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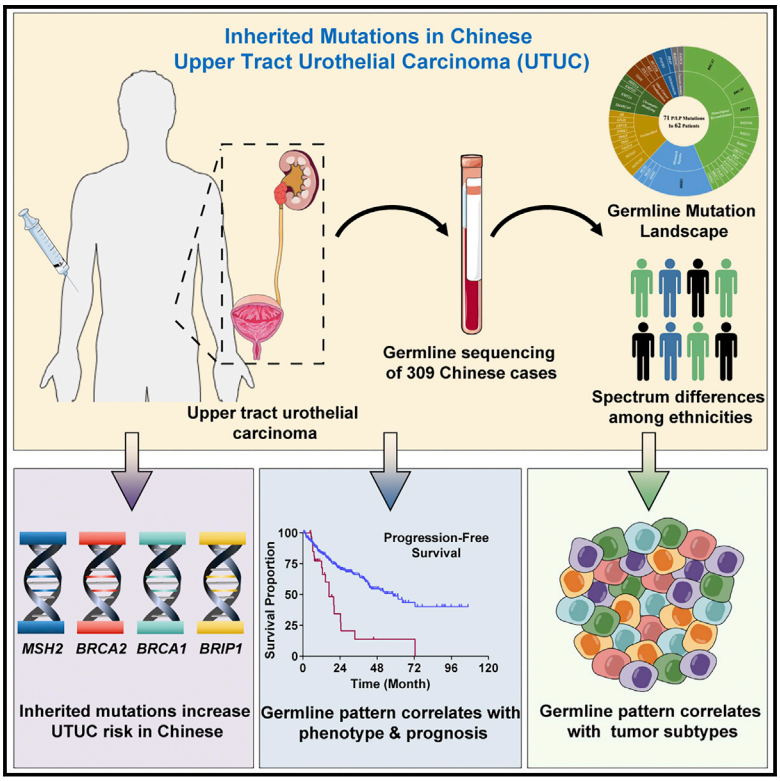
Inherited mutations in Chinese patients with upper tract urothelial carcinoma

Graphical abstract



Highlights

- Inherited mutation landscape is elucidated in Chinese patients with UTUC
- Germline mutation in *BRCA1/2*, *MSH2*, or *BRIP1* increases UTUC risk in Chinese
- Germline genotype correlates with patient features and prognosis of UTUC
- Certain germline patterns correlate with specific tumor subtypes

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In brief

Based on germline mutation analysis of 309 Chinese patients with upper tract urothelial carcinoma (UTUC), Wu et al. elucidate inherited mutation landscape, compare mutational spectrum with other ethnicities, and also correlate certain germline genotypes with UTUC predisposition, patient characteristics, prognoses, and specific tumor subtypes.

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Report

Inherited mutations in Chinese patients with upper tract urothelial carcinoma

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SUMMARY

Upper tract urothelial carcinoma (UTUC) accounts for 10% of urothelial carcinomas (UCs) and has a substantial hereditary component. However, the majority of our knowledge of germline spectrum comes from bladder cancer (BCa) data in White populations. Here, we sequence 309 Chinese UTUC cases and identify 71 germline pathogenic/likely pathogenic (P/LP) mutations in 62 patients (20.1%). Compared with White cases, we observe disparities and similarities in inherited mutational profiles. Association analysis reveals that germline P/LP mutations in *MSH2*, *BRCA2*, *BRCA1*, and *BRIP1* significantly increase UTUC risk in Chinese populations. Furthermore, germline P/LP mutation in homologous recombination genes indicates poor prognosis for non-metastatic UTUC. Finally, we perform paired sequencing and observe significant correlations between germline mutation patterns and tumor subtypes. This study highlights the importance of genetic testing in patients with UTUC and calls for germline data from various ethnicities to better understand this disease.

INTRODUCTION

Urothelial carcinoma (UC) is one of the most common cancers worldwide with a substantial hereditary component, consisting of bladder cancer (BCa; ~90%) and upper tract UC (UTUC; ~10%).[1] Because of lower incidence and paucity of research, systemic management of UTUC has long been formulated with reference to BCa. However, emerging data have revealed poorer outcome and different genomic characteristics of UTUC compared with BCa.[2–4] This emphasizes the need for molecular characterization and precision therapy research specifically for UTUC.

Beyond the role in heritability and increased risk of tumor pathogenesis, therapeutic implications and prognostic value of germline variants in malignancies are increasingly recognized. Carlo et al. and Nassar et al. described germline pathogenic/likely pathogenic (P/LP) mutations in cancer susceptibility genes in UC and found that *BRCA1/2* and *MSH2* harbored most pathogenic mutations. These two studies were landmarks underscoring the importance of germline testing in UC.[5,6] Despite important advances, our understanding of UC genetics is mainly based on data from White populations, with limited data from other ethnicities. Moreover, the proportion of BCa patients in both studies exceeded 80%, which may not represent genetic characteristics of UTUC. Considering the higher UTUC incidence in Chinese populations,[7] and the difference in molecular features between UTUC and BCa, the inherited mutation landscape of Chinese UTUC needs to be elucidated.

Herein, we retrospectively analyzed genetic testing data of 520 cancer-related genes in 309 Chinese patients with UTUC to elucidate the inherited mutation landscape, compare the genetic profile with that in White populations, and correlate germline genotype with tumor phenotypes. We also analyzed UTUC risk-increasing genes and potential actionable variants to anchor clinical implications.

Table 1. Clinical and pathologic characteristics of 309 Chinese patients with upper tract urothelial carcinoma

Characteristics	n = 309	Proportion (%)
Age at diagnosis, years, median (range)	63 (25–88)	–
Follow up, months, median (range)	16.9 (0.5–106.9)	–
Gender		
Male	197	63.8
Female	112	36.2
Laterality		
Left	156	50.5
Right	153	49.5
Location		
Pelvis	160	51.8
Ureter	128	41.4
Both	21	6.8
Smoking history		
Yes	102	33
No	207	67
Personal bladder cancer history		
Yes	38	12.3
No	271	87.7
Personal malignancy history other than bladder cancer		
Yes	39	12.6
No	270	87.4
First-degree relative malignancy history		
Yes	64	20.7
No	245	79.3
T stage		
1	111	35.9
2	74	24
3	114	36.9
4	10	3.2
N stage		
0	277	89.6
1	11	3.6
2	21	6.8
Tumor grade		
High	279	90.3
Low	30	9.7
Carcinoma *in situ*		
Yes	34	11
No	275	89
Standard adjuvant chemotherapy		
Yes	31	10
No	278	90

"n" means number of patients in certain category. Detailed data for each case are presented in Table S1.

RESULTS

Patient characteristics

Demographics on 309 Chinese patients with UTUC were analyzed and are presented in Table 1 (details in Table S1). Median age of onset was 63 years (range 25–88 years). Early-onset ($\leq$45 years) patients accounted for 4.9%. Patients were mostly men (197, 63.8%). The primary tumor site was the renal pelvis in 160 (51.8%) cases and the ureter for 128 (41.4%). Only 30 (9.7%) patients had low-grade UTUC, and 34 (11%) had carcinoma *in situ*. Thirty-eight patients (12.3%) had a personal history of BCa, and 39 (12.6%) had a history of malignancy other than BCa. Sixty-four patients (20.7%) had a positive family cancer history. Only 31 patients (10%) received standard adjuvant chemotherapy. The median follow-up time was 16.9 (0.5–106.9) months. Approximately one-third (109/309, 35.3%) of patients developed relapse or metastasis during follow up.

Spectrum of germline variants

In this study, pathogenicity determination was defined according to the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines. In total, we identified 71 germline P/LP mutations in 62 of 309 (20.1%) Chinese patients with UTUC. Within germline P/LP mutation carriers, 6 patients harbored more than 1 germline P/LP mutation. The most frequently mutated genes were *MSH2* (10/309, 3.2%, 95% confidence interval [CI]: 1.3%–5.2%); *BRCA2* (10/309, 3.2%, 95% CI: 1.3%–5.2%); *BRCA1* (4/309, 1.3%, 95% CI: 0.03%–2.6%); and *BRIP1* (3/309, 1%, 95% CI: 0%–2.1%). The 71 germline P/LP mutations occurred in 39 different genes, which can be divided into 7 groups according to molecular pathways (Figure 1A; Table S2), including homologous recombination (HR; 31/71, 43.7% of total P/LP mutations); mismatch repair (MMR; 12/71, 18.3%); Fanconi anemia (2/71, 2.8%); RTK/RAS/MAPK (3/71, 4.2%); chromatin modifying (6/71, 8.5%); other cancer predisposition (5/71, 7%); and unclassified (11/71, 15.5%).[1,4,8]

Correlation between germline genotype and patient characteristics

We found that patients with personal cancer (excluding BCa) history ($p = 0.005$) and family cancer history ($p < 0.001$) had significantly higher germline P/LP mutation rates (Figure 1B). Further investigation revealed that the higher mutation rate in patients with personal cancer (excluding BCa) history may attribute to significantly higher mutation frequency in MMR genes ($p < 0.001$), and a higher mutation rate in patients with family cancer history may attribute to significantly higher mutation frequencies in both MMR ($p = 0.001$) and HR ($p = 0.015$) genes (Table S3). In addition, we did not find any significant association between P/LP carrier status and age, gender, side, personal BCa history, T stage, N stage, tumor grade, and tumor location (Figure 1B).

Comparison of inherited mutation profiles between Chinese and Western populations

We further investigated the similarity and disparity of inherited mutational spectrum between Chinese and Western populations. Germline P/LP variants in DNA damage repair genes

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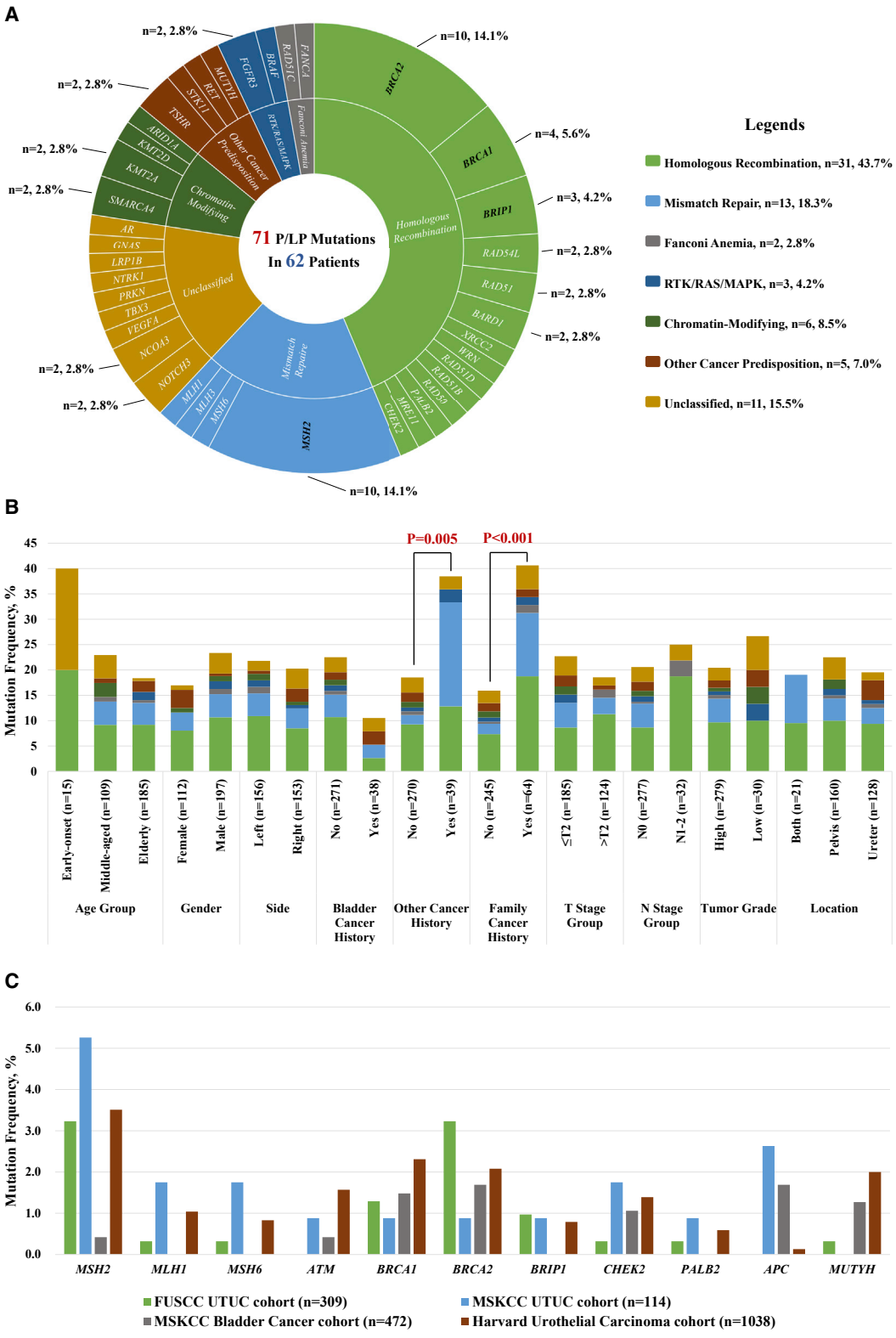

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(DRGs; including HR, MMR, and Fanconi anemia pathway genes) account for 63.4% (45/71) of all pathogenic variants detected in Chinese population. Although the proportion of patients with UTUC carrying an inherited P/LP mutation in DRGs was similar between Chinese UTUC (44/309, 14.2%) and Memorial Sloan Kettering Cancer Center (MSKCC) UTUC (17/114, 14.9%) cohorts, we observed a relatively higher *BRCA2* mutation frequency (3.2% versus 0.88%) in Chinese populations. In addition, we also observed relatively lower germline P/LP mutation frequencies in MMR genes (4.2% versus 8.8%), *CHEK2* (0.32% versus 1.7%), and *APC* (0% versus 2.6%) in Chinese populations (Figure 1C). Moreover, comparison with BCa confirmed the difference in mutation spectrum of UTUC and BCa, especially in MMR genes. This may be related to higher penetrance of Lynch syndrome in UTUC than BCa (Figure 1C). Similar findings were also observed by comparing Fudan University Shanghai Cancer Center (FUSCC) UTUC cohort with Harvard Urothelial Carcinoma Cohort (>90% BCa; Figure 1C).[5]

Estimate of UTUC risk associated with observed variants

We identified more than 2 inherited P/LP variants in *MSH2*, *BRCA1/2*, and *BRIP1* genes in the Chinese UTUC cohort (Figure 2A). Thus, we further studied whether P/LP mutations in these 4 genes can significantly increase UTUC risk in Chinese populations. By comparing it with East Asian non-cancer population data from GnomAD, we observed that inherited P/LP mutations in *MSH2* ($p < 0.001$, odds ratio [OR]: 169.5, 95% CI: 37.0–777.3); *BRCA2* ($p < 0.001$, OR: 13.6, 95% CI: 6.5–28.5); *BRCA1* ($p < 0.001$, OR: 8.5, 95% CI: 2.8–25.5); and *BRIP1* ($p = 0.012$, OR: 4.6, 95% CI: 1.4–15.5) genes were significantly associated with UTUC.[9] Association analysis using the China Metabolic Analytics Project[10] controls also confirmed significant UTUC-risk-increasing effects in all these 4 genes: *MSH2* ($p < 0.001$, OR: 124.2, 95% CI: 34.0–453.9); *BRCA2* ($p < 0.001$, OR: 10.4, 95% CI: 5.1–21.1); *BRCA1* ($p < 0.001$, OR: 7.8, 95% CI: 2.7–23.3); and *BRIP1* ($p = 0.005$, OR: 5.9, 95% CI: 1.7–20) (Figure 2B). Thus, *MSH2*/*BRCA1*/*BRCA2*/*BRIP1* genes were designated as UTUC predisposition genes in Chinese populations.

Potential actionable germline variants in UTUC

Finally, to explore potential therapeutic implications of inherited P/LP mutations in UTUC, we classified potential actionable variants/genes according to the highest evidence levels from OncoKB database.[11] We found that 63.4% P/LP variants detected in our cohort were potentially therapeutic mutations, occurring in 44/309 (14.2%) Chinese patients with UTUC (Table S4).

Correlation between germline genotype and prognosis

Progression occurred in 109 of 309 patients (35.3%) during follow up. We found a trend toward shorter progression-free survival (PFS) but did not reach statistical significance for germline P/LP mutation carriers ($p = 0.084$; Figure 3A). However, germline HR mutation carriers had significantly worse prognosis than that of other patients ($p < 0.001$; Figure 3B). Multivariate cox regression analysis also showed significantly poor prognosis for Chinese patients with UTUC carrying a germline HR mutation after adjusting other clinicopathological factors (hazard ratio: 2.746, 95% CI: 1.611–4.683, $p < 0.001$; Figure 3C). However, we did not find a statistically significant association between germline P/LP mutation status ($p = 0.389$) or germline HR gene mutation status ($p = 0.779$) and prognosis with adjuvant chemotherapy (Figure S1).

Correlation between germline genotype and tumor phenotype

We found that of 24/62 patients with germline P/LP mutations had sufficient archived tumor tissue for somatic sequencing (38.7%). Loss of heterogeneity (LOH) or second hit[12] was detected in 12 of 24 patients (50%; Table S5). We also performed Catalog of Somatic Mutations in Cancer (COSMIC) signature analysis to define mutational scars associated with specific mutational processes on these 24 patients based on the somatic sequencing results (Table S6).

Twelve patients carrying classic germline MMR P/LP mutations (10 with *MSH2*, 1 with *MLH1*, and 1 with *MSH6*). Five had somatic sequencing data, and they all had confirmed microsatellite instability (MSI)-high status (Table S5). Additionally, 11 of these 12 patients have documented slides for immunohistochemistry (IHC) staining. Corresponding protein loss was confirmed in all 11 patients by IHC (Figure S2).

To investigate the association of germline mutations with tumor-variant-based molecular subtypes, we subtyped these 24 patients according to a previous study (Figure 3D).[4] We found that certain correlations between germline mutational signatures and tumor subtypes of UTUC (Figure 3E). Germline MMR genes mutation, *BRCA1/2* mutation, and chromatin-modifying gene mutation were significantly associated with hypermutated ($\varphi = 0.889$), TP53/MDM2-mutated ($\varphi = 0.662$), and RAS-mutated ($\varphi = 0.674$) subtypes, respectively. Non-*BRCA1/2* HR-mutated subtypes showed no significant trend in their tumor molecular subtype. In addition, other germline tumor susceptibility gene mutation characteristics were associated with FGFR3-mutated subtype ($\varphi = 0.700$) (Figure 3F).

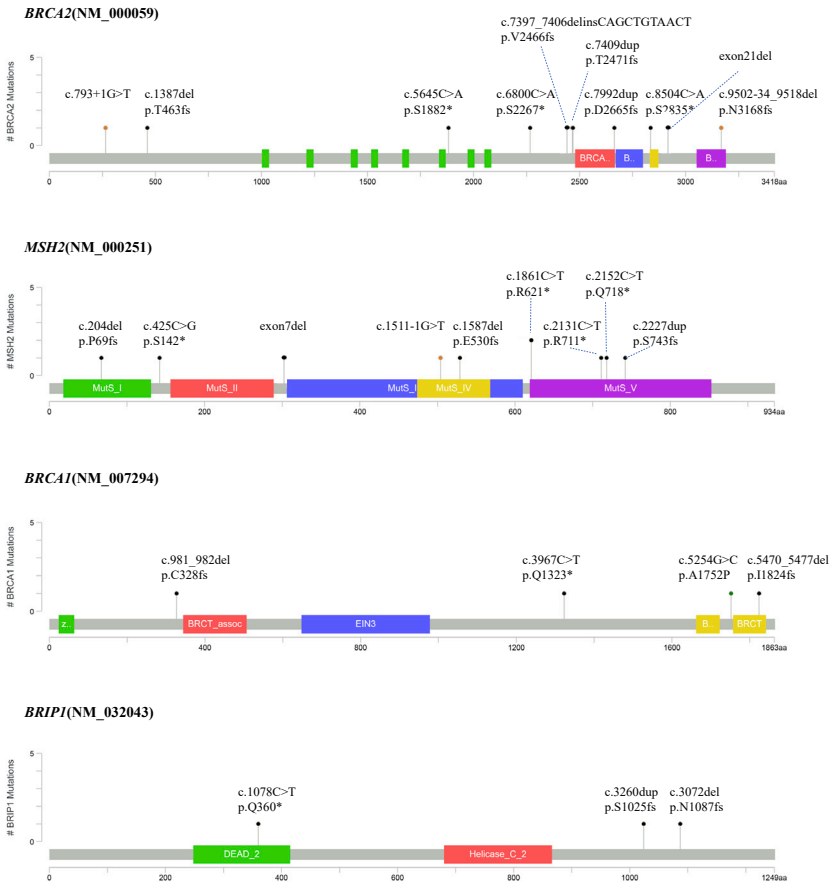
DISCUSSION

Using this 309 Chinese UTUC cohort, we aimed to elucidate inherited mutation landscape and correlate germline genotypes with patient characteristics, prognoses, and tumor subtypes. About 1/5 (20.1%) of patients with UTUC carried pathogenic germline variants, occurring predominantly in HR and MMR

Figure 1. Inherited mutation landscape in Chinese patients with UTUC from FUSCC cohort

(A) Distribution of 71 identified germline pathogenic/likely pathogenic mutations and classification according to 7 molecular pathways. See also Tables S2 and S4.
(B) Proportions of Chinese patients with UTUC with germline pathogenic/likely pathogenic mutations by specific clinical characteristics. See also Table S3.
(C) Comparison of pathogenic/likely pathogenic mutation frequencies among FUSCC UTUC cohort (n = 309), MSKCC UTUC cohort (n = 114), MSKCC BCa cohort (n = 472), and Harvard Urothelial Carcinoma cohort (n = 1,038). FUSCC, Fudan University Shanghai Cancer Center; UTUC, upper tract urothelial carcinoma; MSKCC, Memorial Sloan Kettering Cancer Center; BCa, bladder cancer.

A



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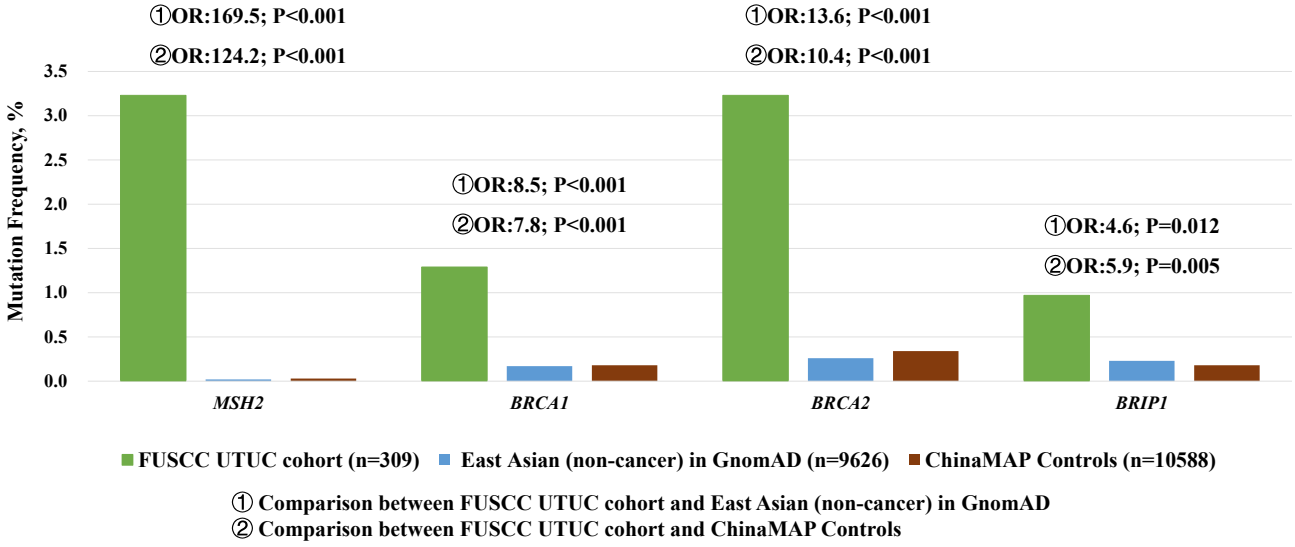

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pathways. Our data confirmed the similarity in germline DRG mutation frequency between Chinese and Western patients with UTUC (~15%), along with mutation disparities in specific genes like *BRCA2* and MMR genes. In addition, we observed that 63.3% inherited P/LP mutations were potential actionable, and P/LP mutations in *BRCA2*/*MSH2*/*BRCA1*/*BRIP1* increased UTUC risk in Chinese populations. Furthermore, we found that germline HR gene mutation indicated significantly poor prognosis (hazard ratio: 2.746, $p < 0.001$). Paired germline and somatic sequencing in patients with germline P/LP mutations revealed loss of heterozygosity and second hit and correlated germline mutation patterns with tumor subtypes. Our study highlights the importance and clinical utility of genetic testing in UTUC and calls for germline data from various ethnicities to better understand this disease.

It is worth noting that although the overall mutation rate of DRGs is similar, the specific mutated genes are different between ethnicities. Chinese patients with UTUC have a higher *BRCA2* mutation rate, a finding similar to that in other cancer types.[13,14] Lower germline P/LP mutation frequency in MMR genes may be related to a higher proportion of aristolochic-acid-associated UTUC in Chinese populations, thereby diluting the proportion of MMR-related disease.[15]

Furthermore, we also observed quite a few patients carrying germline mutations in DNA damage response (DDR)-related genes. In recent years, PARP inhibitors have been applied to patients with UC carrying mutations in DDR-related genes. Variants underlining HR deficiency represented 8.41% in our study, which were potential targets of poly(ADP-ribose) polymerase inhibitors.[16] Results from the BAYOU trial demonstrated a potential PFS benefit with durvalumab plus olaparib in patients with UC with mutations in HR-related genes.[17] In addition, final analysis of ATLANTIS trial demonstrated that use of rucaparib following chemotherapy could extend PFS in patients with metastatic UC (mUC) with DNA damage repair deficiency.[18] These preliminary results indicated that PARP inhibitors might play an important role in treating UCs with HR mutations. Additionally, we detected MSI-high status or loss of MMR-corresponding proteins (using IHC) in all patients with MMR germline mutations that could be tested. Our findings are in good agreement with a previous study.[6] This suggests that germline P/LP mutations in MMR genes often lead to deficient MMR function or MSI-high status, which may be a potentially modifiable population benefiting from PD-1/PD-L1 immunotherapy.

Finally, we also revealed the poor prognosis of patients with germline HR-mutated UTUC in this study and found a surprising correlation between germline mutational signatures and tumor molecular subtypes. The biological reasons behind these relationships need to be further explored.

Limitations of the study

This study was a retrospective, single-center study and only enrolled Chinese patients with UTUC with no distant metastasis. Although UTUC is an uncommon tumor, the sample size of 309 patients to describe the germline mutation profile is still kindly insufficient. Subsequent studies should increase the sample size and include metastatic UTUC so that the germline mutation spectrum can be more representative. Secondly, not all germline mutation carriers have paired tumor tissues sequenced. This limited our ability to study tumorigenesis and clarify the correlation between germline mutational characteristics and tumor molecular subtypes. Thirdly, because most of our patients were enrolled before the POUT trial,[7] only 10% of patients received standard adjuvant chemotherapy. This limited our study on the association between germline mutational signatures and adjuvant chemotherapy response. Finally, our research has not been able to further clarify the biological characteristics of these mutations. Although we have identified many inherited pathogenic variants and correlated germline mutations with patient characteristics, prognoses, and tumor subtypes, the absence of experimental validation reduces credibility. Follow-up research is still needed.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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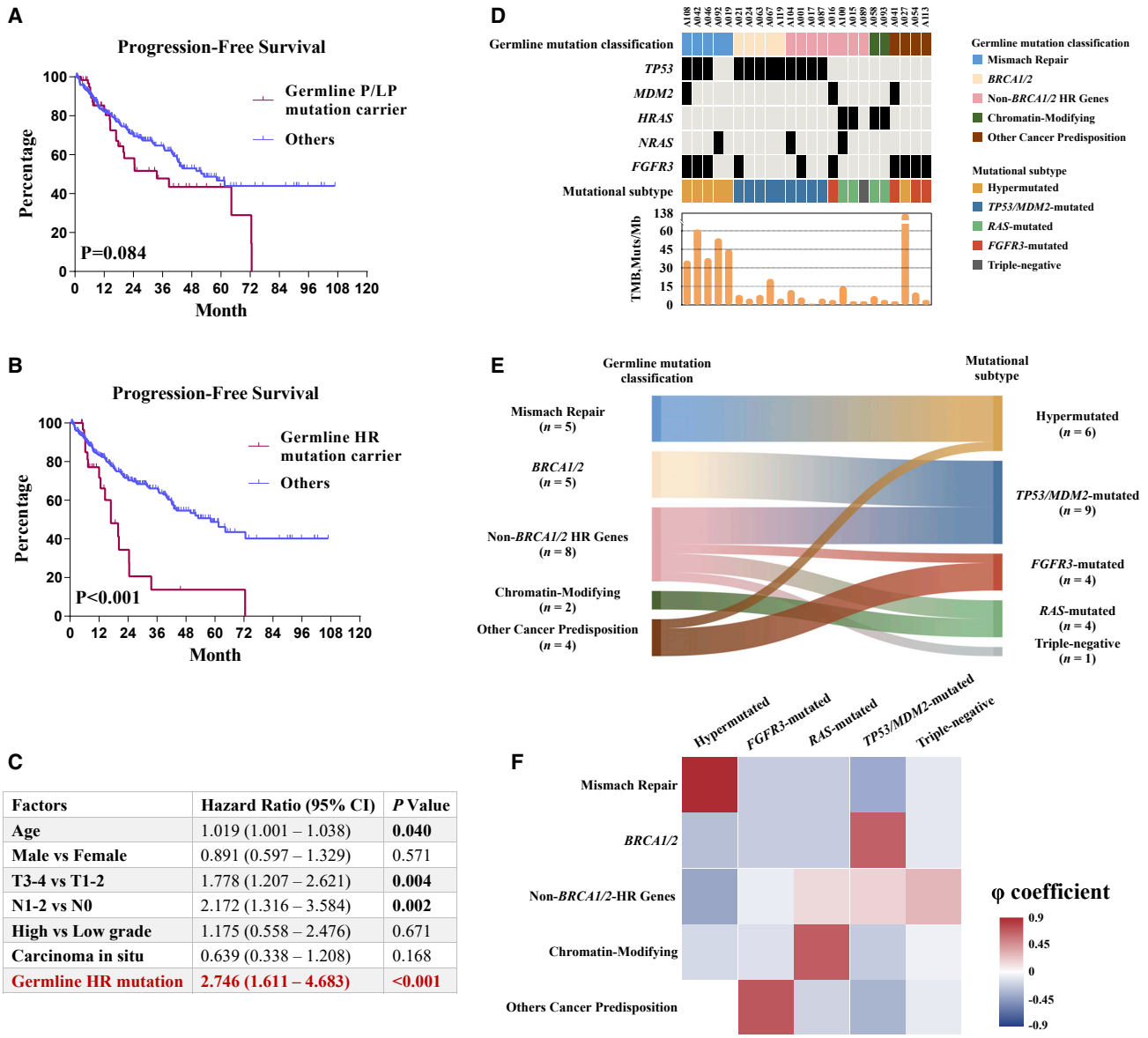
SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2022.100883.

Figure 2. Most frequently mutated genes with germline pathogenic/likely pathogenic variants in Chinese patients with UTUC

(A) Locations of pathogenic/likely pathogenic germline mutations in *MSH2*, *BRCA2*, *BRCA1*, and *BRIP1*. Each mutation identified is shown by a lollipop.

(B) Case-control association analysis results indicate that inherited pathogenic/likely pathogenic mutations in *MSH2*, *BRCA2*, *BRCA1*, and *BRIP1* can significantly increase UTUC risk in Chinese populations. ① Comparison between FUSCC UTUC cohort and East Asian (non-cancer) in GnomAD. ② Comparison between FUSCC UTUC cohort and ChinaMAP controls. Statistically significant cutoff with Bonferroni-corrected $\alpha = 0.05/4 = 0.0125$. FUSCC, Fudan University Shanghai Cancer Center; UTUC, upper tract urothelial carcinoma; GnomAD, Genome Aggregation Database; ChinaMAP, China Metabolic Analytics Project; OR, odds ratio.



C

Factors	Hazard Ratio (95% CI)	*P* Value
Age	1.019 (1.001 – 1.038)	**0.040**
Male vs Female	0.891 (0.597 – 1.329)	0.571
T3-4 vs T1-2	1.778 (1.207 – 2.621)	**0.004**
N1-2 vs N0	2.172 (1.316 – 3.584)	**0.002**
High vs Low grade	1.175 (0.558 – 2.476)	0.671
Carcinoma in situ	0.639 (0.338 – 1.208)	0.168
Germline HR mutation	**2.746 (1.611 – 4.683)**	**<0.001**

Figure 3. Correlation between germline genotype and patient survival/phenotypes

(A) Progression-free survival of 309 Chinese patients with UTUC comparing cases with germline P/LP mutations (62 patients) with others (247 patients). See also Figure S1.

(B) Progression-free survival of 309 Chinese patients with UTUC comparing cases with germline HR gene P/LP mutations (30 patients) with others (279 patients). See also Figure S1.

(C) Multivariate cox regression analysis in 309 Chinese patients with UTUC indicates that carrying the germline HR gene P/LP mutation is an independent prognostic factor predicting shorter progression-free survival even after adjusting clinicopathological factors.

(D) Heatmap describing somatic gained mutations, tumor mutation burden, and tumor subtype in 24 Chinese patients with UTUC with germline P/LP mutations and paired somatic sequencing data. See also Table S5 and Figure S2.

(E) Sankey plotting showing correlation between germline mutation classification and tumor mutational subtype in the 24 Chinese patients with UTUC with germline P/LP mutations and paired somatic sequencing data. See also Table S5 and Figure S2.

(F) Phi plotting showing correlation between germline mutation classification and tumor mutational subtype using the data from the 24 Chinese patients with UTUC above. See also Table S5. HR, homologous recombination; TMB, tumor mutation burden; CI, confidence interval.

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AUTHOR CONTRIBUTIONS

J.W. and D.Y. designed the study; J.W., C.G., Yao Zhu, B.D., H.Z., G.S., Yu Zhu, Y.S., and Yiping Zhu collected samples; J.W., C.G., and D.Y. performed sequencing; J.W., S.J., Y.W., and J.P. analyzed the data; Yao Zhu and H.G. reviewed pathology; Yao Zhu, A.N., and S.F.S. supervised and performed critical review; all authors provided discussion, participated in revising the manuscript, and agreed to the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mismatch Repair rabbit monoclonal antibody panel	Abcam	cat#: ab252190
Rabbit monoclonal anti-MLH1	Abcam	cat#: ab92312; RRID:AB_2049968
Rabbit monoclonal anti-MSH2	Abcam	cat#: ab227941
Rabbit monoclonal anti-MSH6	Abcam	cat#: ab92471; RRID:AB_2144959
Rabbit monoclonal anti-PMS2	Abcam	cat#: ab110638; RRID:AB_10858491
Biological samples		
Human upper tract urothelial carcinoma samples	This paper	N/A
Human peripheral white blood cell samples	This paper	N/A
Critical commercial assays		
OncoScreen Plus (520-gene Sequencing Panel)	Burning Rock Biotech	Item name: OncoScreen Plus. https://www.brbiotech.com/service/a1
QIAamp DNA Mini Kit	Qiagen	Cat No. 51306
TruSeq Exome Enrichment kit	Illumina	Cat No. FC-121-1024
Envision detection kit	Dako	Cat No. GK500710
Deposited data		
Germline and somatic DNA sequencing data of UTUC	This paper	National Genomics Data Center (NGDC), BioProject: PRJCA013430
Germline data of non-cancer East-Asian people	Karczewski et al.[9]	https://gnomad.broadinstitute.org/(GnomAD)
Germline data of Chinese people	Cao et al.[10]	http://www.mbiobank.com/(ChinaMAP)
Software and algorithms		
GraphPad Prism 6.0	GraphPad Prism	https://www.graphpad.com/
SPSS Statistics 22.0	IBM	https://www.ibm.com/
R version 4.2.0		https://www.r-project.org
R studio version 0.94.102		https://www.rstudio.com/
ComplexHeatmap R package v 2.8.0	Gu et al.[19]	https://github.com/jokergoo/ComplexHeatmap
NetworkD3 R package v 0.3		https://github.com/christophergandrud/networkD3/issues
Corrplot R package v 0.92		https://github.com/taiyun/corrplot
ggplot2 R package v. 3.3.3		https://cran.r-project.org/web/packages/ggplot2/index.html
MutationalPatterns R package v 3.0.1	Blokzijl et al.[20]	https://bioconductor.org/packages/release/bioc/html/MutationalPatterns.html

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dingwei Ye (dingwei_ye1963@163.com).

Materials availability

All unique/stable reagents generated in this study are available from the lead contact with a completed Materials Transfer Agreement.

Data and code availability

The germline and somatic DNA sequencing data generated during this study are all available at National Genomics Data Center (NGDC), under the accession numberBioProject: PRJCA013430.

This paper does not report original code.

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

We retrospectively included Chinese UTUC patients received partial ureterectomy or radical nephroureterectomy at Department of Urology, Fudan University Shanghai Cancer Center (FUSCC) from January 2012 to March 2019. All of the patients had a diagnosis of UTUC with no distant metastasis and were not selected based on age at diagnosis, family history, or genetic background knowledge. Included patients should have undergone germline genetic testing or have sufficient archived white blood cell for next-generation sequencing. In total, 309 patients were included. Because we retrospectively included all eligible UTUC patients in FUSCC, no sample size estimation was involved in this study. Germline DNA sequencing (commercial targeted panel of 520 cancer-related genes or whole-exome) was performed in all 309 patients, so the assignment of experimental and control groups was also not involved in this study. Electronic medical records were used to retrieve information about demographics, tumor location, histology, and prognosis. A full description of the patients' demographics including age at diagnosis, gender, tumor stage, tumor grade, family cancer history and other clinicopathological characteristics are provided in Table S1. Please note that positive family cancer history is only restricted to first-degree and second-degree relatives. No significant influence of gender was observed on our study. This study was approved by Ethics Committee of FUSCC and signed informed consents were obtained from all patients or corresponding family members.

Within these 309 cases, 234 UTUC patients had germline testing data from a commercial targeted panel of 520 cancer-related genes (OncoScreen Plus, Burning Rock Biotech, Guangzhou, China) and germline mutational data were collected from reports. The remaining 75 patients were sequenced using whole exome sequencing using archived white blood cell isolated from peripheral blood sample. In addition, of the 62 carriers of germline pathogenic variants, 24 had matched urothelial carcinoma samples for the same targeted panel sequencing of 520 cancer-related genes. Peripheral blood samples and urothelial carcinoma samples were collected in accordance with the guidelines of the Institutional Human Research Ethics Committee at the Fudan University Shanghai Cancer Center, Shanghai, China. Peripheral white blood cells were isolated from peripheral blood samples and kept in liquid nitrogen. The tumor samples were obtained during surgery after obtaining informed consent from UTUC patients. The fresh tumor tissue specimens were immediately transferred to the laboratory, extensively washed with phosphate buffered saline (PBS) to remove excess blood, snap frozen, and kept in liquid nitrogen until use.

METHOD DETAILS

Germline whole exome sequencing

Genomic DNA was extracted from white blood cells using a QIAamp DNA kit (Qiagen), and libraries were prepared using protocols recommended by Illumina. Briefly, 1 μg of DNA was sheared into short fragments of 200–300 bp with a Covaris S220 ultrasonicator. The DNA fragments were repaired to generate adenylated 3′ ends, which were then ligated with adaptors. Adaptors with barcode sequences were then ligated to both ends of the fragments. DNA fragments of the targeted size were selected via gel electrophoresis, underwent 10 amplification cycles, purified, and subjected to whole exome capture with a TruSeq Exome Enrichment kit (Illumina) per kit instructions and sequenced on an Illumina HiSeq 2500.

Capture-based targeted sequencing

All wet-lab procedures were performed in a College of American Pathologists (CAP)-accredited/Clinical Laboratory Improvement Amendments (CLIA)-certified clinical laboratory at Burning Rock Biotech. DNA library construction was prepared with a minimum of 50 ng DNA, subjected to capture with a panel of 520 cancer-related genes (Burning Rock Biotech, Guangzhou, China) and sequenced with a Nextseq500 sequencer (Illumina, Inc., USA) with pair-end reads. Sequence data were analyzed using proprietary computational algorithms optimized for identifying gene variants and sequencing artifacts. Variants with a population frequency >0.1% based on the ExAC, 1000 Genomes, dbSNP, and ESP6500SI-V2 databases were grouped as single-nucleotide polymorphisms and excluded from downstream analysis.

Interpretation of inherited variants

All genetic annotations and nomenclature were based on GRCh37/hg19 build. The interpretation of germline variants followed the standards and guidelines of American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP).[21] Variants were divided into the following five classes: pathogenic (P; Class 5), likely pathogenic (LP; Class 4), variants of uncertain significance (VUS, Class 3), likely benign (Class 2) and benign (Class 1). An in-house pipeline with a combination of

population data, computational and predictive data, and function data was constructed to assist the interpretation, and the pipeline was updated every 3 months. Two genetic consultants evaluated the interpretation independently and searched for newly published data manually.

We used consistent methodologies to detect germline alterations regardless of sequencing platform. That means for germline WES data, we only acquired and interpreted variants in the same coverage area as targeted-panel sequencing in this study. This allows the analysis of germline variant data independent of platform and sequencing scope. We combined these results in order to increase the sample size, enabling the detection of more germline P/LP mutations, which in general are not frequent. On the other hand, germline P/LP mutations are less frequent, but more stable. Previous studies have shown that the germline variation data obtained by different sequencing platforms and calling methods have very high consistency.[22]

Microsatellite instability (MSI) status determination

Five patients with germline MMR P/LP mutations also underwent somatic sequencing (OncoScreen Plus Panel) using tumor tissues. MSI-status was analyzed based on the somatic sequencing data.

MSI status was determined with a read count distribution-based approach. The MSI status of a sample was determined by the percentage of unstable loci among 245 present marker loci. For each marker locus, a read-count histogram was constructed, and those with coverage ratio < [mean – 3 × SD] of the reference ratio was considered ''length-instable''. Percentage of length-unstable marker loci was set at > 40% for MSI-High samples.

OncoScreen Plus Panel has a small range and is not suitable for the MSI algorithm for WES. Therefore, we developed a targeted MSI algorithm for data from sequencing panel, which is highly consistent with the results of gold standard PCR. The accuracy of this algorithm for sequencing panel was 99.0%, the sensitivity was 98.1%, and the specificity was 100%. The tissue MSI algorithm is also included in the consensus of Chinese experts on MSI detection, and there are corresponding patent endorsements for both tissue and blood tests (201710061152.6; 201811149011.0); This algorithm for sequencing panel was reported in relevant published literature.[23,24]

Tumor mutation burden (TMB) estimation

TMB was estimated based on the somatic sequencing data. TMB was calculated as number of non-synonymous somatic alterations on the coding regions of the targeted genes per million base pairs after excluding variants with allelic frequency <2% from tissue samples or <0.2% from liquid biopsy samples.

TMB count was defined as the number of somatic single-nucleotide variants (SNVs) and small insertions and deletions (InDels) located at the coding region and its 20 bp upstream/downstream region after the removal of known and probably oncogenic driver events and germline SNPs. Copy number variations (CNV), fusions and large genomic rearrangements were excluded from the mutation count. Tissue samples with maximum mutation AF (maxAF) $\geq$ 5% and plasma samples with maxAF $\geq$0.5% were screened for TMB calculation. TMB was calculated according to the following equation:

$$TMB = \frac{Mutation\ count[except\ for\ copy\ number\ variant\ and\ fusion]}{Coding\ region\ size}$$

and for sample(s) sequenced by WES procedure in germline, only genes in OncoScreen Plus Panel (520 genes) were included when evaluating TMB.

In addition, we also compared the consistency of sequencing data based on 520-gene-panel with WES sequencing data in TCGA public database. 6704 samples were used to calculate TMB with based on 520-gene-panel results and WES results respectively. The correlation between them is very good (R^2 = 0.98; Correlation coefficient = 0.99).

Loss of heterozygosity analysis

Method for analyzing loss of heterozygosity (LOH) in the same documented in our previously published paper.[12] LOH of the pathogenic variant was estimated by analyzing the frequency of the patient's SNVs or InDels within the mutated gene and checked manually. If the mutation frequency in somatic sequencing data increased in comparison germline sequencing and represented more than 60% of the reads, it was proposed that the mutation demonstrated LOH, assuming a certain degree of contamination from normal tissues.

Immunohistochemistry (IHC) analysis

Immunostaining of MLH1, MSH2, MSH6 and PMS2 was performed using a mismatch repair rabbit monoclonal antibody panel (Cat No. ab252190, Abcam) with a 1:500 dilution for MSH2 and MSH6, 1:200 for PMS2 and MLH1, along with the Envision detection kit (Dako). We defined loss of MMR proteins (MLH1, MSH2, MSH6, PMS2) expression as a complete loss of specific nuclear staining in cell nuclei of carcinoma according to previous studies.[25,26]

Mutational subtype determination

Mutational subtype classification was determined according to the criteria reported in previous published paper.[4] We first ranked 24 UTUC somatic sequencing samples according to their tumor mutational burden in descending order. A huge change of mutational

burden was observed in Figure 3D. We chose TMB>30 Muts/Mb as a cutoff to discriminate hypermutated and non-hypermutated samples. For non-hypermutated cases, we examine somatic sequencing results for the presence of *TP53* mutations. Patients are classified as *TP53/MDM2*-mutated subtype if they have *TP53* mutation(s). If no *TP53* mutation is present, we will look for the presence of a *RAS* or *FGFR3* alteration, thereby classifying as a *RAS*-mutated or *FGFR3*-mutated subtype. When the patient has no mutation in *TP53*, *RAS* and *FGFR3*, if there is *MDM2* alteration, this case will still be classified as *TP53/MDM2* mutated subtype; if there is no alteration in *MDM2*, this case will be deemed as a Triple Negative subtype.

Mutational signature analysis of tumor somatic alterations

Mutational signature was analyzed using the R package MutationalPatterns (version 3.0.1)[20] in all 24 germline P/LP mutated UTUC patients with paired somatic sequencing data. Briefly, single base substitution patterns archived in the Catalog of Somatic Mutations in Cancer (COSMIC; http://cancer.sanger.ac.uk/cosmic/signatures) were identified in the somatic genomic profiles from Chinese UTUC patients with somatic sequencing data in this study. Using this analysis method, the value of each signature in CSOMIC Signature 1 to 30 will be output for each sample. A signature value greater than 0 indicates presence of a tumor component with this signature. Among the 30 COSMIC signatures, the signature with the largest value is the dominant signature of the tumor sequenced. Results of COSMIC signature analysis by case were shown in Table S6.

QUANTIFICATION AND STATISTICAL ANALYSIS

The descriptive statistics of Chinese UTUC patients' clinicopathological information are shown in Table 1. Statistical analyses were performed in SPSS Statistics 22.0. Before running SPSS 22.0 for a certain statistical analysis, the software checks the data to ensure that it is suitable for analysis using the statistical method. In this study, Chi-square test was used to compare the proportions of patients with germline P/LP mutations among different age groups and different tumor location groups (Table S3). Fisher exact test was used to compare the proportions of patients with germline P/LP mutations among other different groups classified by clinicopathological factors (details in Figure 1B and Table S3). Survival curves were plotted using GraphPad Prism 6.0. Kaplan-meier plotting method (Figures 3A, 3B and S1) and multivariate cox regression analysis (Figure 3C) were used to evaluate prognosis data. Progression-free survival was defined as time from surgery to either first recurrence in the tumor bed or bladder, first metastasis, or death from any cause. New non-muscle-invasive bladder cancer was not regarded as progression to censor according to POUT Trial criteria.[7] Case–control association was assessed by logistic regression. The significance of associations was adjusted for multiple testing by using the Bonferroni correction (Figure 2B). For all figures and tables in this study, "N" presents number of patients in certain group or condition. All tests were two-tailed, and a p value of <0.05 was considered statistically significant.