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A MALDI-TOF mass spectrometry-based method for detection of copy number variations in *BRCA1* and *BRCA2* genes

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Background: Identifying germline mutations in *BRCA1* and *BRCA2* genes (*BRCAs*) would benefit the carriers in multiple aspects. In addition to single-nucleotide variations and small indels, copy number variations (CNVs) is also an indispensable component of identifiable mutations in *BRCAs*. A sensitive, rapid and throughput-flexible method to detect CNVs would be preferred to meet the rising clinical requirements for *BRCAs* testing.

Methods: We developed a MALDI-TOF-MS-based method (MS assay) which included three steps: first, multiplex end-point PCR followed by a single base extension reaction; second, automated analyte transfer and data acquisition; third, data analysis. We applied MS assay to detect CNVs in *BRCAs* in 293 Chinese patients with ovarian or pancreatic cancer. All the samples were examined by targeted next-generation sequencing (TS) simultaneously. Samples were further cross-validated by multiplex ligation-dependent probe amplification (MLPA) if the results from MS assay and TS were inconsistent. Long range PCR was then applied to identify the exact breakpoints in *BRCAs*.

Results: MS assay introduced highly multiplexed panels to detect CNVs of *BRCAs* semi-quantitatively. Simplified on-board data analysis was available for MS assay and no complex bioinformatics was needed. The turnaround time of MS assay was less than 8 hours with a hands-on time of only 40 min. Compared to TS, MS assay exhibited higher sensitivity (100% vs. 75%) and was more flexible in throughput, with the reagent cost per sample remaining constant no matter how many samples were examined per assay. A total of eight CNVs in *BRCAs* were detected from the 293 samples, and the molecular breakpoints were successfully identified in five samples through long-range PCR followed by Sanger sequencing.

Conclusion: Our results suggested that MS assay might be an effective method in primary screening for CNVs in genes such as *BRCAs*, especially when short turnaround time and/or high sensitivity is a top priority.

KEYWORDS

MALDI-TOF mass spectrometry, targeted sequencing, copy number variation, *BRCA1*, *BRCA2*

Background

BRCA1 and *BRCA2* genes are recognized as the primary inherited causes of breast and ovarian cancer since they were discovered in 1990s (Miki et al., 1994; Wooster et al., 1995). Germline mutations in *BRCA1* and *BRCA2* genes not only increase the risk of breast and ovarian cancer, but also contribute to the susceptibility of pancreatic and prostate cancer (Breast Cancer Linkage Consortium, 1999; Thompson et al., 2002). For carriers of *BRCA1* mutations, the risk of developing breast and ovarian cancer by age of 80 years is estimated to be 72% and 44% respectively, while it is 69% and 17% respectively for *BRCA2* carriers (Kuchenbaecker et al., 2017). Mutations in *BRCA2* also cause a 5–10% lifetime risk for pancreatic cancer, while *BRCA1* carriers might have two to four times risks compared to non-carriers (van Asperen et al., 2005; Ferrone et al., 2009; Mocci et al., 2013; Zhen et al., 2015; Roberts et al., 2016). In the case of prostate cancer, *BRCA1* and *BRCA2* mutations would lead to 8.6% and 15% cumulative risks respectively by age of 65 years (Thompson et al., 2002; Venkitaraman, 2002; Kote-Jarai et al., 2011; Leongamornlert et al., 2012). Identifying germline mutations in *BRCA1* and *BRCA2* genes would benefit the carriers by taking risk-reducing interventions before they get a cancer, as well as providing valuable information on the therapeutic application of Poly (ADP-ribose) polymerase inhibitors after a cancer occurs (Fong et al., 2009; Domchek et al., 2010).

Genetic testing for *BRCA1* and *BRCA2* mutation has now become a useful tool for both clinical and healthcare management. Multiple guidelines on genetic testing have been published emphasizing the necessity of identifying *BRCA* carriers for preventive and therapeutic purposes (US Preventive Services Task Force et al., 2019; Daly et al., 2020; Pujol et al., 2021). Although the majority of pathogenic variants identified in *BRCA1* and *BRCA2* are single-nucleotide variations (SNVs) and small insertions/deletions (indels), large genomic rearrangements (LGRs) accounts for up to 21% of all pathogenic variants, indicating that LGRs is an indispensable component of identifiable mutations (Judkins et al., 2012). The NCCN Guidelines also emphasized the need for comprehensive testing of all types of mutations including LGRs based on full length sequencing of *BRCA1* and *BRCA2* genes (Daly et al., 2021a). However, LGRs could not be detected by conventional PCR-based methods, and alternative methods such as long-range PCR, fluorescent *in situ* hybridization, comparative genomic hybridization, multiplex ligation-dependent probe amplification (MLPA) and targeted next-generation sequencing (TS) have been developed (Lambros et al., 2007; Walsh et al., 2010; Hernan et al., 2012; Stuppia et al., 2012; Biesma et al., 2015; Kwong et al., 2015). These approaches have contributed a lot in identifying LGRs, but they are either complicated, time-consuming or expensive, which hinders their applications in large scale screening. A simple, time-saving and throughput-flexible method to detect LGRs would be preferred to meet the rising clinical requirements for *BRCA* testing.

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) is an outstanding platform for

single nucleotide polymorphisms (SNPs) detection, featured by simple assay design, high throughput, high accuracy, excellent resolution, and short analysis times (Storm et al., 2003). In this study, we developed a MALDI-TOF-MS-based method (MS assay) for rapid detection of copy number variations (CNVs), which are due to LGRs, in *BRCA1* and *BRCA2* genes. The major advantage of this method is introducing highly multiplexed panels to detect CNVs of targeted genes semi-quantitatively, characterized by short turnaround time, flexible throughput and simplified on-board data analysis. Using this method, we examined CNVs in *BRCA1* and *BRCA2* in 293 Chinese patients with ovarian or pancreatic cancer. MS assay exhibited higher sensitivity compared to TS, suggesting that it might be an effective method in primary screening for CNVs, especially when shorter turnaround time and/or high sensitivity is a top priority.

Materials and methods

Samples

Leftover peripheral blood samples after routine clinical tests were collected from patients with ovarian cancer ($n = 289$) or pancreatic cancer ($n = 4$) in Shanghai General Hospital. Genomic DNA (gDNA) was extracted from blood using the QIAamp Blood Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The concentration of genomic DNA was measured by Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA) using dsDNA HS assay (Q32854). The study was performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Shanghai General Hospital (No. 2022KY019). Requirement for informed consent was waived by the ethics committee.

MS assay for CNVs detection

Three steps were included in MS assay: first, multiplex end-point PCR followed by a single base extension reaction, which took about 5.5 h with a hands-on time of 25 min; second, automated analyte transfer and data acquisition, which took about 100 min with a hands-on time of only 5 min; third, data analysis which took a hands-on time of 5–10 min. More specifically, each exon of *BRCA1* (NM_007294) and *BRCA2* (NM_000059) genes, as well as fragments of internal reference genes including *RNaseP*, *EIF2C1* and *ALB* genes, were amplified following by single base extension in multiplexed panels. Both primers for amplification and unextended primers (UEP) were designed using Assay Design Suite (version 2.2, Agena Bioscience). To be noted, primer sequences should not contain any SNPs with minor allele frequency >0.5%. Oligonucleotides that were only one base different from the corresponding gDNA fragments, namely competitors, were amplified and extended in parallel with gDNA using the same primers (Figure 1A). The sequences of primers for multi-plex PCR and UEP were listed in Supplementary Table S1.

All the exons of *BRCA1* and *BRCA2* were examined in a total of four multiplex PCR amplification and extension reactions

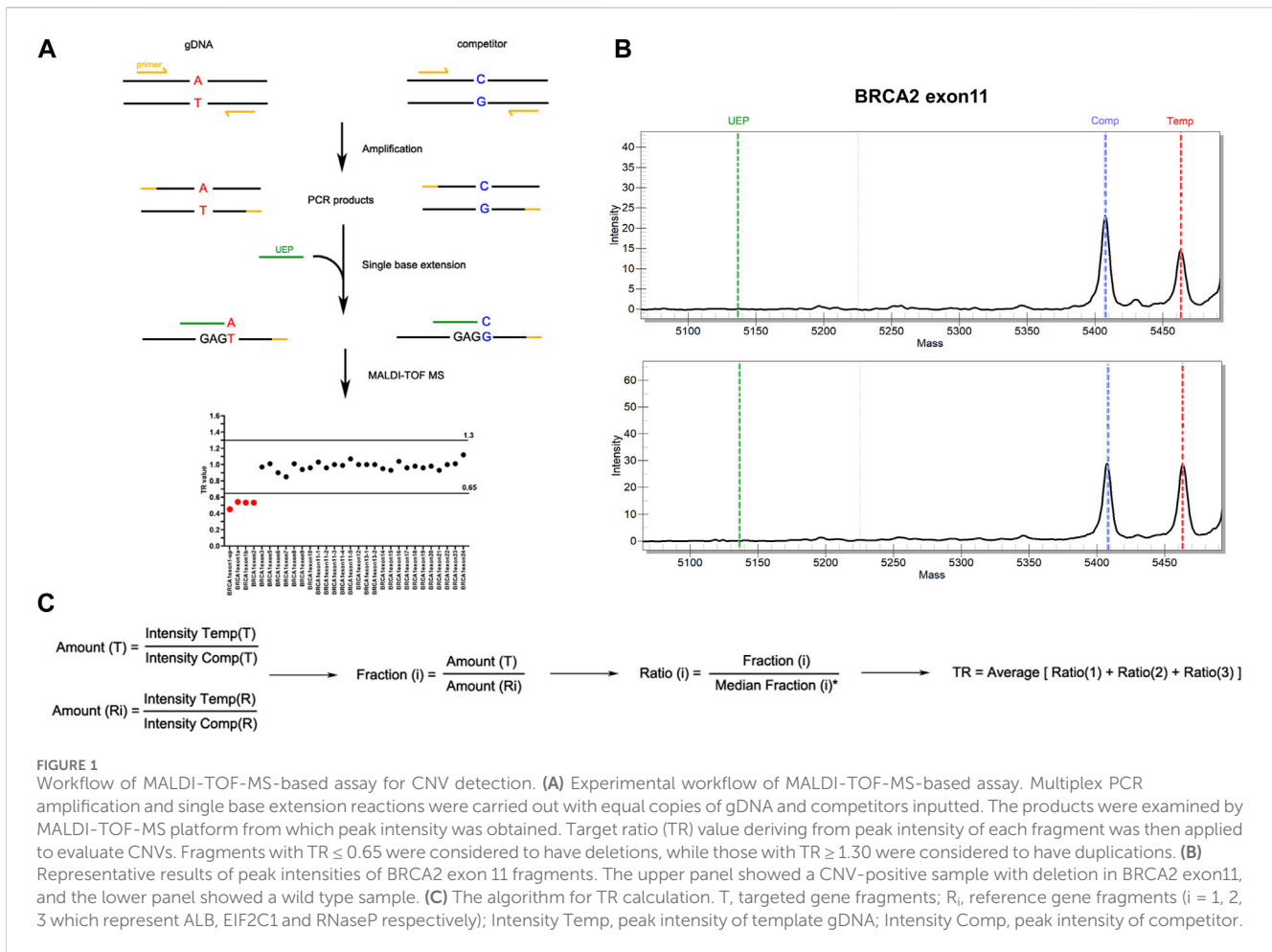


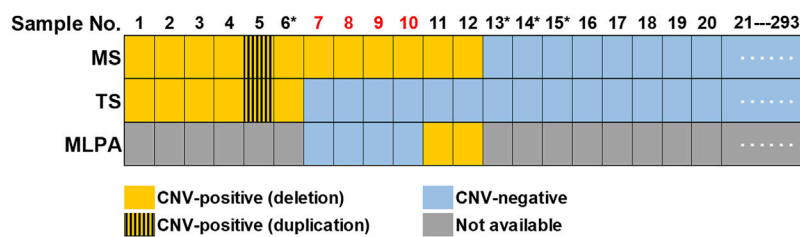
FIGURE 1 Workflow of MALDI-TOF-MS-based assay for CNV detection. **(A)** Experimental workflow of MALDI-TOF-MS-based assay. Multiplex PCR amplification and single base extension reactions were carried out with equal copies of gDNA and competitors inputted. The products were examined by MALDI-TOF-MS platform from which peak intensity was obtained. Target ratio (TR) value deriving from peak intensity of each fragment was then applied to evaluate CNVs. Fragments with $TR \leq 0.65$ were considered to have deletions, while those with $TR \geq 1.30$ were considered to have duplications. **(B)** Representative results of peak intensities of BRCA2 exon 11 fragments. The upper panel showed a CNV-positive sample with deletion in BRCA2 exon11, and the lower panel showed a wild type sample. **(C)** The algorithm for TR calculation. T, targeted gene fragments; R_i, reference gene fragments (i = 1, 2, 3 which represent ALB, EIF2C1 and RNaseP respectively); Intensity Temp, peak intensity of template gDNA; Intensity Comp, peak intensity of competitor.

which were carried out using iPLEX Pro Reagents Kit (Agena Bioscience) according to the manufacturer’s instructions. Briefly, equal copies of gDNA and competitors were inputted into one tube and PCR amplification was performed as followings: 30°C for 10 min followed by 96°C for 2 min, then 5 cycles of 96°C 45 s, 65°C 30 s and 72°C 1 min, 40 cycles of 96°C 45 s, 60°C 30 s and 72°C 1 min, with a final step of 72°C 5 min. PCR products were then treated with shrimp alkaline phosphatase, followed by a single-base extension which was performed as followings: 97°C 45 s, 97°C 5 s plus 5 repetitions of 52°C 5 s and 80°C 5 s for 40 cycles, and then 72°C 3 min. After extension, the products were desalted and spotted onto a SpectroCHIP (Agena Bioscience) using the MassARRAY Chip Prep Module (Agena Bioscience). SpectroCHIPS were scanned using the MassARRAY™ Analyzer 4 (Agena Bioscience) and the spectra were processed using the TYPER™ (Agena Bioscience) software to get the peak intensity for each allele. Target ratio (TR) value deriving from peak intensity of each fragment was applied to evaluate CNVs (Figure 1B). The algorithm for TR calculation was shown in Figure 1C. The cut-off of TR value was established using both CNV-positive standard samples purchased from the Coriell Institute (Camden, NJ) (Supplementary Table S2) and negative control samples which was proved to have diploid gene copy of BRCA1 and BRCA2 by MLPA. Fragments with $TR \leq 0.65$ were

considered to have deletions, while those with $TR \geq 1.30$ were considered to have duplications. Batch calculation of TR value for multiple samples was performed in RStudio. The R code is available upon reasonable request.

Hybrid capture-based targeted next-generation sequencing

The gDNA was fragmented and ligated to adaptors according to the protocol of NanoPrep™ DNA Library Preparation Kit (Nanodigmbio, Nanjing, China). Targeted fragments were captured by xGen® Predesigned Gene Capture Pools (IDT, IA, United States) which covered all coding exons and flanking noncoding regions of BRCA1 and BRCA2 genes, and hybridization was carried out using xGen® Hybridization and Wash Kit and Universal Blockers (IDT, IA, United States). Then the DNA library was sequenced on MGISEQ-2000 instrument using MGISEQ-2000RS High-throughput Sequencing Kit (PE 150) (MGI, Shenzhen, China). Sequencing data was processed by GATK (<https://gatk.broadinstitute.org/>). Briefly, adaptor sequences in raw sequencing data were marked using MarkIlluminaAdapters, then aligned to human reference genome (version hg19) using BWA-MEM. Duplicated reads were identified using



* represents samples from patients with pancreatic cancer. Other samples were from patients with ovarian cancer. Sample No. in red indicates there is SNVs in primer sequences of MS (No.7-10).

FIGURE 2

Summary of CNVs detected in 293 samples. Consistent results were obtained by MS assay and TS in 287 samples (No.1-6 and No.13-293), with six (No.1-6) being CNV-positive and 281 being CNV-negative (No.13-293). Sample No.7-12 were validated as CNV-negative, while sample No.11-12 were validated as CNV-positive by MLPA. Sample No. in red (No.7-10) indicated there were SNVs in the primer sequences of MS assay. "*" represented samples from patients with pancreatic cancer, while all the other samples were from patients with ovarian cancer. MS, MALDI-TOF-MS-based assay; TS, targeted sequencing; MLPA, multiplex ligation-dependent probe amplification.

MarkDuplicates and base quality score recalibration was performed using BaseRecalibrator. Germline variants including point mutation and small indels were called by HaplotypeCaller, while germline CNVs was called by GermlineCNVCaller.

MLPA assay for CNVs detection

CNVs in *BRCA1* and *BRCA2* genes were also examined using SALSA MLPA Probemix P002-D1 *BRCA1* and P090-C1 *BRCA2* (MRC-Holland, Amsterdam, Netherlands) respectively according to the manufacturer's instructions. Genomic DNA was denatured at 98°C for 5 min, then the hybridization of probes to genomic DNA was performed at 95°C 1 min and 60°C 16 h. Hybridized probes were then ligated with a Ligase-65 master mix at 54°C 15 min and 98°C 5 min. PCR amplification of ligated probes was performed as followings: 35 cycles of 95°C 30 s, 60°C 30 s and 72°C 60 s, and then 72°C 20 min. Electrophoresis was performed on ABI 3500 (Thermo Fisher Scientific, Waltham, MA), and data analysis was done by Coffalyser.NET software (MRC-Holland, Amsterdam, Netherlands).

Identification of breakpoints by long range PCR

Long range PCR was applied to explore and identify the exact breakpoints for samples with CNVs in *BRCA1* or *BRCA2* genes detected by MS-based assay. PCR was performed with TaKaRa LA Taq (Takara Bio, Otsu, Japan) in a final volume of 50 µL as followings: 94°C 1 min, 30 cycles of 98°C 10 s and 68°C 15 min, and then 72°C 10 min. PCR products were purified with the DiaSpin DNA Gel Extraction Kit (Diamond, Shanghai, China) and subjected to Sanger sequencing. Data analysis were performed by Sequencing Analysis v5.2 software (Thermo Fisher Scientific, Waltham, MA). Repetitive elements, such as LTR, LINE, Alu and MIR were identified by RepeatMasker and annotated through searching UCSC Genome Browser (<http://genome.ucsc.edu>).

Results

MS assay for detection of CNVs in *BRCA1* and *BRCA2* genes

A MS assay was developed for detection of CNVs in *BRCA1* (NG_005905.2) and *BRCA2* (NG_012772.3) genes, of which the turnaround time costed less than 8 hours, with a hands-on time of only 40 min. The throughput of MS assay was quite flexible, varying from one to more than a thousand samples per day, while the reagent cost per sample remained constant no matter how many samples were examined per assay. Besides, simplified on-board data analysis was available for MS assay and no complex bioinformatics was needed, which would greatly facilitate its application in clinical labs. More details of MS assay were described in Methods.

Blood samples from 289 patients with ovarian cancer and four patients with pancreatic cancer were collected and subjected to CNVs detection through MS assay. The characteristics of enrolled patients were shown in [Supplementary Table S3A](#). A total of 281 samples were found to be CNV-negative, while 11 patients with ovarian cancer (No. 1–5, 7–12) and one patient with pancreatic cancer (No. 6) were identified to carry CNVs in *BRCA1* or *BRCA2* genes by MS assay ([Figure 2](#)).

Detection of CNVs in *BRCA1* and *BRCA2* genes by TS and MLPA

All the 293 samples mention above were examined by TS simultaneously. Through TS, a total of 47 unique pathogenic SNVs were identified in 56 individuals ([Supplementary Table S3B](#)). CNVs analysis showed that 287 samples (including No. 7–12) were CNV-negative, while five patients with ovarian cancer (No. 1–5) and one patient (No. 6) with pancreatic cancer were identified to carry CNVs in *BRCA1* or *BRCA2* genes ([Figure 2](#)). Thus, the results of six samples (No. 7–12) were inconsistent between TS and MS assay.

We then performed MLPA to validate whether there were CNVs or not in sample No. 7–12. Two samples (No. 11-12) were validated by MLPA as CNV-positive, while the other four (No. 7–10) were CNV-negative. Further analysis of the TS results indicated that in the cases of

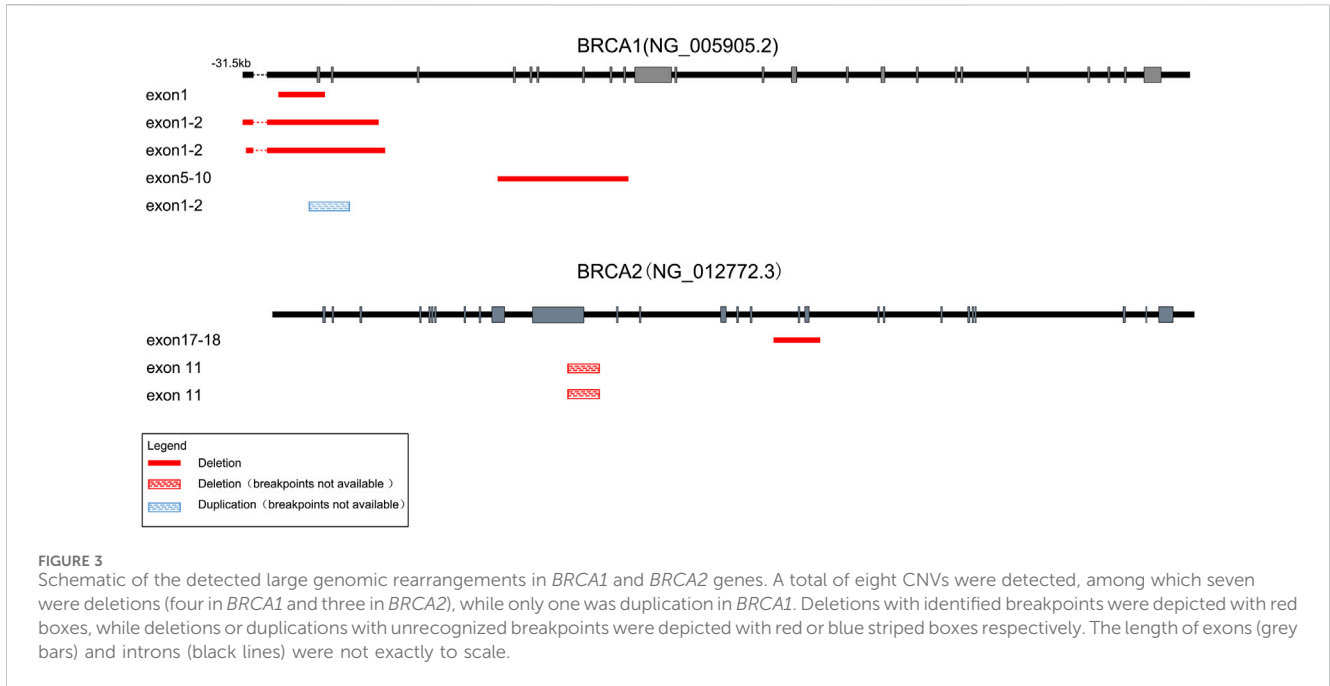


TABLE 1 The breakpoints and mechanisms of CNVs detected in this study.

Sample no.	CNVs	Size (bp)	Breakpoints	Mechanisms
<i>BRCA1</i> (NG_005905.2)				
1	exon 1-2 deletion	36934	g.61,101_98,034del	NAHR (ΨBRCA1/BRCA1)
2	exon 1 deletion	19857	g.73,379_93,235del	NHEJ (Non-Alu/AluSx)
3	exon 1-2 deletion	36934	g.61,422_98,355del	NAHR (ΨBRCA1/BRCA1)
5	exon 1-2 duplication	NA	NA	Unknown
6	exon 5-10 deletion	12117	g.110,371_122,487del	NAHR (AluSz6/AluJb)
<i>BRCA2</i> (NG_012772.3)				
4	exon 17-18 deletion	7892	g.48,409_56,300del	NAHR (AluSx/AluSz)
11	part of exon 11 deletion	NA	NA	Unknown
12	part of exon 11 deletion	NA	NA	Unknown

NA, not available; NAHR, non-allelic homologous recombination; NHEJ, non-homologous end-joining.

sample No. 7–10, there were SNVs in the amplification primer binding sites of MS assay (Figure 2; Supplementary Table S4), which might lead to false positive results in MS assay. Meanwhile, sample No. 11-12 that were CNV-positive identified by both MS assay and MLPA was recognized as CNV-negative in the TS sequencing results, indicating the results of TS were false negative.

Characterization of CNVs in *BRCA1* and *BRCA2* genes

Totally, we detected CNVs in *BRCA1* and *BRCA2* genes in eight (2.7%) out of 293 samples. Consistent with previous reports (Sluiter and van Rensburg, 2011; Su et al., 2018), there were more deletions

than duplications among the eight CNVs we identified. As shown in Figure 3 and Table 1, seven out of the eight CNVs were deletions (four in *BRCA1* and three in *BRCA2*), while only one CNV was duplication in *BRCA1*. Recurrent CNVs included deletions in exon 1-2 of *BRCA1* and in exon 11 of *BRCA2*. In fact, three samples were found to harbor CNVs in exon 1-2 of *BRCA1*, including two samples with deletions and one sample with duplication, suggesting that exon 1-2 of *BRCA1* might be a potential hotspot for CNV occurrence.

Moreover, we successfully identified the molecular breakpoints in five out of the eight CNV-positive samples by applying long-range PCR followed by Sanger sequencing, among which deletions of *BRCA1* exons 1-2 including g.61,242_98,355del and g.61,101_98,034del have been identified as recurrent CNVs in Chinese

TABLE 2 Sensitivity and specificity of MS assay and TS.

Methods	False positive	False negative	True positive	True negative	Sensitivity (%)	Specificity (%)
MS	n = 4	n = 0	n = 8	n = 281	100	98.6
TS	n = 0	n = 2	n = 6	n = 285	75	100

MS, MALDI-TOF-MS-based assay; TS, targeted sequencing.

patients with breast cancer (Su et al., 2018). As shown in Table 1, four CNVs were caused by Alu-mediated or Ψ BRCA1-mediated non-allelic homologous recombination (NAHR), while another one was caused by non-homologous end-joining (NHEJ).

Sensitivity and specificity of MS assay

To sum up, consistent results were obtained from 287 samples (No. 1–6 and No. 13–293) by both MS assay and TS, with six being CNV-positive and 281 being CNV-negative. Another six samples (No. 7–12) turned out to be CNV-negative in the result of TS, but CNV-positive in that of MS assay. Further validation by MLPA indicated that sample No. 11–12 were CNV-positive while sample No. 7–10 were CNV-negative. Thus, the sensitivity for MS assay and TS was 100% and 75%, while the specificity was 98.6% and 100% respectively (Table 2). MS assay showed higher sensitivity while the specificity was slightly lower when compared to that of TS.

Discussion

It was suggested that unselected BRCA genetic testing should be carried out in both ovarian and breast cancer patients regardless of family history and histopathology (Wu et al., 2017; Sun et al., 2022). Improvement in progression-free survival as well as in overall survival with maintenance Olaparib (a Poly (ADP-ribose) polymerase inhibitor) have been observed in patients with newly diagnosed advanced ovarian cancer and a BRCA variant (Moore et al., 2018; DiSilvestro et al., 2023). Furthermore, patients with HER2-negative early breast cancer and germline BRCA1 or BRCA2 variants could also benefit from adjuvant Olaparib treatment (Tutt et al., 2021). For non-cancer individuals, carriers of a BRCA1 or BRCA2 pathogenic variants was recommended to take more intensive screening and preventive strategies for breast, ovarian, prostate and pancreatic cancer (Elezaby et al., 2019; Daly et al., 2021b). The clinical demand for BRCA testing keeps increasing, which calls for fast, throughput-flexible and cost-effective technologies to detect SNVs as well as CNVs. In this study, we developed the MS assay, which was featured by sensitive, time-saving and throughput-flexible, for CNV detection of BRCA1 and BRCA2.

MALDI-TOF MS technology has been utilized for single nucleotide polymorphisms (SNPs) genotyping for two decades (Tost and Gut, 2002). In a recent study, the MS assay was also applied in CNV detection of SMN1 and SMN2 genes for spinal muscular atrophy genetic testing, showing highly concordant results with MLPA (Jin et al., 2022). In this study, we developed a MS assay for CNV detection of BRCA1 and BRCA2 genes. MLPA has also been widely applied in clinical labs for CNV detection of BRCA genes (Lim et al., 2007; Lips et al., 2011). However, the experimental procedure of MLPA usually takes 2 days and the throughput is

relatively low (45 testing samples plus three reference samples per batch), which might hinder its wide application in clinical practice. Although the throughput of TS is much higher, it is also time-consuming when compared to MS assay. The highly-automatized procedure of MS assay only takes less than 8 hours to generate results for 96 testing samples per batch, with a hands-on time of only 40 min. Besides, our results indicated that the sensitivity of MS assay is higher than TS (100% vs. 75%), implying that MS assay might be more effective in primary screening for CNV-carriers.

Among the 293 patients involved in this study, we identified a total of 64 carriers (21.8%) of pathogenic variants of BRCA1 and BRCA2 genes, including 56 patients (19.1%) carrying pathogenic SNVs/small indels, and eight patients (2.7%) carrying CNVs through TS and MS assay. Our results indicated that CNVs accounted for 12.5% (eight out of 64) of all pathogenic mutations in BRCA1 and BRCA2 genes, suggesting that examination of CNVs should also be carried out to comprehensively identify pathogenic mutation carriers of BRCA genes in clinical practice. To be noted, mismatches in the primer binding sites of MS assay may lead to a false-positive result of deletion. In fact, we found four false-positive samples (sample No. 7–10) from the results of MS assay which were caused by SNVs in the primer binding sites as revealed by TS. Similarly, SNVs located on the MLPA probe binding sites have been observed to cause false positive results (Agaoglu et al., 2022). Another independent approach, such as TS, long-range PCR or fluorescent *in situ* hybridization is recommended to further validate the CNV-positive results derived from MS assay or MLPA.

It was reported more than 40% of the intronic sequences of BRCA1 consist of Alu elements which were responsible for recombinational “hot spots”. Meanwhile, there were only 17% Alu sequences in BRCA2, which might explain why the incidence of rearrangements was lower in BRCA2 than in BRCA1 (Ewald et al., 2009). In addition, rearrangement involving Ψ BRCA1 might also constitute a “hot spot” for recombination (Puget et al., 2002). Consistently, we observed Alu-mediated genomic rearrangements in both BRCA1 and BRCA2 in two out of the eight CNV-positive samples, and Ψ BRCA1-mediated rearrangement in another two samples. Two CNV-positive samples harbored BRCA1 exon 1–2 deletion, which has been reported to be recurrent in Chinese patients with breast cancer (Su et al., 2018). Our results further suggested BRCA1 exon 1–2 deletion might be a “hot spot” for recombination mediated by either Alu element or Ψ BRCA1.

The MS assay has several limitations. First, although three internal reference genes (*RNaseP*, *EIF2C1* and *ALB*) as well as a highly homologous competitor were included for double calibration of gene dosage in the MS assay, the quality of mass spectrometric signal might impact its accuracy for dosage quantification. Thus, the MS assay is recommended for detection of germline CNVs in high-quality samples, while it is not suitable for low-quality samples or samples with low tumor purity. Another limitation of MS assay is its

lower specificity than TS, which is caused by mismatches in the primer binding sites and could not be completely avoided. In order to minimize potential mismatches, we excluded any SNPs with minor allele frequency >0.5% from primer sequences. However, as *BRCA1* and *BRCA2* variation is ethnic-specific, the minor allele frequency data are mainly derived from Caucasian populations of Europe and North America and might not be accurate in the case of Chinese population (Bhaskaran et al., 2019). Further improvement in primer design might be made by drawing information from Chinese-specific reference database such as dbBRCA-Chinese (<https://genemutation.fhs.um.edu.mo/dbbrca-chinese/>). Besides, the specificity of MS assay would also be improved by simultaneously applying another primer set avoiding mismatches in the current set, which is now under development.

In summary, we developed a MALDI-TOF MS-based assay for CNV detection of *BRCA1* and *BRCA2* genes, which was characterized by high sensitivity, time-saving, and flexible throughput. Our results indicated that MS assay might be an effective method in primary screening for CNV-carriers, especially when short turnaround time and/or high sensitivity is a top priority. Application of MS assay is expected to satisfy the requirements of increasing demand for genetic testing of *BRCAs*, and could be easily expanded to the detection of CNVs in other genes in clinical practice and population screening.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Shanghai General Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from a by-product of routine care or industry. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

HZ: Conceptualization, Data curation, Investigation, Methodology, Writing–original draft, Writing–review and editing.

XH: Conceptualization, Data curation, Methodology, Writing–original draft, Writing–review and editing. JZ: Investigation, Methodology, Software, Writing–original draft. ZM: Methodology, Resources, Validation, Writing–original draft. XZ: Methodology, Validation, Writing–original draft. WY: Methodology, Validation, Writing–original draft. HD: Funding acquisition, Supervision, Writing–original draft, Writing–review and editing.

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Conflict of interest

XH is an employee of Agena Bioscience (Shanghai) Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmolb.2023.1301652/full#supplementary-material>

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