

# Mutation analysis of circulating tumor DNA and paired ascites and tumor tissues in ovarian cancer

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Received March 10, 2022; Accepted May 20, 2022

DOI: 10.3892/etm.2022.11479

**Abstract.** Circulating tumor DNA (ctDNA) is one conventional type of liquid biopsy that can be collected to dynamically monitor disease status. However, its potential clinical value and concordance with ascites samples or tumor biopsy needs to be evaluated further for patients with ovarian cancer. Therefore, the present study compared the mutation profiles among ctDNA, paired tumor tissue and ascites samples to explore their possible clinical value in ovarian cancer. Targeted next-generation sequencing was used to screen for mutations in 18 peripheral blood samples, six paired ascites samples and eight paired tumor tissues collected from patients with ovarian cancer. Functional analyses were performed using public databases. WebGestalt was used to perform Gene Ontology and pathway enrichment analyses. The cBioPortal for Cancer Genomics was used to assess therapeutic targets. Chilibot and Search Tool for the Retrieval of Interacting Genes/Proteins were used to obtain key genes and their functional interactions. Comparative analysis was performed among the three types of samples using Venn diagram. A total of 104 cancer-associated mutant genes in ctDNA samples, 95 genes in tumor tissues and 44 genes in ascites samples were found. A cluster covering 10 genes, namely NOTCH2, NOTCH3, lysine methyltransferase 2A, PTEN, androgen receptor, DNA-activated protein kinase catalytic subunit, hepatocyte nuclear factor 1 homeobox A, SRC, insulin receptor substrate 2 and SRY-box transcription factor 10, was obtained by Chilibot analysis. This gene panel may have the potential to monitor metastasis and identify therapeutic targets in ovarian

cancer. Taken together, the present study focused on the mutant genes in ctDNA, ascites and tumor tissues, and suggested that the integrated information of different samples could be examined to comprehensively reflect the mutational landscape in ovarian cancer. However, procedures and protocols to interpret and utilize the integrated information obtained from various forms of liquid biopsies will require optimization prior to their use for future clinical applications.

## Introduction

According to cancer statistics of 2022, ovarian cancer ranks as the fifth major cause of cancer-associated mortality for females in the United States, with a 5-year survival rate <50% for all stages (1). In addition, ~60% of newly diagnosed patients typically present with distant metastasis, resulting in a 30% 5-year survival rate (2). Due to the anatomical location of the ovaries in the deep pelvic cavity, symptoms of this cancer include abdominal or pelvic pain or distension, bloating, urinary frequency and urgency (3,4). However, they are only perceptible after metastatic malignancy has been developed or after the volume of the primary tumor has reached a size that can obstruct the gastrointestinal or pelvic organs (3,4). Genetic heterogeneity is widespread in ovarian cancer (5-7). In ovarian cancer, genomic variation, invasive ability and sensitivity to platinum profiles are all heterogeneous among intratumor subclones, subtypes or between primary and recurrent malignancy (8-10). This poses a challenge to the development of applicable genetic biomarkers for early diagnosis, dynamic disease monitoring and therapeutic target identification in the clinic. Identification of novel biomarkers and deeper understanding of the genomic profile of ovarian cancer will likely increase the survival rate from this disease through metastasis monitoring and finding potential mutations for guiding individualized treatment.

Compared with traditional tissue biopsies, liquid biopsy is a relatively non-invasive sample collection method. Liquid biopsy specimens can be obtained with ease and used to monitor diseases in real time dynamically at multiple sampling times (11-13). The most common type of liquid biopsy is circulating tumor DNA (ctDNA), which is a fragmented form of single-stranded or double-stranded DNA that is released into the bloodstream following the necrosis and apoptosis of

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**Key words:** ovarian cancer, circulating tumor DNA, liquid biopsy, tumor heterogeneity

primary, metastatic or recurrent tumor cells (14,15). ctDNA application is promising in the field of precision oncology due to its potential for use in reflecting the tumor genetic profile and the wide availability of techniques, such as PCR and next-generation sequencing technology (NGS) (16).

However, the accuracy of using ctDNA to reflect all mutational information in the primary tumor lacks direct experimental evidence. Previous studies compared the genetic information between ctDNA and primary or metastatic tumor tissues. Comparison analysis of genetic profiles in prostate, breast and lung cancer showed that the concordance between ctDNA and tissues might depend on alteration types, with considerable concordance in driver DNA alterations but limited concordance in gene amplification and hotspot mutations (17-20). Furthermore, previous analyses suggested that the concordance of hotspot mutations or specific mutations in ctDNA and tumor tissues depends on gene types, for example, 90% concordance of the PIK3CA mutation and 78% concordance of the KRAS mutation were observed in colorectal cancer, and 50% concordance of EGFR mutation was observed in lung cancer (21-24). DNA methylation status and intratumor heterogeneity were also examined in ctDNA. ctDNA could partially reflect the methylation status of a specific gene in breast cancer but had limited value of reflecting intratumor heterogeneity in lung cancer (25,26). These studies revealed that the integrated analysis with both liquid and tumor biopsy might help to obtain a more comprehensive genetic profile of tumors.

ctDNA has exhibited a considerable clinical value as a tumor biomarker in diagnosis and disease monitoring of ovarian cancer (27). However, few studies compared the sequencing results of ctDNA, paired ascites and tumor tissue samples in ovarian cancer (28,29). A parallel analysis of these three types of samples mainly explained the potential of ascites and ctDNA to reflect the mutational landscape in 10 patients (30), although these results require further validation.

Therefore, the present study aimed to detect potential mutant genes in ctDNA samples for clinical application, the data of which were then used to compare against the mutational spectrum in plasma, matching ascites and tumor tissue samples using NGS. The rationale was to further understand the underlying mechanism of the inter-tumor and intra-tumor heterogeneity in ovarian cancer, in addition to its genomic landscape. Overall, the utility of ctDNA to dynamically reflect the overall condition of ovarian cancer and its prospective value in clinical applications, such as metastasis and targeted therapy, were examined.

## Materials and methods

*Patient enrollment and sample collection.* Patients with suspected ovarian cancer were recruited at the Obstetrics and Gynecology Hospital of Fudan University (Shanghai, China) between October 2016 and June 2017. Patients with pathologically confirmed epithelial ovarian cancer were included. Patients with borderline ovarian tumors, malignancies in other organs and uncontrollable infection, and those without consent were excluded. A total of 18 patients were included. In total, 8-10 ml peripheral blood was collected for ctDNA from these 18 patients, ~10 ml matched ascites from 6 of these patients,

and 0.5-cm<sup>3</sup> tumor tissues from 8 of these patients. The present study was approved by the Ethical Committee of Obstetrics and Gynecology Hospital of Fudan University (approval no. 2016-48; Shanghai, China).

*Nucleic acid isolation and quality assessment.* Peripheral blood samples were first centrifuged at 800 x g for 10 min at room temperature within 4 h of blood collection to obtain the plasma. The plasma samples were further centrifuged at 16,000 x g for 10 min at room temperature to remove the remaining cellular debris. ctDNA was extracted from the plasma using the QIAamp Circulating Nucleic Acid kit (cat. no. 55114; Qiagen China Co., Ltd.). The quantity and quality of the extracted DNA were assessed using an UV-vis spectrophotometer (Thermo Fisher Scientific, Inc.) and the Agilent 2100 Bioanalyzer.

DNA was extracted from tumor tissue or ascite samples after cell lysis and digestion with the QIAamp DNA Mini Kit (cat. no. 51304; Qiagen China Co., Ltd.) according to the manufacturer's protocols. The quantity and quality of the extracted DNA were assessed as for ctDNA. The extracted DNA from tumor tissues and ascites was sheared using Covaris sonicator (Covaris LLC) with the following settings: 4-8°C, 10% duty factor, 200 cycles per burst and 360 sec, after further quantification using a Qubit dsDNA HS Assay Kit (cat. no. Q32851; Thermo Fisher Scientific, Inc.). The extracted DNA was stored at -80°C.

*Detection of mutations in DNA sequences using NGS.* The SureSelectXT Reagent Kit (cat. no. G9611A; Agilent Technologies, Inc.) was used to construct the NGS libraries according to the SureSelect Target Enrichment workflow instructions. Briefly, after end repair, A-tailing and ligation with adaptors using a SureSelect Library Prep Kit (cat. no. G9684A; Agilent Technologies, Inc.), the DNA was amplified by pre-capture PCR (1 cycle of 98°C for 2 min; 10 cycles of 98°C for 30 sec, 65°C for 30 sec and 72°C for 1 min; and then 72°C for 10 min). The products were then hybridized and captured using the reagents in the aforementioned SureSelectXT Reagent Kit. The captured DNA fractions were amplified by post-capture PCR (1 cycle at 98°C for 2 min; 16 cycles at 98°C for 30 sec, 57°C for 30 sec and 72°C for 1 min; and then 72°C for 10 min) using a Herculase II Fusion DNA Polymerase kit (cat. no. 600677; Agilent Technologies, Inc.). Agencourt AMPure XP Beads (cat. no. A63881; Beckman Coulter, Inc.) were used to purify the samples throughout these processes. The libraries were assessed with the DNA 1000 Assay (cat. no. 5067-1504; Agilent Technologies, Inc.) on the Agilent 2100 Bioanalyzer. After quality assessment, the concentration of PCR products was quantified using an Agilent qPCR NGS Library Quantification Kit (cat. no. G4880A; Agilent Technologies, Inc.).

The generated libraries were sequenced with a concentration of 8 pM for 150-bp paired end reads on an Illumina MiSeq sequencer using a MiSeq Reagent Kit (cat. no. MS-102-2002; Illumina, Inc.) according to the manufacturer's recommendations. All samples were sequenced via the OncoGxOne cancer panel by Shanghai Liwen Biotechnology Co., Ltd. After Illumina sequencing, the reads were aligned to the human HG19 reference genome (GRCh37; <https://www.ncbi>.

nlm.nih.gov/grc) using Burrows-Wheeler Aligner v0.7.12 (<http://bio-bwa.sourceforge.net>). Variant calling was performed using the Genome Analysis Toolkit v3.4 (<https://gatk.broadinstitute.org>), and was annotated using ANNOVAR (last update, 06/01/17; <https://annovar.openbioinformatics.org>). The variants with a minor allele frequency <5% were filtered out.

**Functional analysis of the mutant genes.** Functional analysis of the potential mutant genes was performed using public bioinformatics tools and databases. WebGestalt (2019 version; <http://www.webgestalt.org>) was used with the following parameters: The functional databases including Gene Ontology (GO), Wikipathway and Kyoto Encyclopedia of Genes and Genomes (KEGG) were selected. The enrichment method was over-representation analysis, where the false discovery rate (FDR) was determined using the Benjamini-Hochberg method. The top 10 categories with FDR <0.05 were identified to be among the enriched categories. The cBioPortal for Cancer Genomics (version 2.0.1; <http://www.cbioportal.org/>) was used to analyze the gene and drug-target interaction network. The network, which showed the query genes, neighbor genes and drug-target information, was generated by submitting the query genes and adding the drug data in the Network tab (31). ‘metastasis’ and ‘ovarian cancer’ were used as key words in Chilibot (last update 06/18/17; <http://www.chilibot.net/>) to analyze mutant genes, and the overlap of these two sets was considered as the genes related to ovarian cancer metastasis. Search Tool for the Retrieval of Interacting Genes/Proteins (STRING; version 11.0; <https://string-db.org>) was used to analyze functional interactions with a medium confidence of 0.4. Comparative analysis of the peripheral blood, tumor tissue and ascites samples was performed using Venn diagram (version 2.1; <https://bioinfo.cnb.csic.es/tools/venny/index.html>).

**Statistical analysis.** The data were analyzed using GraphPad Prism 6.0 (GraphPad Software, Inc.) and SPSS 16.0 (SPSS, Inc.). Pearson correlation was used to identify the correlation coefficient and significant difference. P<0.05 was considered to indicate a statistically significant difference.

## Results

**Patient characteristics.** The clinicopathological characteristics of the 18 patients with ovarian cancer are summarized in Table I. The mean age of patients was 51.9±11.1 years. Preoperative serum cancer antigen 125 levels were >500 IU/l in 72.2% of the patients. In addition, >80% of the recruited patients were diagnosed with advanced ovarian cancer, at International Federation of Gynecology and Obstetrics stage III or IV. Serous ovarian carcinoma (77.8%) was the most common pathological type, followed by 2 patients with clear cell carcinoma, 1 with mucinous carcinoma and 1 with endometrioid carcinoma. In total, 22.2% of the patients were found with lymph node metastasis.

**Mutant genes detected in patients with ovarian cancer.** A panel of 333 potential oncogenes was used for the targeted NGS of DNA isolated from 18 peripheral blood samples, eight tumor tissue samples and six ascites samples. This panel covers a

Table I. Summary of clinicopathological characteristics of patients with ovarian cancer.

Clinicopathological characteristics	Value
Age range, years	24-70
FIGO stage, n (%)	
II	1 (5.6)
III	15 (83.3)
IV	2 (11.1)
Histological type, n (%)	
Serous carcinoma	14 (77.8)
Clear cell carcinoma	2 (11.1)
Endometrioid carcinoma	1 (5.6)
Mucinous carcinoma	1 (5.6)
Lymph nodes metastasis, n (%)	
Positive	4 (22.2)
Negative	4 (22.2)
Unknown	10 (55.6)
Preoperation cancer antigen 125 level, n (%)	
>1000 IU/l	8 (44.4)
500-1000 IU/l	5 (27.8)
<500 IU/l	5 (27.8)

FIGO, International Federation of Gynecology and Obstetrics.

number of reported genes associated with tumor initiation, those in the clinical investigation phase of cancer drug application, such as AZD6738 against ATR, and those that have been clinically applied for targeted therapy, such as larotrectinib against neurotrophin receptor kinase (Data S1). For example, the small-molecule or antibody drugs targeting MET, SRC, KRAS and BRCA1/2 were approved or in clinical trials. In the present study, for the target regions of the included samples, the coverage reached >99%, whereas the mean sequencing depth was >200X and the alignment rate was >95%.

A total of 104 genes were found to be mutated in the ctDNA samples from ≥1 patient (Fig. 1A). Of these genes, 29 were detected in ≥3 patients, 40 were detected in ≥2 patients, whereas the remaining 64 genes were mutated in only 1 patient. In the ovarian cancer tissue samples, 95 mutant genes were found, among which mutations in 34 genes were found in ≥2 patients (Fig. 1B). A total of 44 mutant genes were found in ascites samples, 23 of which were shared by ≥2 patients (Fig. 1C).

**Mutational heterogeneity in patients with ovarian cancer.** The mutant genes found in the peripheral blood, tumor tissue and ascites samples were compared to understand the concordance among the mutation spectra and genomic landscapes of the different samples from patients with ovarian cancer. Mutational heterogeneity was widely observed among the specimens and patients. The mutant genes shared by different patients and different sample types within the same individuals are shown in Figs. 1 and 2. The highest similarity was found between the ascites and tumor tissues (51.2%), followed by that between peripheral blood and tumor tissue samples (41.7%).

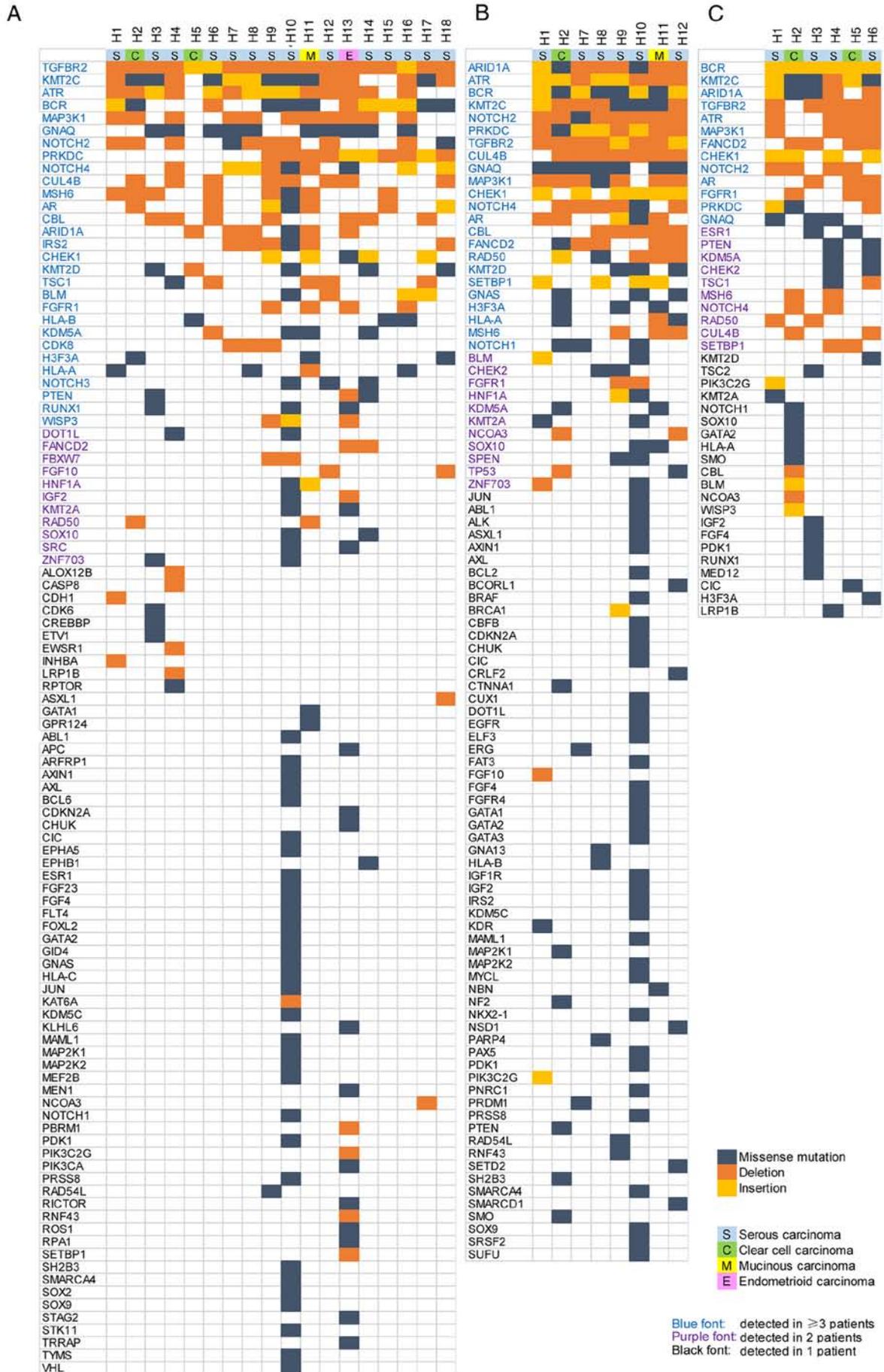


Figure 1. Mutant genes and mutation patterns detected in the patients with ovarian cancer. Mutant genes found in the (A) circulating tumor DNA of peripheral blood samples (n=18), (B) tumor tissue samples (n=8) and (C) ascite samples (n=6).

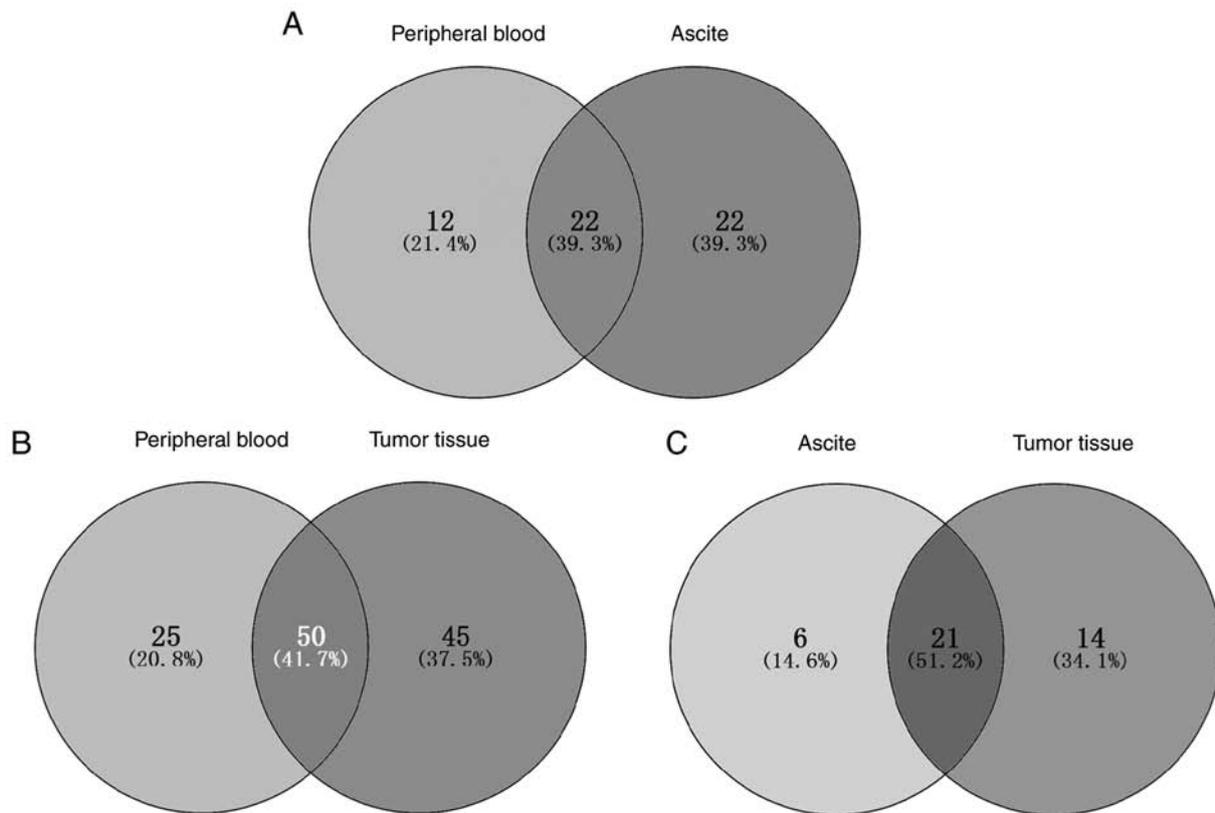


Figure 2. Venn diagram of the mutant genes shared among the peripheral blood, tumor tissue and ascite samples in patients with ovarian cancer. The comparative analysis between any two types of samples was only performed in patients for which both types of samples were collected. The percentage was calculated by dividing the number of genes shared in the two types of samples by the total number of genes in these two types of samples. (A) Common mutant genes found in the peripheral blood and ascite samples isolated from the same patient (n=6). (B) Common mutant genes found in the peripheral blood and tumor tissue samples isolated from the same patient (n=8). (C) Common mutant genes found in the tumor tissue and ascite samples isolated from the same patient (n=2).

The similarity between peripheral blood and ascite samples was the lowest (39.3%).

In addition, the mutation sites and types in the same gene were different among patients. For example, a missense mutation (D268E) was detected in the *PTEN* gene in the ctDNA from patients H3 and H14, whilst a deletion mutation (T321fs) in the same gene was detected in the ctDNA from patient H13.

**Functional analysis of the mutant genes.** To further explore the potential functions of these mutated genes in ovarian cancer samples, the mutant genes found in  $\geq 2$  patients were analyzed using the WebGestalt tool. GO analysis revealed that the mutant genes in peripheral blood, tumor tissue and ascites samples mainly participated in the biological processes of 'Response to stimulus', 'biological regulation', 'metabolic process', 'cell communication' and 'cell proliferation' (Fig. 3A-C). These mutant genes were also analyzed for KEGG and Wikipathway enrichment. The enriched pathways in peripheral blood, tumor tissue and ascites samples were similar and mainly included 'integrated cancer pathway', 'DNA IR-DSBs and cellular response via ATM', 'DNA damage response', 'Cellular senescence' and 'Notch signaling pathway netpath' (Fig. 3D-F).

**Potential of the mutant genes for metastasis evaluation.** To find the potential of using mutant genes in the ctDNA samples to monitor metastasis, 'metastasis' and 'ovarian cancer' were used as key words in Chilibot to analyze the 40 mutant genes

presented in  $\geq 2$  patients. A cluster covering 10 genes, namely NOTCH2, NOTCH3, lysine methyltransferase 2A (KMT2A), PTEN, androgen receptor (AR), DNA-activated protein kinase catalytic subunit (PRKDC), hepatocyte nuclear factor 1 homeobox A (HNF1A), SRC, insulin receptor substrate 2 (IRS2) and SRY-box transcription factor 10 (SOX10), was obtained. This cluster was then designated as the mutant gene panel for performing correlation analysis between tumor dissemination and metastasis in patients with ovarian cancer. The coverage of this mutant gene panel in the 18 patients who underwent ctDNA sequencing in the present study was 94.4% (17/18). This count was based on tumor lesions  $>1$  cm in the abdominal and pelvic cavities during intraoperative exploration, although miliary lesions were not counted. If the number of tumor lesions was  $>10$  in a patient, then the number of lesions was counted as 10. Pearson correlation analysis found that the number of mutant genes in this mutant gene panel was positively correlated with the number of tumor lesions with a correlation coefficient of 0.851 ( $P < 0.001$ ). This suggests that this panel derived from ctDNA samples may have the potential to be used in reflecting tumor dissemination and metastasis. Furthermore, an interaction network of the mutant genes within this panel was obtained using STRING (Fig. 4A), which indicated the direct or indirect interactions among the mutant genes.

In addition, a validation analysis of this mutant gene panel was performed in matching tumor tissue and ascites

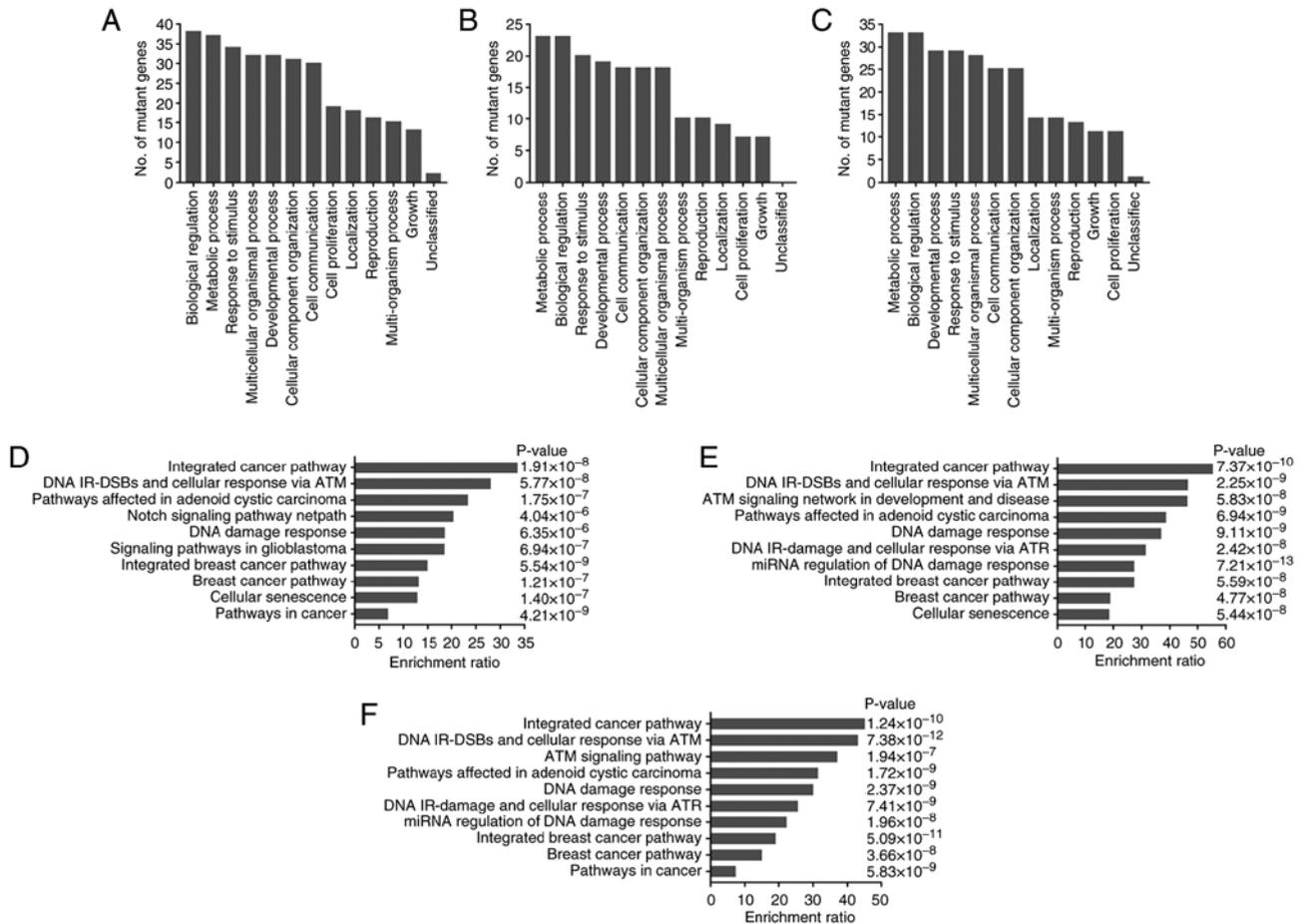


Figure 3. Functional analysis of the mutant genes presented in  $\geq 2$  patients with ovarian cancer. Gene Ontology summary for biological process categories of the mutant genes in the (A) peripheral blood, (B) ascites and (C) tumor tissue samples. Kyoto Encyclopedia of Genes and Genomes pathway and Wikipathway enrichment analyses of the mutant genes in (D) peripheral blood, (E) ascites and (F) tumor tissue samples. IR-DSBs, ionising radiation-induced DNA double-strand breaks; ATM, ataxia-telangiectasia mutated kinase; ATR, Rad3-related protein; miRNA, microRNA.

samples isolated from the same patient. The coverage of this panel in the paired tumor tissues and ascites was 100%. In total, mutations in six of the genes in the panel, namely NOTCH2, KMT2A, PTEN, AR, PRKDC and SOX10, were found in ascites. By contrast, mutations in eight genes in the panel, specifically NOTCH2, KMT2A, PTEN, AR, PRKDC, HNF1A, IRS2 and SOX10, were found in the ovarian cancer tissues.

*Further exploration of mutations associated with targeted therapy.* To assess whether the mutant genes had the potential to become targets for ovarian cancer therapy, the cBioPortal online tool was used for exploration. This integrates gene-centric drug-target information. It was found that the genes, except SOX10, in the present panel are either currently under investigation as a target or can already be targeted therapeutically (Fig. 4B). The hormone therapy class of therapeutic agents, such as cyproterone and enzalutamide, can be used for antitumor therapy by targeting AR, whereas there are numerous small-molecule drugs that can target SRC (32,33). NOTCH2 and NOTCH3 can also be targeted for ovarian cancer, since NOTCH3 knockdown has been found to increase the chemosensitivity of paclitaxel-resistant ovarian cancer cells (34,35).

## Discussion

Previous studies on the utility of ctDNA of ovarian cancer have mainly focused on early detection and screening (36,37), treatment response evaluation (38-40) and prognosis prediction (41). Higher levels of somatic mutations in ctDNA have been associated with decreased clinical benefit from treatment and shorter progression-free survival or overall survival (40,42-44). By applying NGS or PCR, various types of genetic alterations in ctDNA and/or ovarian cancer tumor tissues have been found, including chromosomal rearrangements (45), chromosomal instability (46), DNA methylation (47,48), and gene mutation and amplification (43,49,50). In the present study, NGS and public database resources based on patient characteristics were used to analyze the profile and function of the mutant genes and mutation patterns in ctDNA, paired ascites and tumor tissue samples from patients with ovarian cancer. By investigating the possible concordance and differences among patients and types of specimens, the mechanism underlying the heterogeneity of ovarian cancer was studied from multiple angles.

ctDNA may have the ability to reveal information regarding the mutation status of all genes involved in the tumor features (51,52). In the present study, ctDNA

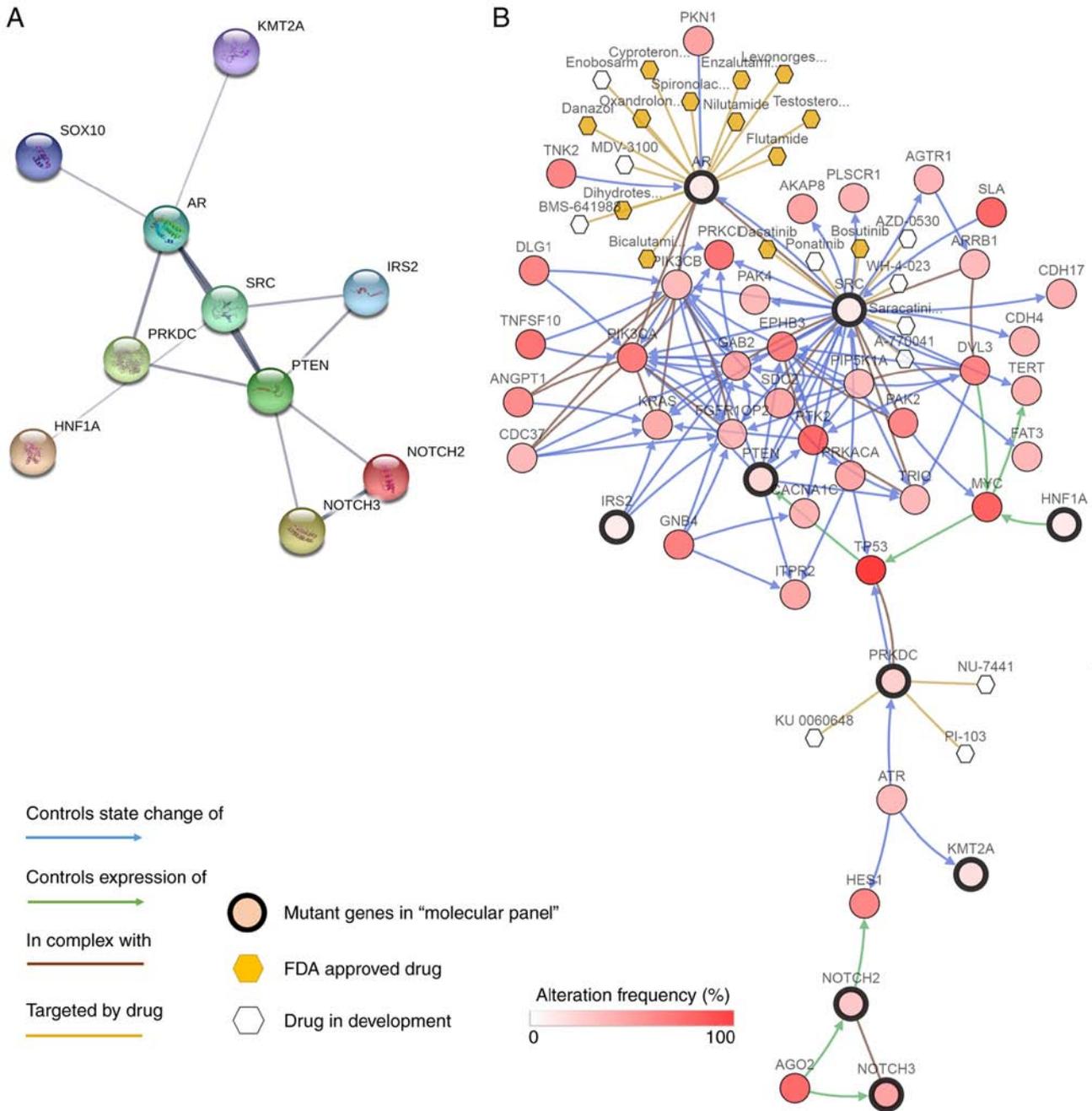


Figure 4. Exploration of the mutant genes associated with ovarian cancer metastasis and targeted therapy. (A) Functional interaction network of the mutant genes in the molecular panel. The network was built using the Search Tool for the Retrieval of Interacting Genes/Proteins database. Network nodes indicate proteins and edges indicate functional interactions. The thickness of the edges represents the strength of data support. (B) Potential drugs targeting the mutant genes in the molecular panel. The query genes, the genes with high frequency mutations in ovarian cancer and the corresponding targeted drugs were integrated using the cBioPortal tool. The genes are color-coded by their alteration frequency, and the query genes are displayed with a bold border.

sequencing was performed in patients with ovarian cancer, where 104 mutant genes were found in all plasma samples. Subsequently, a mutant gene panel that associated the most with tumor metastasis and dissemination was established after analyzing the 40 genes present in  $\geq 2$  patient among the ctDNA samples. These results suggest that ctDNA, as a conventional type of non-invasive liquid biopsy, may have the potential for application in monitoring the tumor metastasis process dynamically. However, the dynamic changes in ctDNA expression and mutation profile before and after tumor relapse were not explored in the present study. In

addition, the dynamic changes in the ctDNA profile and their association with imaging data and CA125 levels were not analyzed. Therefore, this mutant gene panel requires further validation in future studies.

Through the mutual verification and comparative analysis of sequencing data from ctDNA, ascites and tumor tissues, an understanding of the overall mutational landscape and the heterogeneity of ovarian cancer was at least partially revealed. The heterogeneity of gene mutations in ovarian cancer is commonly observed, where they are manifested as differences in the mutation locations and mutation types (53). In

the present study, this heterogeneity was observed from three angles; the inter-tumor heterogeneity among different patients, the intra-tumor heterogeneity among different lesions within the same patient and the clonal heterogeneity in different subclones within the same lesion were all considered. It was then predicted that gene mutations in different types of specimens from the same patient were also heterogeneous in the present study. In particular, some of the gene mutations that could not be detected in tumor tissues could be detected in ctDNA samples, which can be explained by the existence of tumor subclonal heterogeneity and due to the space restriction of tumor sampling. However, it should be noted that the source of ctDNA is not only tumor cells. The cells of the tumor microenvironment, including stromal cells, endothelial cells and immune cells, may release DNA into the circulatory system in patients with cancer (54,55).

ctDNA confers advantages in being able to reflect the overall tumor information compared with the space restrictions of tissue biopsy, particularly in disease monitoring and management (52,56). However, this does not suggest that ctDNA can be used to fully represent all tumor information. Comparative analysis results showed that mutation detection data from ctDNA cannot completely cover the mutant genes found in ascites and tumor tissues. Plasma mutation detection typically has low positive predictive value, meaning that more sensitive ctDNA assays are needed due to the low quantity of ctDNA in a number of patients (57). As a result, parallel sequencing analysis of liquid biopsies, tumor tissues and ascites can be used to unravel the overall tumor information more comprehensively. Combination of clinical, surgical, pathological and molecular features all facilitates the transformation of linear treatments into multidisciplinary and integrated approaches, in turn promoting the advent of individualized therapy to improve the survival outcome (58-60). Therefore, comprehensive analysis into the genetic landscape will likely contribute to the realization of multidisciplinary approaches and personalized medicine in the field of ovarian cancer treatment.

The present study has a number of limitations. A small sample size of matched ctDNA, ascite and tumor tissue samples was evaluated. Low quantities of ascites, insufficient remaining tumor tissues after pathological sampling and quality problems of the tissue samples all contributed to the exclusion of some samples in the present study. As a form of non-invasive examination, peripheral blood samples for ctDNA detection can be easily collected. This maybe one of the reasons why the number of cases with available ctDNA was larger compared with those with paired tumor tissues or ascites. The correlation analysis between mutations in the gene panel in the ctDNA samples and patient survival was lacking in the present study. The cancer panel used in this study included 333 tumor-related genes covering the exons and some introns of coding genes, meaning that mutations in non-coding regions were not explored. This also meant that the ctDNA sequencing data in the present study were not comparable to the sequencing data in TCGA. The sequencing data in TCGA were based on tumor tissues. Therefore, only TCGA was used to assist in screening for mutations due to the difference in subjects between the present study and the external dataset. In addition, since all data in public

databases were based on existing reported studies, relying on public databases to assist in screening for genes would run the risk of overlooking significant mutations that were not previously reported.

In conclusion, the present study analyzed the mutant genes of ctDNA and found a molecular panel that can be used to reflect the dissemination and metastasis process of ovarian cancer. In addition, this panel of mutant genes was validated against external data sets, whereas the sequencing data of this panel of mutant genes were compared among ctDNA, matching ascites and tumor tissue samples. A deeper understanding of the overall mutational landscape and heterogeneity was obtained in ovarian cancer. Results from the present study suggest that it is not sufficient to rely solely on the information provided by a specific type of specimens for investigating the genomic landscape and screening for mutations in cancers. Combining the information obtained from multiple types of samples with other diagnostic information facilitates more comprehensive understanding of the pathophysiology of cancer. For future clinical applications of liquid biopsy, a standardized and optimized method for interpreting and utilizing NGS and PCR data is required.

#### **Acknowledgements**

Not applicable.

#### **Funding**

The present study was supported by grants from the National Natural Sciences Foundation of China (grant no. 82172747) and the Shanghai Medical Center of Key Programs for Female Reproductive Diseases (grant no. 2017ZZ01016).

#### **Availability of data and materials**

The datasets generated and/or analyzed during the current study are not publicly available due to patient privacy protection and the Regulation of the People's Republic of China Administration of Human Genetic Resources (Document Number: Order No. 717 of the State Council of the People's Republic of China) but are available from the corresponding author on reasonable request.

#### **Authors' contributions**

CX and XZ designed the study. XJie performed the experiments and analyzed the data. MD and MZ performed data collection and analyzed the data. XJin performed data collection and part of the data analysis. QC performed part of the data analysis. XJie, CX and XZ confirmed the authenticity of the raw data. All authors have read and approved the final manuscript.

#### **Ethics approval and consent to participate**

The present study was approved by the Ethical Committee of Obstetrics and Gynecology Hospital of Fudan University (approval no. 2016-48; Shanghai, China). Written informed consent was obtained prior to initiation of the study.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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