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Homologous recombination repair gene mutations in Chinese localized and locally advanced prostate cancer patients

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ABSTRACT

Background: Homologous recombination repair gene (HRR) mutations have been proven to be effective biomarkers for PARP inhibitor therapy for metastatic castration resistant prostate cancer. However, the frequency of HRR mutations in patients with localized and locally advanced prostate cancer is still unclear. This study investigated the profile of HRR gene mutations in Chinese localized and locally advanced prostate cancer patients.

Materials and Methods: 74 patients with localized and locally advanced prostate cancer patients in Beijing Chaoyang Hospital between May 2018 and September 2019 were retrospectively included. Matched prostate cancer and histologically normal tissues were subjected to next-generation sequencing. Pathogenic alterations of 19 HRR genes were examined.

Results: Ten deleterious and suspected deleterious mutations (4 germline and 6 somatic mutations) were detected in 9 of 74 (12.16 %) patients, occurred in seven HRR-related genes, including *CDK12*, *NBN*, *ATM*, *ATR*, *BRCA2*, *PALB2* and *RAD51C*. The mutation frequency of HRR genes in this study (12.16 %) was higher than TCGA cohort (7.29 %), and the mutation sites in 7 HRR genes detected in this cohort were different from those of TCGA data. Patients with HRR gene mutations had higher Gleason grade ($\geq$ 3) ($P = 0.03$) and risk level (very-high) ($P = 0.03$). Postoperative prostate specific antigen level and positive surgical margin rate was not associated with HRR gene mutation status.

Conclusions: This study illustrated the mutation patterns of HRR genes in Chinese population with localized and locally advanced prostate cancer. These results provide further evidence that HRR gene mutations were more prevalent in patients with higher Gleason grade, or with very-high-risk level. Patients with these clinicopathologic characteristics may need more precise stratification through molecular detection.

1. Introduction

Prostate cancer (PCa) is one of the mostly occurred types of cancer in male [1]. Men with T1b-4, N0-Nx (N not allowed), M0 PCa can be defined early PCa, including localized (T1−2, M0, N0-Nx) and locally advanced (T3−4, any N; or any T, N+) PCa (all M0) [2–5]. Most of the patients with localized disease will be cured with radiation therapy, surgery and/or endocrine therapy, while, intermediate- and high-risk localized PCa patients (20 %–35 % of total localized disease) will suffer from disease recurrence, metastasis and unfavored prognosis [6]. And men with locally advanced PCa are at a significant risk of disease recurrence and PCa-specific mortality rates [7,8]. Therefore, it is urgent to improve the accuracy of PCa diagnosis, predict patients at highest risk of relapse, and ultimately achieve the goal of improving the treatment effect.

With the development of genome sequencing technology, the molecular and genetic profiles of a variety of cancers, including PCa [9–12], have been explored and understood, providing guidance for using

Abbreviations: PCa, prostate cancer; HRR, homologous recombination repair; FFPE, formalin-fixed paraffin-embedded; PSA, prostate specific antigen; NCCN, National Comprehensive Cancer Network; TCGA, The Cancer Genome Atlas; HRRm, homologous recombination repair gene mutation.

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genomics to risk-stratify patients and to select patients for precise targeted therapies. Notably, DNA repair deficiency contributes to the development of prostate cancer, and the incidence of genetic mutations differs among different disease states. The percentage of patients with germline mutations in DNA repair genes ranges from 4.6 % in patients with localized PCa to 11.8 % in patients with metastatic PCa [13,14]. In Chinese population, Wei et al. displayed 11.8 % of 246 PCa patients carried germline homologous recombination repair (HRR) gene mutations [15].Identifying PCa patients harboring HRR gene mutations at localized and locally advanced stage may indicate the early introduction of targeted therapies (such as PARP inhibitors) that could improve clinical outcome as well as life quality in patients.

However, information on somatic and germline HRR gene mutations in patients with localized and locally advanced PCa is still limited, especially in Chinese population. Herein, we sought to investigate the profile of HRR gene mutations and their relationship with clinicopathological characters of Chinese localized and locally advanced PCa patients.

2. Materials and methods

2.1. Patients

A total of 74 patients with localized and locally advanced PCa (all M0) at the Beijing Chaoyang Hospital between May 2018 and September 2019 were enrolled in this study. Routine sampling for standard pathological examination by H&E and immunostaining was performed, allowing for tumor classification, Gleason grading and staging. The inclusion criteria were: the histologic diagnosis of PCa was confirmed by a pathologist specializing in genitourinary tumors; prostatectomy specimens were obtained and matched histologically normal tissues was drawn for germline DNA; archival samples were preserved as formalin-fixed paraffin-embedded (FFPE) samples. Patient data, including age, smoking history, alcoholism history, family history, Gleason grade and prostate specific antigen (PSA) level were obtained from clinical records. This study was approved by the Ethics Committee of Beijing Chaoyang Hospital (No.: 2020-8-17−1). Informed consent was waived because of the retrospective nature of this study and anonymized clinical data was used for analysis.

2.2. Sample preparation, next-generation sequencing and data processing

Ten 5 μm tumor slices cut from FFPE samples were used for genomic DNA extraction using Amoy extraction kit (Amoy Diagnostics, Xiamen, China) according to the manufacturer's instructions. DNA fragments were used for library construction using the HRR gene combination detection library preparation kit (Amoy Diagnostics, Xiamen, China) according to the manufacturer's protocol. Genomic DNA samples were sequenced with a HRR 32-gene panel (including 19 HRR genes: *ATM*, *ATR*, *BRCA1*, *BRCA2*, *BARD1*, *CHEK1*, *CHEK2*, *FANCA*, *FANCL*, *PALB2*, *RAD51B*, *RAD51C*, *RAD51D*, *RAD54L*, *CDK12*, *NBN*, PPP2R2A, *BRIP1*, *MRE11A*, and 13 genetic and therapy related genes: *AR*, *BRAF*, *CDH1*, *ESR1*, *HDAC2*, *KRAS*, *TP53*, *NRAS*, *PIK3CA*, *HOXB13*, *ERBB2*, *PTEN*, *STK11*) (Amoy Diagnostics, Xiamen, China). DNA sequencing was then performed on the NextSeq500 Illumina platform (Illumina, San Diego, CA, USA) at an average depth of 1000×, the effective sequencing depth is greater than 300×, and the proportion of Q30 bases is ≥ 75 %. The types of detected mutations include Single nucleotide variants (SNVs) and small indels (<50 bp). For patients with HRR gene mutations detected in tumor tissue, paracancerous tissue was also tested to distinguish between germline and somatic mutations. Sequencing data was analyzed by Sequencing Data Analysis Software (Amoy Diagnostics, Xiamen, China), the deleterious and suspected deleterious of gene mutations were scrutinized and interpreted according to American College of Medical Genetics and Genomics (ACMG) guidelines and/or in ClinVar. [16].

Germline variant data [13] and somatic variant data (https://www.cbioportal.org/) of localized and locally advanced PCa patients from The Cancer Genome Atlas (TCGA) database were analyzed for comparison with the data in this study.

2.3. Statistical analysis

Chi-square test or Fisher's exact test was used to compare categorical variables, including Gleason grade, risk level, smoking and alcoholism status, and positive surgical margins. The independent *t*-test and Mann-Whitney *U* test were used to compare continuous variables, including age at diagnosis, PSA, PSA decline rate. Significance was determined by a P-value < 0.05. All statistics were performed using SPSS software (SPSS version 24.0 for Windows, IBM Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of study participants

Of the 74 patients, 3 did not undergo radical surgery and only received endocrine therapy. The remaining 71 patients underwent laparoscopic radical prostatectomy, of which 13 received neoadjuvant endocrine therapy (total androgen blockade) before surgery, and 28 received adjuvant endocrine therapy +/- radiotherapy after surgery. No patients had biochemical recurrence at the time of data collection. 70 (94.60 %) patients were acinar adenocarcinoma, 3 (4.05 %) patients were acinar adenocarcinoma with ductal adenocarcinoma, and 1 (1.35 %) patient was acinar adenocarcinoma with intraductal carcinoma. None of the patients had a family history of PCa, other detailed characteristics of the study patients are presented in Table 1.

3.2. Genomic alterations in HRR genes and other oncogenic genes

Variants in HRR genes were identified in 9 of 74 (12.16 %) patients (5.41 %, 4/74 carried germline mutations and 6.76 %, 5/74 carried somatic mutations). A total of 10 deleterious or suspected deleterious mutation sites, 4 germline mutations and 6 somatic mutations, occurred in seven HRR genes, including *CDK12* (3/10, 30 % of total mutations), *NBN* (2/10, 20 %), *ATM* (1/10, 10 %), *ATR* (1/10,10 %), *BRCA2* (1/10, 10 %), *PALB2* (1/10, 10 %), and *RAD51C* (1/10, 10 %) (Fig. 1A). One of these patients had two *CDK12* mutation sites, and two patients had the same *NBN* mutation. In addition to HRR gene mutations, the panel also detected 4 other oncogene mutations in 4 patients, including 1 case with *BRAF* mutation (NM_004333 exon11 c.1406 G > C p.G469A) and 3 cases with *TP53* mutations (NM_000546 intron4 c.376−1G > A, exon6 c.610 G > T p.E204*, and exon6 c.586C > T p.R196*, respectively) (Supplementary Table 1 and Supplementary Fig. 1).

To determine the difference of mutation landscape in HRR genes between Chinese populations and data documented in TCGA, the localized and locally advanced cases in the TCGA cohort were analyzed (pT ≤ 3, N0−1, M0) (Fig. 1B). Compared with this cohort, the HRR gene mutation rate was lower (7.29 %, 28/384) in TCGA cohort (2.08 %, 8/384 carried germline mutations, and 5.21 %, 20/384 carried somatic mutations), but the result was not statistically significant ($p = 0.17$). Among the 7 HRR genes that were mutated in this cohort, 6 genes were also mutated in TCGA data. Although the mutation frequency of each gene was different, there was no significant difference between this cohort and TCGA data (Supplementary Table 2). Two genes with relatively high mutation frequency in this cohort were *CDK12* (2/74, 2.70 %) and *NBN* (2/74, 2.70 %), while *ATM* (9/384, 2.34 %) and *BRCA2* (5/384, 1.30 %) had relatively high mutation frequency in TCGA cohort. *BRCA1*, *BRIP1*, *CHEK2* and *RAD51D* gene mutations were also detected in the TCGA database, but they were not detected in this cohort.

Table 1
Clinical characteristics of patients with prostate cancer.

Parameter	Total (n = 74)	Any HRRm (n = 9)	No HRRm (n = 65)	*p* value
Median age, yr	68 (48–84)	60 (56–79)	68 (48–84)	0.15
Smoking history, n (%)				
Never	48 (64.86 %)	5 (55.56 %)	43 (66.15 %)	
Current or former	25 (33.78 %)	4 (44.44 %)	21 (32.31 %)	
Unknown	1 (1.35 %)	0	1 (1.54 %)	0.48
Alcoholism history, n (%)				
Never	59 (79.73 %)	8 (88.89 %)	51 (78.46 %)	
Current or former	14 (18.92 %)	1 (11.11 %)	13 (20.00 %)	
Unknown	1 (1.35 %)	0	1 (1.54 %)	1
Median baseline PSA, ng/mL	14.93 (3.88–516.77)	20.86 (4.66–516.77)	12.49 (3.88–254.16)	0.10
Histology, n (%)				
acinar adenocarcinoma	70 (94.59 %)	8 (88.89 %)	62 (95.38 %)	
acinar adenocarcinoma with ductal adenocarcinoma/intraductal carcinoma	4 (5.41 %)	1 (11.11 %)	3 (4.62 %)	0.41
Gleason grade group, n (%)				
1 + 2	34 (45.95 %)	1 (11.11 %)	33 (50.77 %)	
3 + 4+5	26 (54.05 %)	3 (88.89 %)	14 (49.23 %)	0.03
Risk group*, n (%)				
Intermediate-High	39 (52.70 %)	2 (22.22 %)	37 (56.92 %)	
Very high	29 (39.19 %)	7 (77.78 %)	22 (33.85 %)	0.03#
Regional	2 (2.70 %)	0	2 (3.08 %)	
Unknown	4 (5.41 %)	0	4 (6.15 %)	

PSA: prostate specific antigen; HRRm: homologous recombination repair gene mutation.
p value of the Intermediate-High compared with Very high by Chi-square test.
* Risk group is categorized according to the National Comprehensive Cancer Network (NCCN) guideline risk criteria.

Furthermore, the mutation sites in 7 HRR genes detected in this cohort were different from those of TCGA data (Fig. 1C).

3.3. Clinico-pathological features and outcomes

In this cohort, there were no statistically significant associations between HRR gene mutations and age of onset, smoking and alcoholism history, PSA value at diagnosis or histology. To obtain more precise risk stratification of poor prognosis category prostate cancer, we further analyzed HRR gene mutations in PCa patients with Gleason grade and risk level. Our analysis showed that HRR mutation carriers tend to have higher Gleason grade ($\geq$ 3) (P = 0.03). Moreover, patients with HRR mutations were more likely to be very-high risk (P = 0.03) (Table 1).

Postoperative PSA level data was collected from 53 patients who underwent laparoscopic radical prostatectomy and without neoadjuvant endocrine therapy. The postoperative median PSA level (0.07 vs. 0.01 ng/mL) and the rate of positive resection margin (80.00 % vs. 52.08 %) were higher in HRR-mutant patients than that in wild-type patients, but there were no statistical differences (Table 2). Furthermore, we analyzed the changes of PSA and the rates of positive resection margin in all patients undergoing laparoscopic radical prostatectomy and patients with neoadjuvant endocrine therapy, but there were no statistical differences between HRR gene mutations carriers and non-carriers (Supplementary Table 3 and 4).

4. Discussion

In this retrospective investigation, we examined the prevalence of germline and somatic alterations in HRR genes in Chinese localized and locally advanced PCa patients. To our knowledge, this is the first study to focus on Chinese localized and locally advanced PCa patients through genomic screening to identify potentially targetable alterations. The current study showed that mutations in HRR genes are enriched in localized and locally advanced PCa with higher Gleason grade (Gleason grade group $\geq$ 3) and higher risk level (very-high). There were no statistically significant association between HRR gene mutations and histology, which was different to the observations in Isaacsson Velho et al. and Lozano et al's reports [17,18], it may be due to the low number of patients resulting in an insufficient statistical potency. Moreover, these data showed that patients with HRR gene mutations may have a poor prognosis after radical surgery, which is reflected in the postoperative PSA level (0.07 vs. 0.01 ng/mL) and the positive rate of resection margin (80.00 % vs. 52.08 %) for HRR gene mutations carriers and noncarriers.

With the continuous exploration and development of tumor precision medicine, the diagnosis and treatment of PCa has also entered a new stage of precision diagnosis and treatment. Description of the genomic profiling of PCa and development of targeted therapy has gradually become breakthroughs in the treatment of PCa. Recently, two PARP inhibitors, olaparib and rucaparib, have been approved by FDA for the treatment of advanced PCa, heralding the onset of precision medicine for this disease [19]. However, more researches were focused on genetic testing, especially DNA damage repair gene mutations detection, in patients with advanced PCa [13,20,21]. And there is no consensus on whether genetic testing is necessary in localized and locally advanced PCa patients. Additionally, it is also unclear which patients with localized and locally advanced PCa should undergo molecular mutation testing [14,22,23]. Our primary motivation in choosing localized and locally advanced PCa patients as subjects in this study was to accumulate data for more precise molecular stratification and provide information for clinical trial design for targeted therapy in localized and locally advanced PCa patients.

The somatic and germline mutation frequency of HRR genes in this study was both higher than TCGA cohort (5.41 % vs. 2.08 % and 6.76 % vs. 5.21 %, respectively). In this cohort, somatic mutated gene were *CDK12* (2.70 %), *ATM* (1.35 %), *BRCA2* (1.35 %) and *RAD51C* (1.35 %). In Marshall et al.'s study, 8.0 % of localized PCa patients (57/710) had any somatic DNA repair gene mutation [22], and the five most commonly mutated genes were *ATM* (2.7 %), *BRCA2* (2.0 %), *CDK12* (1.5 %), *BRCA1* (0.7 %), and *PALB2* (0.7 %). Abida et al. reported somatic mutated genes in Locoregional PCa patients were *BRCA2* (6%), *CDK12* (4%), *BRCA1* (1%), *ATM* (2%), and *FANC* (1%) [14]. Additionally, Wei et al. 's study showed that the rates of germline pathogenic mutations were 9.8 % in 18 DNA repair genes in 316 Chinese PCa patients (*BRCA2* 62.5 %, *BRCA1* 6.25 %, and *ATM* 6.25 %, etc.). Of note, in these 316 Chinese patients, the rates of cancer family history and metastatic cases were 27 % and 59 %, respectively [24]. Similarly, in another study, 11.8 % of 246 Chinese PCa patients carrying germline pathogenic HRR gene mutations were detected, and cancer family

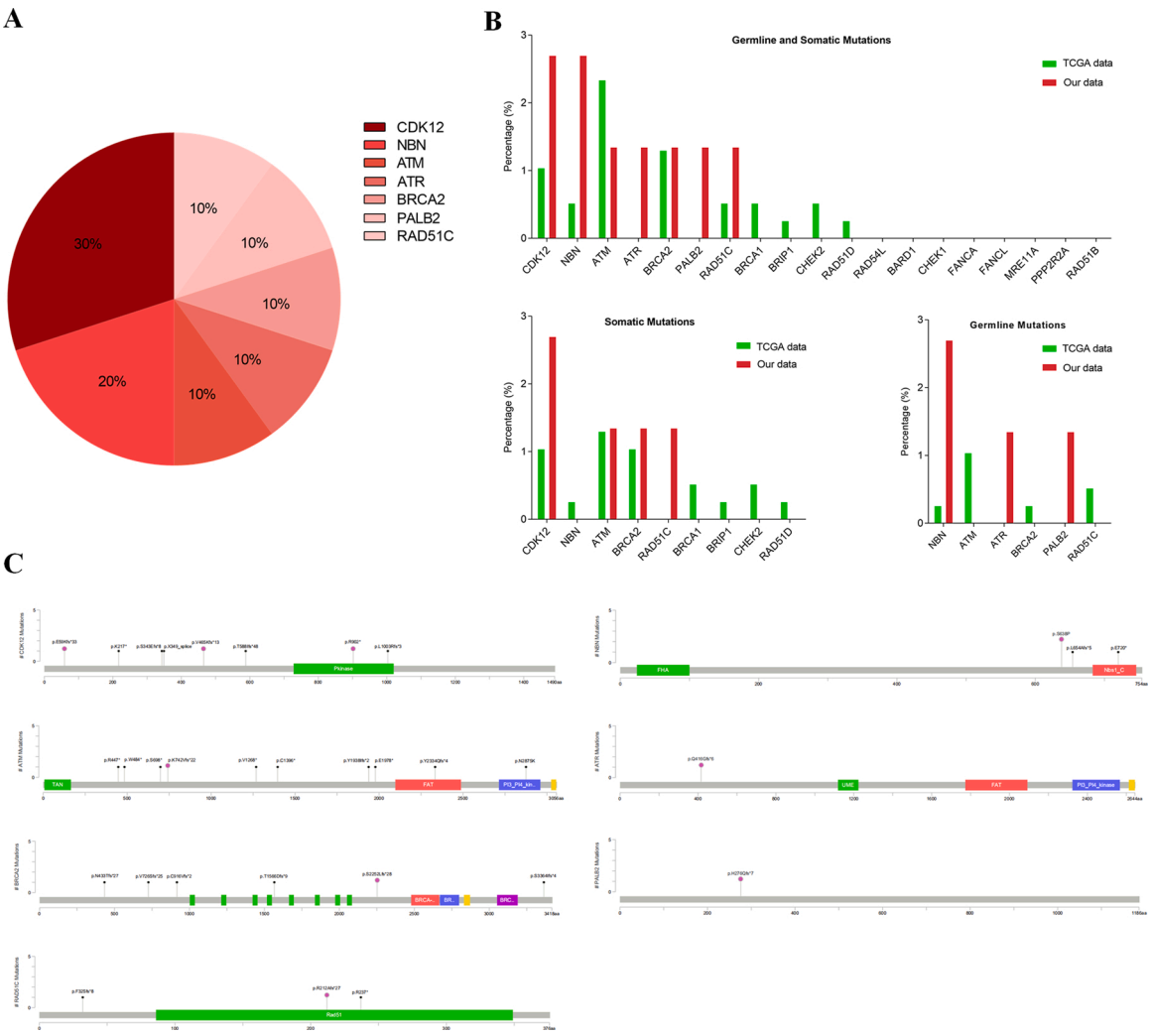

Fig. 1. HRR gene mutations in this cohort. **A** Distribution of deleterious and suspected deleterious mutations that occurred in seven HRR genes; **B** Comparison of germline and somatic (top), somatic (bottom left) and germline (bottom right) HHR genes mutation spectrum in this data and TCGA data; **C** Locations of deleterious or suspected deleterious alterations in the seven HRR genes. The red lollipop plot represents our date and the black lollipop plot represents TCGA date.

Table 2
Changes of PSA in patients undergoing laparoscopic radical prostatectomy without neoadjuvant endocrine therapy.

Parameter	Total (n = 53)	Any HRRm (n = 5)	No HRRm (48)
Median baseline PSA, ng/mL	10.80 (3.88–95.18)	14.86 (4.66–20.86) *p* value = 0.78	10.23 (3.88–95.18)
Median PSA after surgery, ng/mL	0.03 (0.00–6.03)	0.07 (0.00–6.03) *p* value = 0.44	0.01 (0.00–5.87)
positive surgical margins, %	54.72 % (29/53)	80.00 % (4/5) *p* value = 0.36	52.08 % (25/48)

history and metastatic cases were 69 % and 21 %, respectively [15]. Collectively, the difference mutation rates in the present study may be related with ethnic backgrounds, the family history of cancers and metastatic cases, and suggest the importance of population selection for molecular profiling study. Furthermore, compared with the previous reports that only performed germline or somatic testing, more patients with HRR gene mutations may be screened out by simultaneous testing for germline and somatic mutations.

The relationship between HRR gene mutations and clinicopathological features of patients were also analyzed in this study. Higher Gleason grade is usually associated with poorly differentiated tumors and a worse prognosis [25]. Our data suggest that patients with Gleason grade ≥ 3, or with very-high-risk level were more likely to have HRR gene mutations. Although molecular profiling has not yet been considered as a standard care for patients with localized and locally advanced PCa, early understanding of the specific molecular environment of these patients may provide the basis for genomics-based risk stratification to identify which subgroup that needs aggressive management. By comparing the postoperative PSA level and positive rate of resection margin between HRR gene mutations carriers and noncarriers, we found that localized and locally advanced PCa patients with HRR gene mutations may have a poor postoperative prognosis. Although lack statistical differences in the results, these estimates can serve as a reference for the future research.

There are several limitations of this study. Firstly, it is a retrospective analysis and includes only a single institution. Secondly, the NGS detection panel contained only 19 frequently mutated HRR genes instead of all HRR genes, and the types of detected mutations only include SNVs and small indels (< 50 bp). These may cause omission of important findings (such as HRR gene somatic Loss of heterozygosity and PTEN deep deletion). Furthermore, the sample size was still limited. Larger sample studies were further warranted.

5. Conclusions

In conclusion, the frequency of HRR gene mutations in Chinese localized and locally advanced PCa patients is higher than the populations of TCGA. And HRR gene mutations in this study are more prevalent in patients with higher Gleason grades (Group 3 and higher), or with very-high-risk level. Thus, patients with these clinicopathologic characteristics may be obtain more precise risk stratification through molecular detection.

CRediT authorship contribution statement

Xingran Jiang: Data curation, Data analysis, Writing - original draft.**: Xiumei Hu:** Data curation, Data analysis.**: Yajuan Gu:** Data curation, Data analysis:. **Yunlong Li:** Methodology, Investigation:. **Mulan Jin:** Supervision:. **Hongying Zhao:** Data analysis, Methodology:. **Ruixia Gao:** Methodology, Investigation:. **Zhan Huang:** Methodology, Investigation:. **Jun Lu:** Supervision, Writing - review & editing.

Data availability

Data used to support the results of this study can be obtained from the corresponding author.

Declaration of Competing Interest

Ruixia Gao and Zhan Huang are employees of AmoyDx. The other authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2021.153507.

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