, et al. Comprehensive cancer predisposition testing within the prospective Master trial identifies hereditary cancer patients and supports treatment decisions for rare cancers. *Ann Oncol*. 2022;33(11):1186-1199. http://doi.org/10.1016/j.annonc.2022.07.008
7. Fox S, Speirs V, Shaaban AM. Male breast cancer: an update. *Virchows Arch*. 2022;480(1):85-93. http://doi.org/10.1007/s00428-021-03190-7
8. Ferzoco RM, Ruddy KJ. The epidemiology of male breast cancer. *Curr Oncol Rep*. 2016;18(1):1. http://doi.org/10.1007/s11912-015-0487-4
9. Xu S, Liu Y, Zhang T, et al. The global, regional, and national burden and trends of breast cancer from 1990 to 2019: results from the global burden of disease Study 2019. *Front Oncol*. 2021;11:689562. http://doi.org/10.3389/fonc.2021.689562
10. Kurian AW, Abrahamse P, Furgal A, et al. Germline genetic testing after cancer diagnosis. *JAMA*. 2023;330(1):43-51. http://doi.org/10.1001/jama.2023.9526
11. Ding YC, Steele L, Kuan C-J, Greilac S, Neuhausen SL. Mutations in BRCA2 and PALB2 in male breast cancer cases from the United States. *Breast Cancer Res Treat*. 2011;126(3):771-778. http://doi.org/10.1007/s10549-010-1195-2
12. Ottini L, Masala G, D'Amico C, et al. BRCA1 and BRCA2 mutation status and tumor characteristics in male breast cancer: a population-based study in Italy. *Cancer Res*. 2003;63(2):342-347.
13. Samadder NJ, Riegert-Johnson D, Boardman L, et al. Comparison of universal genetic testing vs guideline-directed targeted testing for patients with hereditary cancer syndrome. *JAMA Oncol*. 2021;7(2):230-237. http://doi.org/10.1001/jamaoncol.2020.6252
14. Xiao W, Ren L, Chen Z, et al. Toward best practice in cancer mutation detection with whole-genome and whole-exome sequencing. *Nat Biotechnol*. 2021;39(9):1141-1150. http://doi.org/10.1038/s41587-021-00994-5
15. Staaf J, Glodzik D, Bosch A, et al. Whole-genome sequencing of triple-negative breast cancers in a population-based clinical study. *Nat Med*. 2019;25(10):1526-1533. http://doi.org/10.1038/s41591-019-0582-4
16. Fang LT, Zhu B, Zhao Y, et al. Establishing community reference samples, data and call sets for benchmarking cancer mutation detection using whole-genome sequencing. *Nat Biotechnol*. 2021;39(9):1151-1160. http://doi.org/10.1038/s41587-021-00993-6
17. Pipis M, Rossor AM, Laura M, Reilly MM. Next-generation sequencing in Charcot-Marie-Tooth disease: opportunities and challenges. *Nat Rev Neurol*. 2019;15(11):644-656. http://doi.org/10.1038/s41582-019-0254-5
18. Turro E, Astle WJ, Megy K, et al. Whole-genome sequencing of patients with rare diseases in a national health system. *Nature*. 2020;583(7814):96-102. http://doi.org/10.1038/s41586-020-2434-2
19. Chen B, Zhang Y, Dai S, et al. Molecular characteristics of primary pulmonary lymphoepithelioma-like carcinoma based on integrated genomic analyses. *Signal Transduct Target Ther*. 2021;6(1):6. http://doi.org/10.1038/s41392-020-00382-6
20. Souche E, Beltran S, Brosens E, et al. Recommendations for whole genome sequencing in diagnostics for rare diseases. *Eur J Hum Genet*. 2022;30(9):1017-1021. http://doi.org/10.1038/s41431-022-01113-x
21. Boycott KM, Hartley T, Biesecker LG, et al. A diagnosis for all rare genetic diseases: the horizon and the next frontiers. *Cell*. 2019;177(1):32-37. http://doi.org/10.1016/j.cell.2019.02.040
22. Gong J, Gregg JP, Ma W, et al. Squamous cell transformation of primary lung adenocarcinoma in a patient With EML4-ALK fusion variant 5 refractory to ALK Inhibitors. *J Natl Compr Canc Netw*. 2019;17(4):297-301. http://doi.org/10.6004/jnccn.2019.7291
23. Liu Y, Wang H, Wang X, et al. Prevalence and reclassification of BRCA1 and BRCA2 variants in a large, unselected Chinese Han breast cancer cohort. *J Hematol Oncol*. 2021;14(1):18. http://doi.org/10.1186/s13045-020-01010-0
24. Chen N, Zhao S, Jolly A, et al. Perturbations of genes essential for Müllerian duct and Wölffian duct development in Mayer-Rokitansky-Küster-Hauser syndrome. *Am J Hum Genet*. 2021;108(2):337-345. http://doi.org/10.1016/j.ajhg.2020.12.014
25. Zhao S, Zhang Y, Chen W, et al. Diagnostic yield and clinical impact of exome sequencing in early-onset scoliosis (EOS). *J Med Genet*. 2021;58(1):41-47. http://doi.org/10.1136/jmedgenet-2019-106823
26. Wen W, Zhao Z, Zheng Z, et al. Rare variant association analyses reveal the significant contribution of carbohydrate metabolic disturbance in severe adolescent idiopathic scoliosis. *J Med Genet*. 2024;61(7):666-676. http://doi.org/10.1136/jmg-2023-109667

27. Robinson JT, Thorvaldsdóttir H, Winckler W, et al. Integrative genomics viewer. *Nat Biotechnol*. 2011;29(1):24-26. http://doi.org/10.1038/nbt.1754
28. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405-424. http://doi.org/10.1038/gim.2015.30
29. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet Med*. 2020;22(2):245-257. http://doi.org/10.1038/s41436-019-0686-8
30. Dreos R, Ambrosini G, Périer RC, Bucher P. The Eukaryotic Promoter Database: expansion of EPDnew and new promoter analysis tools. *Nucleic Acids Res*. 2015;43(Database issue):D92-D96. http://doi.org/10.1093/nar/gku1111
31. Amberger JS, Hamosh A. Searching Online Mendelian Inheritance in Man (OMIM): a knowledgebase of human genes and genetic phenotypes. *Curr Protoc Bioinformatics*. 2017;58:1.2.1-1.2.12. http://doi.org/10.1002/cpbi.27
32. Maguire S, Perraki E, Tomczyk K, et al. Common susceptibility loci for male breast cancer. *J Natl Cancer Inst*. 2021;113(4):453-461. http://doi.org/10.1093/jnci/djaa101
33. Kramer I, Hooning MJ, Mavaddat N, et al. Breast cancer polygenic risk score and contralateral breast cancer risk. *Am J Hum Genet*. 2020;107(5):837-848. http://doi.org/10.1016/j.ajhg.2020.09.001
34. Giorgi FM, Ceraolo C, Mercatelli D. The R language: an engine for bioinformatics and data science. *Life*. 2022;12(5):648. http://doi.org/10.3390/life12050648
35. Blokzijl F, Janssen R, Van Boxtel R, Cuppen E. MutationalPatterns: comprehensive genome-wide analysis of mutational processes. *Genome Med*. 2018;10(1):33. http://doi.org/10.1186/s13073-018-0539-0
36. Tate JG, Bamford S, Jubb HC, et al. COSMIC: the catalogue of somatic mutations in cancer. *Nucleic Acids Res*. 2019;47(D1):D941-D947. http://doi.org/10.1093/nar/gky1015
37. Campos FAB, Rouleau E, Torrezan GT, et al. Genetic landscape of male breast cancer. *Cancers*. 2021;13(14):3535. http://doi.org/10.3390/cancers13143535
38. Pritzlaff M, Summerour P, McFarland R, et al. Male breast cancer in a multi-gene panel testing cohort: insights and unexpected results. *Breast Cancer Res Treat*. 2017;161(3):575-586. http://doi.org/10.1007/s10549-016-4085-4
39. Cancer Association Consortium Breast, Dorling L, Carvalho S, et al. Breast cancer risk genes—association analysis in more than 113,000 women. *N Engl J Med*. 2021;384(5):428-439. http://doi.org/10.1056/NEJMoa1913948
40. Billing D, Horiguchi M, Wu-Baer F, et al. The BRCT domains of the BRCA1 and BARD1 tumor suppressors differentially regulate homology-directed repair and stalled fork protection. *Mol Cell*. 2018;72(1):127-139.e8. http://doi.org/10.1016/j.molcel.2018.08.016
41. Adamovich AI, Banerjee T, Wingo M, et al. Functional analysis of BARD1 missense variants in homology-directed repair and damage sensitivity. *PLoS Genet*. 2019;15(3):e1008049. http://doi.org/10.1371/journal.pgen.1008049
42. Tung N, Battelli C, Allen B, et al. Frequency of mutations in individuals with breast cancer referred for BRCA1 and BRCA2 testing using next-generation sequencing with a 25-gene panel. *Cancer*. 2015;121(1):25-33. http://doi.org/10.1002/cncr.29010
43. Menolfi D, Zha S. ATM, ATR and DNA-PKcs kinases-the lessons from the mouse models: inhibition ≠ deletion. *Cell Biosci*. 2020;10:8. http://doi.org/10.1186/s13578-020-0376-x
44. Di Benedetto A, Ercolani C, Mottolese M, et al. Analysis of the ATR-Chk1 and ATM-Chk2 pathways in male breast cancer revealed the prognostic significance of ATR expression. *Sci Rep*. 2017;7(1):8078.
45. Weber AM, Ryan AJ. ATM and ATR as therapeutic targets in cancer. *Pharmacol Ther*. 2015;149:124-138. http://doi.org/10.1016/j.pharmthera.2014.12.001
46. van Jaarsveld MTM, Deng D, Wiemer EAC, Zi Z. Tissue-specific Chk1 activation determines apoptosis by regulating the balance of p53 and p21. *Iscience*. 2019;12:27-40. http://doi.org/10.1016/j.isci.2019.01.001
47. Jaganathan K, Kyriazopoulou Panagiotopoulou S, McRae JF, et al. Predicting splicing from primary sequence with deep learning. *Cell*. 2019;176(3):535-548.e24. http://doi.org/10.1016/j.cell.2018.12.015
48. Arora S, Huwe PJ, Sikder R, et al. Functional analysis of rare variants in mismatch repair proteins augments results from computation-based predictive methods. *Cancer Biol Ther*. 2017;18(7):519-533. http://doi.org/10.1080/15384047.2017.1326439
49. Helder-Woolderink JM, Blok EA, Vasen HF, Hollema H, Mourits MJ, De Bock GH. Ovarian cancer in Lynch syndrome; a systematic review. *Eur J Cancer*. 2016;55:65-73. http://doi.org/10.1016/j.ejca.2015.12.005
50. Edwards P, Monahan KJ. Diagnosis and management of Lynch syndrome. *Frontline Gastroenterol*. 2022;13(e1):e80-e87. http://doi.org/10.1136/flgastro-2022-102123
51. Luo C, Cen S, Ding G, Wu W. Mucinous colorectal adenocarcinoma: clinical pathology and treatment options. *Cancer Commun (Lond)*. 2019;39(1):13. http://doi.org/10.1186/s40880-019-0361-0
52. Stoll J, Rosenthal E, Cummings S, Willmott J, Bernhisel R, Kupfer SS. No evidence of increased risk of breast cancer in women with Lynch syndrome identified by multigene panel testing. *JCO Precis Oncol*. 2020;4:51-60. http://doi.org/10.1200/PO.19.00271
53. Liotta L, Lange S, Maurer HC, et al. PALLD mutation in a European family conveys a stromal predisposition for familial pancreatic cancer. *JCI Insight*. 2021;6(8):e141532. http://doi.org/10.1172/jci.insight.141532
54. Pogue-Geile KL, Chen R, Bronner MP, et al. Palladin mutation causes familial pancreatic cancer and suggests a new cancer mechanism. *PLoS Med*. 2006;3(12):e516. http://doi.org/10.1371/journal.pmed.0030516
55. Carvalho CMB, Pehlivan D, Ramocki MB, et al. Replicative mechanisms for CNV formation are error prone. *Nat Genet*. 2013;45(11):1319-1326. http://doi.org/10.1038/ng.2768
56. Miyashita T, Krajewski S, Krajewska M, et al. Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene*. 1994;9(6):1799-1805.
57. Sturm I, Papadopoulos S, Hillebrand T, et al. Impaired BAX protein expression in breast cancer: mutational analysis of the BAX and the p53 gene. *Int J Cancer*. 2000;87(4):517-521. http://doi.org/10.1002/1097-0215(20000815)87:4<517::aid-ijc9>3.0.co;2-b
58. Yang YF, Lee YC, Wang YY, Wang CH, Hou MF, Yuan SF. YWHAE promotes proliferation, metastasis, and chemoresistance in breast cancer cells. *Kaohsiung J Med Sci*. 2019;35(7):408-416. http://doi.org/10.1002/kjm2.12075
59. Deb S, Do H, Byrne D, et al. PIK3CA mutations are frequently observed in BRCAX but not BRCA2-associated male breast cancer. *Breast Cancer Res*. 2013;15(4):R69.
60. Deb S, Wong SQ, Li J, et al. Mutational profiling of familial male breast cancers reveals similarities with luminal A female breast cancer with rare TP53 mutations. *Br J Cancer*. 2014;111(12):2351-2360. http://doi.org/10.1038/bjc.2014.511
61. Lee-Six H, Olafsson S, Ellis P, et al. The landscape of somatic mutation in normal colorectal epithelial cells. *Nature*. 2019;574(7779):532-537. http://doi.org/10.1038/s41586-019-1672-7
62. Nik-Zainal S, Davies H, Staaf J, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. *Nature*. 2016;534(7605):47-54. http://doi.org/10.1038/nature17676
63. Gulhan DC, Lee JJ-K, Melloni GEM, Cortés-Ciriano I, Park PJ. Detecting the mutational signature of homologous recombination deficiency in clinical samples. *Nat Genet*. 2019;51(5):912-919. http://doi.org/10.1038/s41588-019-0390-2
64. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*. 2005;434(7035):917-921. http://doi.org/10.1038/nature03445
65. Alsop K, Fereday S, Meldrum C, et al. BRCA mutation frequency and patterns of treatment response in BRCA mutation–positive women with

ovarian cancer: a report from the Australian Ovarian Cancer Study Group. *J Clin Oncol*. 2012;30(21):2654-2663. http://doi.org/10.1200/JCO.2011.39.8545

66. Barnes DR, Rookus MA, McGuffog L, et al. Polygenic risk scores and breast and epithelial ovarian cancer risks for carriers of BRCA1 and BRCA2 pathogenic variants. *Genet Med*. 2020;22(10):1653-1666. http://doi.org/10.1038/s41436-020-0862-x
67. Mars N, Widén E, Kerminen S, et al. The role of polygenic risk and susceptibility genes in breast cancer over the course of life. *Nat Commun*. 2020;11(1):6383. http://doi.org/10.1038/s41467-020-19966-5
68. Hill MC, Kadow ZA, Long H, et al. Integrated multi-omic characterization of congenital heart disease. *Nature*. 2022;608(7921):181-191. http://doi.org/10.1038/s41586-022-04989-3
69. Northcott PA, Buchhalter I, Morrissy AS, et al. The whole-genome landscape of medulloblastoma subtypes. *Nature*. 2017;547(7663):311-317. http://doi.org/10.1038/nature22973
70. Polak P, Kim J, Braunstein LZ, et al. A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. *Nat Genet*. 2017;49(10):1476-1486. http://doi.org/10.1038/ng.3934