Research Article

Challenges in next generation sequencing of homology recombination repair genomic variants in prostate cancer: A nationwide survey and calibration project in China

Huanwen Wu [a], Liqun Zhou [b], Xiaoyan Zhou [c, d, e], Qiang Wei [f], Nengtai Ouyang [g], Jianyong Shao [h], Jian Huang [i, *], Zhiyong Liang [a, **]

[a] Department of Pathology, State Key Laboratory of Complex Severe and Rare Diseases, Molecular Pathology Research Center, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China
[b] Department of Urology, Peking University First Hospital, Beijing 100034, China
[c] Department of Pathology, Fudan University Shanghai Cancer Center, Shanghai 200032, China
[d] Department of Oncology, Shanghai Medical College, Fudan University, Shanghai 200032, China
[e] Institute of Pathology, Fudan University, Shanghai 200032, China
[f] Department of Urology, West China Hospital Sichuan University, Chengdu 610041, China
[g] Cellular & Molecular Diagnostics Center, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou 510288, China
[h] Department of Molecular Diagnostic, Sun Yat-sen University Cancer Center, Guangzhou 510700, China
[i] Department of Urology, Sun Yat-sen Memorial Hospital of Sun Yat-sen University, Guangzhou 510630, China

ARTICLE INFO

ABSTRACT

Background: Homologous Recombination Repair (HRR) is the most reliable and important signaling pathway for repairing DNA damage. We initiated a calibration project to better understand the NGS landscape for HRR gene testing in China, provide indications for testing standardization, and guide clinical practice.

Methods: A questionnaire was used to collect laboratory information, panel design for HRR gene testing, tissue sample test parameters, plasma ctDNA sample test parameters, and procedures for variant interpretation. The testing quality of the participating laboratories was further evaluated by external quality assessment (EQA), which provided 5 FFPE slices and 5 mimic ctDNA samples as standard references for evaluation. Test results and reports were collected to assess laboratory performance.

Results: Our results showed that different laboratories had significant differences in sequencing platforms, library construction technologies, genes in the testing panel, detectable mutation types, probe coverage regions, sequencing parameters, variants interpretation guidelines, and positive test rates. For the EQA test, the overall pass rate was about 60%. The average accuracy for tissue samples and ctDNA samples was 79.55% and 74.13%, respectively. It is worth noting that variants in tandem repetition regions and splice sites, and those with low allele frequency were more prone to misdetection. The most common reasons for misdetection were as follows: the testing panel did not cover the genes or the whole exon and splice sites of the genes; the variants were misclassified as benign or likely benign, and the variants failed the QC criteria.

Conclusions: The discrepancies observed in our survey and EQA test affect the authenticity of HRR gene test results for prostate cancer, underlining the need to establish guidelines for HRR gene testing and variant interpretation in China, and to optimize HRR gene testing in clinical practice to improve management and patient care.

1. Introduction

The incidence of prostate cancer (PCa) is 15.6/100,000, and new cases exceed 110,000, leading to more than 50,000 deaths in China in 2020.[1] Although the majority of patients with locally advanced and

* Corresponding author.
** Corresponding author.
E-mail addresses: Huangjianaz321@163.com (J. Huang), liangzhiyong1220@yahoo.com (Z. Liang).

metastatic disease initially respond to androgen deprivation therapy (ADT), most will progress into metastatic castration-resistant PCa (mCRPC).[2] However, some unique RNA-seq profiles of Pca had been detected,[3] and the instability of RNA limits its application in clinical testing. There had been some previous studies suggesting homology recombination repair (HRR) was the most important pathway to repair DNA double-strand break, which was one of the most geno-toxic forms of DNA damage and tumori-genesis.[4]

In the mCRPC stage, 25%−30% of patients carrying HRR gene mutations are sensitive to poly (ADP-ribose) polymerase (PARP) inhibitors and platinum-based chemotherapy[5],[6] Based on the positive results from PROfound trial Phase III, the PARP inhibitor Olaparib was approved by the United States Drug and Food Administration (FDA) in 2020 for mCRPC patients with deleterious or suspected deleterious HRR gene mutations who progressed on abiraterone or enzalutamide therapy.[7] The 2021 edition of the National Comprehensive Cancer Network (NCCN) and the European Association of Urology (EAU) Prostate Cancer Guidelines recommend that all patients with metastatic PCa should undergo tumor HRR gene testing[8],[9]

Next-generation sequencing (NGS) is increasingly used in cancer screening and early detection[10],[11] Although HRR gene testing has been widely used in China, the concordance of testing results among different labs remains unclear. In fact, a survey of 158 laboratories that provided *BRCA1/BRCA2* sequence analysis revealed significant differences in the platforms and variant detection used in 2018,[12] but there was no Chinese laboratory participated in this survey.

Though the failure rate of tumor tissue HRR gene testing is about one-third,[13] repeated biopsies are often impractical for mCRPC patients. Liquid biopsy is rapidly developing in tumor biomarker detection, with the advantages of easy sampling, minimally invasive, and reproducible.[14],[15] HRR gene testing using plasma circulating tumor DNA (ctDNA) has high consistency with the test using tissue samples, and can be alternatively used in clinical practice when tissue is unavailable, especially in mCRPC patients.[16-20] The technical requirements for ctDNA testing are much stricter than tissue sample testing due to the low concentration and high fragmentation of tumor DNA in plasma.[21] Consequently, uncertainty exists regarding the reliability of ctDNA testing.

Because there are no guidelines or consensus statements available in China, we launched a nationwide HRR gene testing calibration project to better understand its landscape and quality. The study will provide a snapshot of the current status and the proficiency of HRR gene testing in China, and will serve as the basis to establish regulations or guidelines.

2. Methods

2.1. Survey

A total of 32 third-party laboratories that could provide PCa NGS-based HRR gene testing participated in our survey. The questionnaire is available on the following website: https://www.wjx.cn/jq/81953684.aspx. There are 43 questions in the questionnaire, including three sections: (1) basic laboratory information; (2) NGS testing information for PCa with HRR gene mutations (including HRR gene panel design information, tissue test parameters, plasma ctDNA test parameters); and (3) the verification and interpretation of HRR gene mutations.

2.2. External Quality Assessment

External Quality Assessment (EQA) was performed using DNA reference standards from PCa cell lines provided by Cobioer Biosciences (Nanjing, China). Formalin-fixed paraffin-embedded (FFPE) samples (CatLog No. CGD2020082501, CGD2020082502, CGD2020092001, CGD2020082503, CGD2020082504) and mimic ctDNA samples generated by enzyme digestion from genomic DNA to with ~150 bp fragments (CatLog No. CGD2020051801, CGD2020-051802, CGD2020051803, CGD2020051804, CGD2020051805) were used. Each lab received 5 FFPE samples with 20-nm thick slices, and 5 ctDNA mimic samples in a 6 μL volume containing 20 ng/μL DNA, which were all stored at 4°C during transportation.

The mutations in the reference standards were verified by Sanger sequencing. The details are reported in Supplementary Tables 1 and 2.

In summary, there are 22 mutation loci in the FFPE samples, involving 12 HRR genes. And the variant allele frequency (VAF) range is 3.60%-54.17%. The ctDNA mimic samples cover 40 mutation loci with the VAF range of 0.56%-49.58%. Only SNVs and indels were included. The expected results of the reference standards are summarized in Table 1.

2.3. Scoring criteria

The scoring criteria were based on the ability to detect the variants. A maximum of 5 points was assigned for each expected locus in the FFPE samples, and the total maximum score for all 22 mutation loci in the FFPE samples was 110 points (5*22). A maximum of 2.5 points was assigned for each expected variant in the mimic ctDNA samples, and the total maximum score for the mimic ctDNA samples was 100 points (2.5*40).

The participants were required to submit clinical reports and basic test information, quality control information, and variant interpretation information, which classified variants as pathogenic/likely pathogenic variants or variants of uncertain significance (VUS). Since there is currently no specific guidance for the interpretation of HRR variants, this EQA project did not assess the interpretation capability. The information on variant classification was collected for data analysis.

3. Results

3.1. Basic information of NGS laboratories

Among the 32 commercial NGS laboratories that participated in the survey, 43.75% of the laboratories were small-scale laboratories (<50 employees), 43.75% were medium-scale (50-500 employees), and 12.5% were large-scale laboratories (over 500 employees). 43.75% of the laboratories passed CAP (College of American Pathologists) certification, among which 18.75% had passed both CAP and CLIA (Clinical Laboratory Improvement Amendments) certi-

Table 1
Expected results of the EQA reference standards

Gene	Tissue sample		ctDNA mimic sample	
	Loci No.	Variant abundance	Loci No.	Variant abundance
BRCA1	1	5.70%	3	0.83-46.35%
BRCA2	5	10.26-53.71%	9	0.81-21.91%
ATM	3	42.21-46.11%	9	1.36-49.58%
BARD1	-	-	1	2.13%
BRIP1	1	49.69%	4	0.94-9.58%
CDK12	2	46.19-51.09%	4	0.78-16.22%
CHEK1	1	10.80%	1	47.45%
CHEK2	2	41.05-54.17%	2	14.32-26.67%
FANCL	1	46.84%	1	12.66%
PALB2	1	4.70%	-	-
PPP2R2A	2	8.15-37.74%	2	0.56-2.17%
RAD51 B	2	3.60-25.20%	2	10.59-31.31%
RAD54 L	1	44.44%	2	0.79-25.88%

Table 2
Sequencing platforms, library construction and panel gene number for HRR testing

Lab ID	Sequencing platform	Library construction method	Tissue sample test panel gene No.	Plasma sample test panel gene No.
1	Illumina	Hybrid capture	15	15
2	Illumina	Hybrid capture	17	17
3	Illumina	PCR amplicon	24	24
4	Illumina	Hybrid capture	27	27
5	Illumina	Hybrid capture	36	36
6	Illumina	Hybrid capture	36	36
7	Illumina	Hybrid capture	55	55
8	Illumina	Hybrid capture	66	66
9	Illumina	Hybrid capture	72	72
10	Illumina	Hybrid capture	128	128
11	Illumina	Hybrid capture	128	128
12	Illumina	Hybrid capture	150	150
13	Illumina	Hybrid capture	102	102
14	Illumina	Hybrid capture	484	484
15	BGI	Hybrid capture	688	688
16	Illumina	Hybrid capture	324	324
17	Illumina	Hybrid capture	539	539
18	Illumina	Hybrid capture	571	571
19	Illumina	Hybrid capture	599	599
20	Illumina	Hybrid capture	603	603
21	Illumina	Hybrid capture	625	332
22	Illumina	Hybrid capture	808	808
23	Illumina	Hybrid capture	825	825
24	Illumina	Hybrid capture	1021	1021
25	BGI	Hybrid capture	WES	WES
26	Thermo Fisher (Tissue)	PCR amplicon (Tissue)	19	-
	Illumina (ctDNA)	Hybrid capture (ctDNA)	-	557
27	Thermo Fisher	PCR amplicon	19	NA
28	Thermo Fisher	PCR amplicon	19	NA
29	Thermo Fisher	PCR amplicon	19	NA
30	Thermo Fisher	PCR amplicon	15	NA
31	Thermo Fisher	PCR amplicon	19	NA
32	Thermo Fisher	PCR amplicon	15	NA

fications, 12.5% passed ISO15189, and the remaining labs passed other certifications. All institutions had participated in and passed at least one external quality evaluation project for *BRCA* testing capability evaluation, and about 63% passed the EMQN external quality evaluation. In summary, although NGS testing in China is dominated by small and medium-sized facilities with relatively short operation time (<5 years), it puts forward high requirements for quality management.

3.2. Sequencing platform and library construction methodology

In terms of sequencing technology, Illumina was the most commonly used NGS platform (24 laboratories, 75%). 7 (22%) laboratories adopted the ThermoFisher platform, while 2 (6%) adopted the BGI (Beijing Genomics Institute) platform. The details were summarized in Table 2.

Overall, 96% of laboratories using Illumina and BGI NGS platforms adopted hybrid capture methodology to construct the sequencing libraries, and all laboratories using the ThermoFisher platforms adopted PCR amplicon methodology to construct libraries. Both library construction methodologies presented advantages and disadvantages, and some laboratories using the ThermoFisher platform did not provide plasma sample testing services.

Regarding the testing panels for tissue samples, 19 laboratories offered PCa-specific small panels, which were preferred by clinicians due to their higher cost-effectiveness. 13 laboratories used more expensive pan-tumor panels. Only 12 laboratories offered clinicians smaller panels for plasma testing. 24 laboratories used the same size panel for both tissue and plasma testing, although the design and quality control parameters of plasma and tissue samples may be different.

3.3. Panel design of HRR gene testing

In terms of gene coverage and probe design, 3 (9.3%) laboratories covered the 14 genes approved by the FDA for mCRPC in the testing panel, 26 (81.25%) laboratories covered more DDR genes (Fig. 1A).

Regarding the exon coverage of HRR genes, 22 (69%) laboratories covered all the exon regions of HRR genes, and 10 (31%) laboratories sequenced only the hot regions of more commonly mutated exons, based on the literature and clinical evidence (Fig. 1B). With regard to intron regions, 17 laboratories included all the canonical splice sites, of which 11 laboratories covered 20-50 bp downstream and upstream of the coding exons, 3 laboratories covered less than 20 bp of the intron region, and 3 laboratories covered only the canonical splice sites. 15 (47%) laboratories selected the covered intron regions, based on clinical evidence associated with individual genes (Fig. 1C). Since there are no specific hotspot loci or regions in the HRR genes, it is important to cover all the exons and splice sites to avoid potential false-negative results.

Several loss-of-function variant types have been identified, including single nucleotide variation (SNV), insertion-deletion (indel), copy number variation (CNV), and large genome rearrangement (LGR). In the requirements of mutation type detection, all laboratories could detect SNV and indel variants, and about 80% could detect CNV. The LGR detection remained a major challenge for NGS, and only 53% of laboratories could detect it (Fig. 1D).

3.4. HRR positive rate and interpretation guideline

From the survey results, 50% of laboratories did not perform statistical analysis on the HRR gene mutation rates in PCa and other tumor types due to the small sample size. However, some labo-
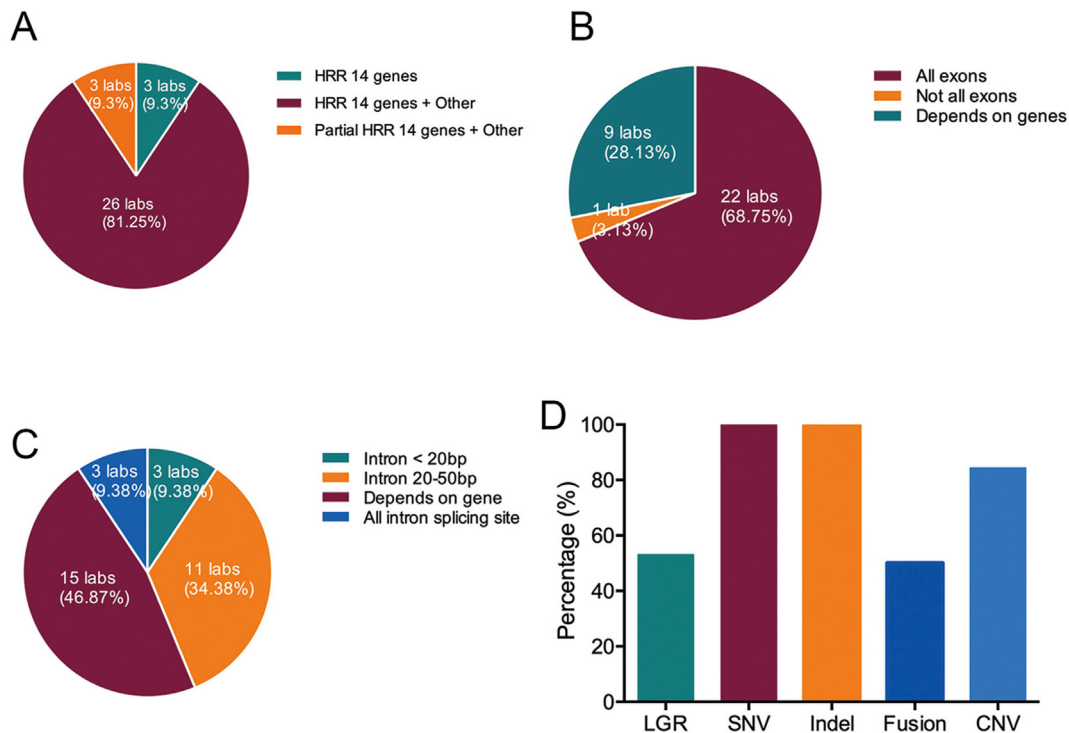
Fig. 1. Panel design for HRR gene testing (A) Genes covered in the testing panel for PCa; (B) Exons coverage of HRR genes in the testing panel (C) Intron coverage of HRR genes in the testing panel; (D) variants type detected in the testing panel.

Table 3
Variant detection rates between sequencing platform or library construction method

Variant type	Sequencing platform			Library construction method	
	Illumina	ThermoFisher	BGI	Hybrid capture	PCR amplicon
SNV	86.7%	79%	96.7%	87.2%	80.8%
Indel	83.2%	10.2%	78.6%	82.7%	19.6%

ratories calculated HRR gene mutation rates ranging widely from 19.89% to 35%. In the registration of PROfound trial Phase III, the positive rate of HRR mutations was 27.9%, and only deleterious or suspected deleterious mutations.[7] The difference in mutation frequency may be due to the inconsistency of gene panels and interpretation. Although there are no specific guidelines on HRR variants interpretation, the standards and guidelines of the American College of Medical Genetics and Genomics (ACMG guidelines) are well-accepted in the practice[22-25]. "Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer" (AMP/ASCO/CAP guideline)[26] is used as a guide for variant classification.

Only 25% of laboratories used only the ACMG guidelines, less than 5% used only the AMP/ASCO/CAP guideline, and 45% of laboratories used both. However, about 15% of laboratories also used other guidelines, and 5% of laboratories used their own standards. Due to the lack of public databases for HRR variant interpretation, the proportion of VUS is 10%-50%. Thus, it is important to establish public databases to facilitate the interpretation. However, only 60% of laboratories were willing to share their internal data.

The test failure rate of tissue and plasma samples was approximately 5%-10%, which was far lower than the PROfound trial.[17] According to the survey, poor sample quality was the main reason, such as less tumor content, DNA degradation, genomic DNA contamination, and insufficient ctDNA extraction. Standardized sample handling is the key to improving the success rate of testing.

3.5. EQA results for HRR testing using tissue samples

We examined the detection rate of 7 indels and 15 SNVs (22 mutation loci in tissue samples) in 32 laboratories according to NGS platforms (23 labs using Illumina [Illumina Inc., San Diego, CA,
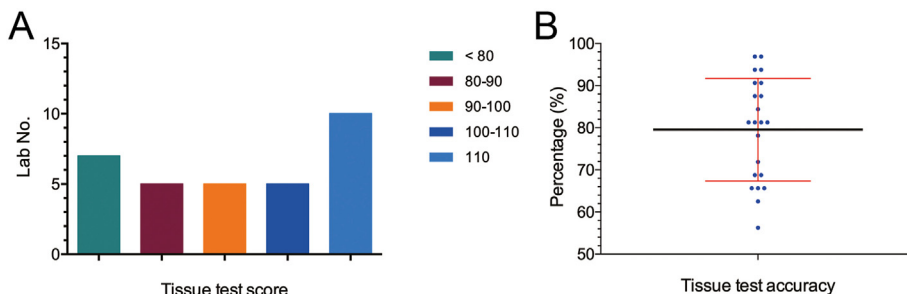

Fig. 2. EQA results of HRR tissue test accuracy (A) the test score distribution of 32 labs in tissue samples; (B) the detection rate of each variant loci in the tissue samples by 32 labs. Data were presented as mean ± SD.

USA], 7 labs using ThermoFisher [ThermoFisher Scientific, Waltham, MA, USA], and 2 labs using BGI [BGI Genomics, Shenzhen, China]) and library construction method (24 labs using hybrid capture, 8 labs using PCR amplicon) in the EQA project (Table 3). All 32 laboratories participated in tissue sample testing, and 62.5% of them passed the assessment (Fig. 2A). 10 laboratories achieved a full score of 110, 15 laboratories achieved more than 100, and 20 laboratories got more than 90 points. The detection rate of each locus is shown in Fig. 2B and the mean is 79.55 ± 12.19%. Additionally, loci with a detection failure rate greater than 30% are listed in Table 4. Small indel variants are likely to be missed. The adjacent genome sequence of these loci were mainly tandem repeat sequences with poly-A or Poly-T regions (3), which may be challenging for some platforms. Sequencing depth and target region coverage rate had no significance on the testing score, while the library construction methodology was closely relevant. In 24 laboratories that adopted the hybrid capture method to construct libraries, the average score was 94.58, and the pass rate was 79%; while in 8 laboratories that adopted the PCR amplicon-based method, the average score was 66.88, and the pass rate was 12.5%.

3.6. EQA results for HRR testing with plasma samples

23 laboratories participated in ctDNA sample testing. 10 laboratories achieved up to 90, 15 got >80 and the pass rate was about 65% (Fig. 3A). The detection rate of each variant loci is shown in Fig. 3B and the mean detection rate is 74.13 ± 17.78%. Information regarding the top missed variants is shown in Table 5. Missing loci were mainly distributed in variants of low allele frequency and indels in tandem repeat regions. These laboratories all adopted Illumina and BGI platforms, and no significance was found between testing score and sequencing depth, or target region coverage rate. The possible reasons for the missed variants were misclassification, low coverage, and below the QC criteria.

4. Discussion

This was the first nationwide survey and EQA involving HRR gene testing laboratories in China to evaluate laboratory practices and challenges in methodology, infrastructure, interpretation, and testing quality. In this survey, we observed inconsistencies in the HRR gene testing laboratories including sequencing platforms, library construction methods, gene panels, probe design, detectable mutation types, HRR positivity rate, variant interpretation, test failure rate, and quality control. In EQA, about 60% of laboratories passed the test. The most challenging variants for detection were small indels and variants located within the tandem repeat regions in tissue samples, as well as splice site and low allele frequency variants in ctDNA samples.

The most common NGS platform was Illumina (75%), followed by Thermo Fisher (22%) and BGI (6%). 96% of Illumina platforms used hybrid capture, while all Thermo Fisher platforms used PCR amplicon approaches. Compared with PCR amplicons, hybrid capture library creation generates higher capture efficiency, specificity, and repeatability, and is more suitable for detecting lower frequency variants, while PCR amplicon-based library preparation requires a much lower DNA loading amount, is easier to handle, has a shorter turn-around time, is more suitable for small panel testing, and is often used in hospital.[27] Indeed, the standard reference testing results showed a higher pass rate via hybrid capture for library construction.

In terms of gene panel design, over 90% of panels covered all the 14 HRR genes to guide Olaparib treatment for mCRPC, but not all laboratories covered all the exons and splice sites of these genes, which may lead to potential false negatives, and misdetection sites in their feedbacks. To avoid this, the panel design should include at least 14 HRR genes, covering all exon regions and splice sites.

With regard to the detectable mutation types, all panels could detect SNV and indel, about 50% of laboratories could detect LGR, and more than 80% could detect CNV. The main limitation of the reference standard was that the capability of LGR and CNV testing was not evaluated in this EQA project. Since LGR and CNV could cause loss of function, it is important to verify its panel testing capability.[28]

For the positive rate, most laboratories did not provide statistical results due to the limited sample volumes and substantial variations. This may be explained by the inconsistencies across testing

Table 4
Top missed variants in HRR tissue sample test

Gene	Reference Transcript	Mutation type	c.HGVS	p.HGVS	VAF
BRCA2	NM_000059.3	Deletion	c.2957delA	p.Asn986Ilefs*5	10.26%
CHEK1	NM_001274.5	Deletion	c.1064delT	p.Leu355*	10.80%
BRCA2	NM_000059.3	Deletion	c.3860delA	p.Asn1287Ilefs*6	48.14%
ATM	NM_000051.3	Insertion	c.1880dupT	p.Gln628Profs*7	46.11%
ATM	NM_000051.3	Insertion	c.4741dupA	p.Ile158Asn1fs*5	42.21%
PALB2	NM_024675.4	Deletion	c.2257delC	p.Arg753Glufs*11	4.70%
BRCA1	NM_007294.3	SNV	c.3172 A > G	p.Ile1058Val	5.70%

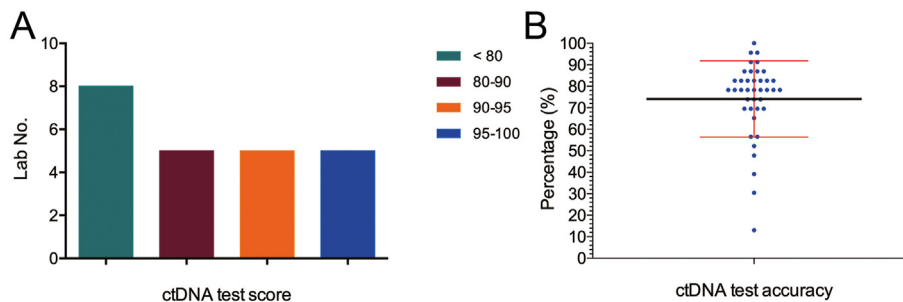HGVS: human genomic variation Society; VAF: variant allele frequency.

Fig. 3. External Quality Assessments result of HRR ctDNA test accuracy (A) the test score distribution of 23 labs in tissue samples; (B) the detection rate of each variant loci in the tissue samples by 23 labs. Data were presented as mean ± SD.

Table 5
Top missed sites in the HRR ctDNA sample test

Gene	Reference Transcript	Mutation type	c.HGVS	p.HGVS	VAF
PPP2R2A	NM_002717.3	Deletion	c.43delT	p.Ser15Leufs*3	0.56%
ATM	NM_000051.3	SNV	c.8584+3A > G	NA	1.59%
PPP2R2A	NM_002717.3	SNV	c.973-2A > G	NA	2.17%
BRCA1	NM_007294.3	SNV	c.2429 A > C	p.Asn810Thr	0.83%
RAD54 L	NM_001142548.2	SNV	c.346 G > T	p.Asp116Tyr	0.79%
RAD54 L	NM_001142548.2	SNV	c.1594 C > A	p.Leu532Met	25.80%

HGVS: human genomic variation Society; VAF: variant allele frequency.

panels, platforms, and interpretation standards. A similar situation was also observed in a worldwide survey study, 47% of the non-US labs used ACMG guidelines and 38% of US labs followed their own measures.[29] The expert consensus in China on BRCA variant interpretation is that ACMG guidelines and AMP/ASCO/CAP guidelines should be combined and only pathogenic or likely pathogenic variants can guide treatment based on the clinical evidence.[30] Laboratories should be encouraged to establish personalized databases and standards based on these rules.

In our survey, 24 labs submitted clinical reports and 19 labs reported VUS, while in a previous survey, 52% of US labs uncalculated VUS.[31] Since the uncertainty of VUS classification, it is challenging to estimate the risk of affected patients during clinical decision-making and genetic counseling[32],[33] Variant interpretation will be improved through the coordination of clinicians, pathologists, and the establishment of a Chinese HRR gene database. Sharing data enables quality-control, peer-reviewed processes, providing opportunities to improve the strategic management of patient care. Unfortunately, our study revealed that over 80% of the data was not shared with public databases due to profit considerations.

Two-thirds of the laboratories carry out plasma ctDNA testing. Lacking matched tissue and plasma samples data is because of the low feasibility of tissue samples. In PROfound and TRITON2, the consistency between tissue samples and plasma samples was approximately 82%-91%[17],[18] Additionally, the quantity and quality of ctDNA are critical[19],[34] Therefore, the extraction protocol of cell-free DNA requires further optimization.[35]

Clinical report standardization is also important for facilitating interpretation. In EQA testing, several issues were raised, including unnecessarily massive information, unacceptable errors, and missing critical information.

In summary, to improve HRR gene testing quality in China, it is necessary to establish standards for involved procedures. Our study is the first to report the nationwide EQA of HRR gene testing. The most common differences across laboratories included NGS platform, gene coverage, probe design, validation method, VUS analysis, interpretation, quality control, and data-sharing. Our results indicate the requirement for national guidelines to improve the clinical practice of HRR gene testing laboratories.

Data availability

The authors declare that all data supporting this study are available in the paper and supplementals.

Conflicts of interest

All authors declare no conflict of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.prnil.2022.07.002.

References

1. Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Pineros M, Znaor A, et al. Cancer statistics for the year 2020: An overview. Int J Cancer 2021;149(4): 778–89.
2. Karantanos T, Corn PG, Thompson TC. Prostate cancer progression after androgen deprivation therapy: mechanisms of castrate resistance and novel therapeutic approaches. Oncogene 2013;32(49):5501–11.
3. Nagaya N, Rosenfeld J, Lee GT, Kim IY. RNA-seq profile of African American men with a clinically localized prostate cancer. Prostate Int 2021;9(3):125–31.
4. Lingg L, Rottenberg S, Francica P. Meiotic Genes and DNA Double Strand Break Repair in Cancer. Front Genet 2022;13831620.
5. Mateo J, Porta N, Bianchini D, McGovern U, Elliott T, Jones R, et al. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-B): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol 2020;21(1):162–74.
6. Abida W, Armenia J, Gopalan A, Brennan R, Walsh M, Barron D, et al. Prospective Genomic Profiling of Prostate Cancer Across Disease States Reveals Germline and Somatic Alterations That May Affect Clinical Decision Making. JCO Precis Oncol 2017;2017.
7. de Bono J, Mateo J, Fizazi K, Saad F, Shore N, Sandhu S, et al. Olaparib for Metastatic Castration-Resistant Prostate Cancer. N Engl J Med 2020;382(22):2091–102.
8. Schaeffer E, Srinivas S, Antonarakis ES, Armstrong AJ, Bekelman JE, Cheng H, et al. NCCN Guidelines Insights: Prostate Cancer, Version 1.2021. J Natl Compr Cancer Netw 2021;19(2):134–43.
9. Cornford P, van den Bergh RCN, Briers E, Van den Broeck T, Cumberbatch MG, De Santis M, et al. EAU-EANM-ESTRO-ESUR-SIOG Guidelines on Prostate Cancer. Part II-2020 Update: Treatment of Relapsing and Metastatic Prostate Cancer. Eur Urol 2021;79(2):263–82.
10. Chen M, Zhao H. Next-generation sequencing in liquid biopsy: cancer screening and early detection. Hum Genom 2019;13(1):34.
11. Chung JS, Morgan TM, Hong SK. Clinical implications of genomic evaluations for prostate cancer risk stratification, screening, and treatment: a narrative review. Prostate Int 2020;8(3):99–106.
12. Wallace AJ. New challenges for BRCA testing: a view from the diagnostic laboratory. Eur J Hum Genet 2016;24(Suppl 1):S10–8.
13. de Bono JS, Fizazi K, Saad F, Shore N, Sandhu SK, Mehra N, et al. 847PD - Central, prospective detection of homologous recombination repair gene mutations (HRRm) in tumour tissue from >4000 men with metastatic castration-resistant prostate cancer (mCRPC) screened for the PROfound study. Ann Oncol 2019;30:v328–9.
14. Bronkhorst AJ, Ungerer V, Holdenrieder S. The emerging role of cell-free DNA as a molecular marker for cancer management. Biomol Detect Quantif 2019;17100087.
15. Andersson D, Kristiansson H, Kubista M, Stahlberg A. Ultrasensitive circulating tumor DNA analysis enables precision medicine: experimental workflow considerations. Expert Rev Mol Diagn 2021;21(3):299–310.
16. Zhang Y, Yao Y, Xu Y, Li L, Gong Y, Zhang K, et al. Pan-cancer circulating tumor DNA detection in over 10,000 Chinese patients. Nat Commun 2021;12(1):11.
17. Chi KN, Barnicle A, Sibilla C, Lai Z, Corcoran C, Williams JA, et al. Concordance of BRCA1, BRCA2 (BRCA), and ATM mutations identified in matched tumor tissue and circulating tumor DNA (ctDNA) in men with metastatic castration-resistant prostate cancer (mCRPC) screened in the PROfound study. J Clin Oncol 2021;39(6_suppl):26, 26.
18. Tukachinsky H, Madison RW, Chung JH, Gjoerup OV, Severson EA, Dennis L, et al. Genomic Analysis of Circulating Tumor DNA in 3,334 Patients with Advanced Prostate Cancer Identifies Targetable BRCA Alterations and AR Resistance Mechanisms. Clin Cancer Res 2021;27(11):3094–105.
19. Wyatt AW, Annala M, Aggarwal R, Beja K, Feng F, Youngren J, et al. Concordance of Circulating Tumor DNA and Matched Metastatic Tissue Biopsy in Prostate Cancer. J Natl Cancer Inst 2017;109(12).
20. Vandekerkhove G, Struss WJ, Annala M, Kallio HML, Khalaf D, Warner EW, et al. Circulating Tumor DNA Abundance and Potential Utility in De Novo Metastatic Prostate Cancer. Eur Urol 2019;75(4):667–75.
21. Volik S, Alcaide M, Morin RD, Collins C. Cell-free DNA (cfDNA): Clinical Significance and Utility in Cancer Shaped By Emerging Technologies. Mol Cancer Res 2016;14(10):898–908.
22. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, 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):405–24.

23. Biesecker LG, Harrison SM. ClinGen Sequence Variant Interpretation Working G. The ACMG/AMP reputable source criteria for the interpretation of sequence variants. Genet Med 2018;20(12):1687–8.

24. Abou Tayoun AN, Pesaran T, DiStefano MT, Oza A, Rehm HL, Biesecker LG, et al. Recommendations for interpreting the loss of function PVS1 ACMG/AMP variant criterion. Hum Mutat 2018;39(11):1517–24.

25. Brnich SE, Abou Tayoun AN, Couch FJ, Cutting GR, Greenblatt MS, Heinen CD, et al. Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. Genome Med 2019;12(1):3.

26. Li MM, Datto M, Duncavage EJ, Kulkarni S, Lindeman NI, Roy S, et al. Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. J Mol Diagn 2017;19(1):4–23.

27. Ballester LY, Luthra R, Kanagal-Shamanna R, Singh RR. Advances in clinical next-generation sequencing: target enrichment and sequencing technologies. Expert Rev Mol Diagn 2016;16(3):357–72.

28. Zakrzewski F, Gieldon L, Rump A, Seifert M, Grutzmann K, Kruger A, et al. Targeted capture-based NGS is superior to multiplex PCR-based NGS for hereditary BRCA1 and BRCA2 gene analysis in FFPE tumor samples. BMC Cancer 2019;19(1):396.

29. Toland AE, Forman A, Couch FJ, Culver JO, Eccles DM, Foulkes WD, et al. Clinical testing of BRCA1 and BRCA2: a worldwide snapshot of technological practices. NPJ Genom Med 2018;3:7.

30. Chinese Society of P, Chinese Pathology Quality Control C. [Chinese expert consensus on BRCA1/2 variant interpretation(2021 version)]. Zhonghua Bing Li Xue Za Zhi 2021;50(6):565–71.

31. Lindor NM, Goldgar DE, Tavtigian SV, Plon SE, Couch FJ. BRCA1/2 sequence variants of uncertain significance: a primer for providers to assist in discussions and in medical management. Oncol 2013;18(5):518–24.

32. Federici G, Soddu S. Variants of uncertain significance in the era of high-throughput genome sequencing: a lesson from breast and ovary cancers. J Exp Clin Cancer Res 2020;39(1):46.

33. Fatkin D, Johnson R. Variants of Uncertain Significance and "Missing Pathogenicity". J Am Heart Assoc 2020;9(3)e015588.

34. Ulz P, Belic J, Graf R, Auer M, Lafer I, Fischereder K, et al. Whole-genome plasma sequencing reveals focal amplifications as a driving force in metastatic prostate cancer. Nat Commun 2016;712008.

35. Mauger F, Dulary C, Daviaud C, Deleuze JF, Tost J. Comprehensive evaluation of methods to isolate, quantify, and characterize circulating cell-free DNA from small volumes of plasma. Anal Bioanal Chem 2015;407(22):6873–8.