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Clinical Characteristics and Mutation Analyses of Ovarian Sertoli-Leydig Cell Tumors

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. *DICER1* • Germline mutations • Somatic mutations • Sertoli-Leydig cell tumors • Whole exome sequencing

Abstract

Background. There are limited studies on Sertoli-Leydig cell tumors (SLCTs) and no data in the population of Chinese patients with SLCTs from the genetic level. In addition, previous studies on SLCTs have focused exclusively on mutations in the *DICER1* gene and no data exists on the genetic landscape of SLCTs.

Methods. Patients with moderately or poorly differentiated SLCTs who underwent surgical resection between January 2012 and October 2018 in our institution were recruited. Whole exome sequencing was performed on formalin-fixed, paraffin-embedded tumor tissue and peripheral blood or normal tissue samples.

Results. Seventeen patients were recruited with 19 tumor samples. The rate of tumor-associated germline mutations was 6 of 17 (35.3%), and that of *DICER1* germline mutations was 4 of 17 (23.5%). Regarding clinical relapse, patients with germline tumor-associated mutations had significantly poorer prognosis than those without (p = .007), and those with germline *DICER1* mutations were relatively more likely to exhibit clinical relapse, although not to a significant degree (p = .069). Regarding somatic mutations, firstly, the subclone evolution analysis demonstrated that the two tumors on the contralateral ovary were primary tumors, respectively. Secondly, somatic mutations were most commonly found in *CDC27* (10/19, 52.6%), *DICER1* (4/19, 21.1%), and *MUC22* (4/19, 21.1%). And the analysis of cancer cell fractions showed that *DICER1* mutations were correlated with tumorigenesis of SLCTs. The rates of germline and somatic *DICER1* mutations were higher in patients who were younger than 18 years than those in older patients (p = .022 and p = .001, respectively).

Conclusion. Our study indicates that genetic testing may have important clinical significance for patients with SLCTs, particularly for younger patients. ***The Oncologist*** 2020;25:e1396–e1405

Implications for Practice: Bilateral ovarian Sertoli-Leydig cell tumors were verified to be primary tumors from the genetic perspective. The rates of germline and somatic *DICER1* mutations were 4 of 17 (23.5%) and 4 of 19 (21.1%), respectively. The rates of germline and somatic *DICER1* mutations were higher in patients who were younger than 18 years than those in older patients (p = .022 and p = .001, respectively).

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Introduction

Ovarian sex cord-stromal tumors, constituting approximately 5% of all ovarian neoplasms, originate from the elements of the sex cord and/or ovarian stroma [1], which could differentiate to ovarian cell types (granulosa and theca cells), testicular types of cells (sertoli and leydig cells), and/or indifferent elements [2, 3]. These various types of cells may be observed separately or in combination [2]. Sertoli-Leydig cell tumors (SLCTs) are composed of sertoli and leydig cells [4] and were once called "arrhenoblastoma" or "androblastoma" [5].

SLCTs occur in women predominantly in the first three decades of life [5, 6], with median ages ranging from 9 to 28 years in various studies [5, 7, 8]. The most common symptoms are related to excessive hormone release, and 34.0% to 62.5% of patients have androgen excess manifestations [5, 7–9], whereas some patients may have increased secretions of estrogen [8, 10, 11] and others do not show endocrine changes [9]. SLCTs are predominantly unilateral (98.0%) and solid tumors, but cystic areas may also be present [4]. The solid components of SLCTs are typically lobulated and are often yellow [5]. Hemorrhagic areas and foci of necrosis are frequently observed [9]. The majority of patients with SLCTs (81.8%–100%) have stage I cancers according to International Federation of Gynecology and Obstetrics (FIGO) [7, 8, 10–16]. Based on the classification of tumors in the female reproductive organs proposed by the World Health Organization, SLCTs can be divided into four histological types: well differentiated, moderately differentiated, poorly differentiated, and retiform [17]; in 20% of tumors of the latter three types, heterologous elements can occur [18].

Well-differentiated SLCTs are benign and do not exhibit recurrence [8]. Poorly-differentiated elements, retiform patterns, or heterologous elements, as well as FIGO stages IC–III are key factors indicating a poor prognosis [7, 19, 20]. The relapse rate of patients with stage IA was reported to be around 7.0%, with a death rate in cases of relapse of 70.0%; the relapse rate with stage IC was around 30.0%, with a death rate in cases of relapse of 54.0%; the prognosis of the advanced-stage disease (stages II to IV) was poor, with a relapse rate of 73.7% and a death rate in cases of relapse of 78.6% [10]. The relapse tends to occur relatively soon after the initial diagnosis and is somewhat rare after 5 years [21]. The tumors of relapse are mostly observed in the pelvic and abdominal cavities [7].

The treatment of patients with SLCTs depends upon the patient's age and cancer stage. Fertility-sparing surgery can be performed for patients with FIGO stage IA and remains to be defined for those with stage IC [10]. For postmenopausal women and patients with FIGO IB or advanced stages, the European Society for Medical Oncology recommends abdominal hysterectomy and bilateral salpingo-oophorectomy with careful surgical staging [22]. The use of postoperative adjuvant chemotherapy for patients with FIGO stage I is controversial, and no clear benefit is found for these patients [8, 23]. However, adjuvant chemotherapy is recommended for the patients with high-risk factors for tumor recurrence, including FIGO stages IC–IV, moderate or poor differentiation, or the presence of retiform patterns or heterologous elements [7, 8, 22]. The most commonly used regimen is the combination of bleomycin, etoposide, and cisplatin (BEP) [8, 24].

Germline loss-of-function mutations in *DICER1* gene encoding an enzyme of the RNase III family are associated with an increased risk of a neoplasm predisposition syndrome, named DICER1 syndrome, and pleuropulmonary blastoma (PPB), SLCTs, and thyroid gland cancer are the three primary categories of malignant tumors in DICER1 syndrome [25]. In addition to the scarcity of data on the germline testing, the rates of germline and somatic *DICER1* mutations in SLCTs have varied dramatically in previous different studies [26–33]. Moreover, previous studies of SLCTs have focused exclusively on *DICER1* mutations, and there are no data regarding whole exome sequencing (WES) of SLCTs [26–33]. And, there are no data on SLCTs on somatic and germline mutations for Chinese populations.

In this study, we conducted WES of tumor samples and paired germline samples from patients with SLCTs in order to explore not only the *DICER1* mutations of SLCTs for Chinese populations but also other genetic variations related to the pathogenesis and prognosis of SLCTs, which could lay a preliminary foundation for genetic counseling.

Materials and Methods

This study and all its protocols were approved by the Peking Union Medical College Hospital ethical committee (approval number JS-1640). All patients signed an informed consent form. To ensure the quality of tissue samples, all patients with SLCTs who underwent surgical resection between January 2012 and October 2018 at the Peking Union Medical College Hospital were included in the study. None of the patients received chemotherapy or radiotherapy 5 years before the first surgery. The histological review conducted by three gynecological pathologists confirmed the diagnosis of moderately or poorly differentiated SLCTs for all the cases investigated.

DNA was extracted from the peripheral blood lymphocytes of 14 patients using the Qiagen Genomic-Tip (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Formalin-fixed, paraffin-embedded (FFPE) tissue sections (5–10 mm) were prepared from 19 tumors and three normal tissues from 17 patients during the surgery. DNA was extracted from FFPE samples using the GeneRead DNA FFPE Tissue Kit (Qiagen), according to the manufacturer's instructions.

DNA quantification, library construction, and WES were performed by the Berry Genomics Group (Beijing, China). Briefly, DNA was extracted and quantified using Qubit 2.0 (Invitrogen, Carlsbad, CA). The DNA was fragmented randomly by a Covaris ultrasonicator (Illumina, San Diego, CA), and the length of the resulting DNA fragments was determined to be 180–250 base pairs. The exon capture was carried out using Agilent SureSelect Human All Exon version 6 (Agilent, Santa Clara, CA). The captured fragments were subsequently purified, amplified, and qualified. High-throughput sequencing of the library products was performed on an Illumina Novaseq 6000 in a paired-end sequencing run; the Q30 was required to be more than 80%. All exons of each target gene were sequenced at the average

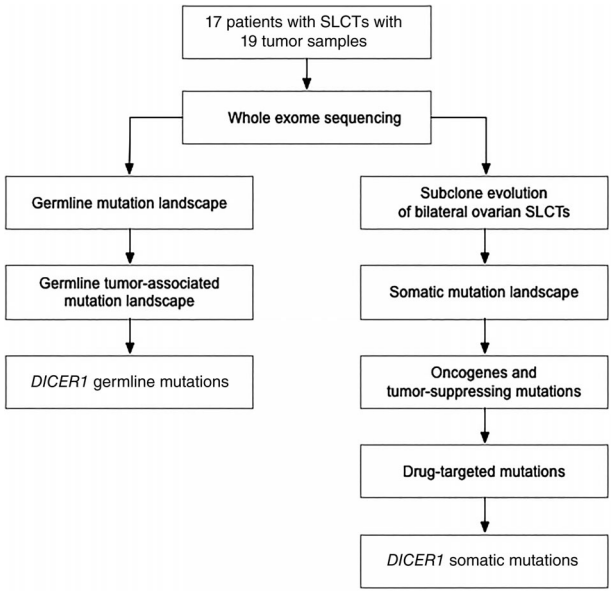


Figure 1. The study flow chart.
Abbreviation: SLCTs, Sertoli-Leydig cell tumors.

depths of 200× and 100× for FFPE tumor tissue and paired germline samples, respectively. Valid sequencing data were mapped to the human reference genome (University of California, Santa Cruz, hg19) using Burrows-Wheeler Aligner software to obtain the original mapping results, which were stored in the BAM format [34, 35].

In terms of the detection and filtering of germline mutations, single nucleotide variants and indels were identified as candidates if they met the following criteria: they were detected by at least two programs (HaplotypeCaller [36], Atlas2 [37], and UnifiedGenotyper for single nucleotide variants [36] or Platypus for indels [38]), and they had a depth of ≥4× and ≤100,000×. Genomic alterations were annotated using ANNOVAR [39], and pathogenic and likely pathogenic mutations were identified based on the American College of Medical Genetics and Genomics (ACMG) standards and guidelines (https://www.acmg.net/).

In terms of the detection and filtering of somatic mutations, GATK4 Mutect2 was used to detect somatic mutations [40], and ANNOVAR was used to annotate the genomic alterations [39]. To identify variations in the candidate genes, filter conditions were set to remove the following: (a) mutations in tumor samples with the depth less than 20×, (b) mutations in tumor samples with variant allele frequency (VAF) <0.03, (c) mutations in normal tissue samples with depth less than 10×, (d) mutations in normal tissue samples with VAF >0.03, (e) variations out of the exon or splicing region, and (f) synonymous mutations. Subsequent analyses of oncogenes and tumor-suppressor genes, cancer cell fractions (CCFs), and potential drug-targeted mutations were based on the above results.

Oncogenic and tumor-suppressing mutations were detected among the identified somatic and germline mutations based on the Catalogue of Somatic Mutations in Cancer (COSMIC) Cancer Gene Census database (https://cancer.sanger.ac.uk/census). The CCF denotes the proportion of cells with a somatic mutation in a population of tumor cells; the analysis takes into consideration several factors, including the VAF, copy number variation, ploidy, and purity [41]. The relevance of the mutated gene to the tumorigenesis was determined based on the CCF value of each mutation site and the number of samples containing this mutation site using the Student's *t* test. In terms of the identification of drug-targeted mutations, mutations that could be potentially targeted with drugs were detected among all identified somatic mutations using the OncoKB database (https://oncokb.org/). The subclone analysis was performed using the SciClone software, which can determine the number and genetic composition of subclones based on the VAF of somatic mutations [42].

Follow-up time was calculated from the date of the initial surgery to the date of death or last contact, and disease-free survival (DFS) was calculated from the date of the initial surgery to the date when cancer progression or clinical relapse was first detected (the metachronous tumor was also included). Categorical variables are presented as frequencies, and continuous variables are presented as the median (range). Frequency distributions were compared using chi-squared or Fisher's exact tests, and median values were compared using Mann-Whitney nonparametric *U* tests. Survival analysis was carried out using the Kaplan-Meier method by log-rank testing. The data were analyzed using SPSS (version 23, IBM, Armonk, NY) or Prism 7 (GraphPad Software, San Diego, CA). A two-tailed value of $p < .05$ was considered statistically significant. The cutoff date for the data collection was August 1, 2019.

Results

In total, 17 patients with 19 tumor tissue samples were included in this study (Fig. 1). FFPE tumor samples and paired peripheral blood samples were collected from 14 patients, and paired normal FFPE samples were obtained from 3 patients. After performing WES, we analyzed the germline mutation landscape, the subclone evolution analysis, the somatic mutation landscape, oncogenic and tumor-suppressing mutations, and potential drug-targeted mutations.

The median age of patients at diagnosis was 39 years (12–72 years; Table 1). Eleven and three patients had androgenic and estrogenic manifestations, respectively, and three patients did not have any endocrine deregulation–associated symptoms. In 11, 5, and 1 patients, respectively, tumors were observed in the right, left, or both ovaries. In the initial surgery, moderately differentiated homogeneous tumors were found in seven patients, and poorly differentiated tumors were observed in four patients; among the latter, two tumors had heterologous elements: mucinous adenoma (parts are borderline) and rhabdomyosarcoma (Fig. 2), respectively. Moderately/poorly differentiated tumors were observed in six patients. Nine, one, six, and one patients, respectively, had tumors of FIGO stages IA, IB, IC, and IIIC. Unilateral salpingo-oophorectomy was performed in ten patients: in eight by laparoscopy and in two by transabdominal surgery. Total hysterectomy and bilateral salpingo-oophorectomy were performed in five patients: in three by laparoscopy and in two by transabdominal surgery. Oophorocystectomy was

Table 1. The clinical characteristics of included patients

Case	Age at diagnosis, years	Endocrine change	Germline tumor-associated mutations	Somatic *DICER1* mutations	Other somatic tumor-associated mutations	Surgery	Pathologic diagnosis	Involved ovary	FIGO stage	Adjuvant therapy (regimen, cycles)	Oncologic outcomes (follow-up time, months)
1	12	Androgenic manifestation	*DICER1* (c.1358_1359delCA:p.T453Sfs)	*DICER1* (c.5125G > A:p.D1709N)		Lap LSO	Moderately/poorly differentiated	Left	IC	TC 3	NED (28)
2	14	Androgenic manifestation	*DICER1* (c.2651-2A > Tc.443_444delTC:p.L148Hfs)	*DICER1* (c.5425G > A:p.G1809R)	*BCL11A*	Lap LSO	Moderately differentiated	Left	IA	No	NED (61)
3	16	Androgenic manifestation		*DICER1* (c.5125G > A:p.D1709N)		Lap RSO	Moderately differentiated	Right	IC	No	NED (37)
4	12	Androgenic manifestation	*DICER1* (c.A4453T:p.K1485X)	*DICER1* (c.5428G > C:p.D1810H) (tumor of clinical relapse)		Tra LSO	Poorly differentiated, with mucinous adenoma, parts of these are borderline	Left	IA	PEB 3	Relapse (right ovary; DFS, 52), tumor section, NED (72)
5	29	Androgenic manifestation			*FGFR1*	Lap LSO	Poorly differentiated	Left	IA	PEB 3	NED (42)
6	30	Androgenic manifestation			*PABPC1*	Lap RSO	Poorly differentiated	Right	IA	No	NED (81)
7	34	Androgenic manifestation				Lap Rcyst	Moderately differentiated	Right	IC	No	NED (57)
8	39	Androgenic manifestation			*NOTCH4*	Lap RSO	Moderately differentiated	Right	IA	No	NED (62)
9	42	Androgenic manifestation	*PMS2*		*MUC16*, *MUC4*	Lap LSO	Moderately/poorly differentiated	Left	IC	No	Relapse (pelvic cavity, focal; DFS, 38), tumor section, NED (74)
10	51	Androgenic manifestation				LH + BSO	Bilateral, moderately differentiated	Bilateral	IB	No	NED (35)
11	54	No	*FOXL2* and *PALB2*		*QKI*	Staging (TAH + BSO + OMEN + LND + Appendectomy)	Moderately/poorly differentiated	Right	IA	PEB 3	NED (11)
12	54	No				TAH + BSO	Moderately differentiated	Right	IA	No	NED (39)
13	57	Estrogenic manifestation				TAH + BSO	Moderately differentiated	Right	IA	No	NED (49)
14	59	No	*DICER1* (c.1786dupA:p.T596Nfs)			TAH + RSO	Poorly differentiated, with heterologous elements (rhabdomyosarcoma)	Right	IIIC	Taxol + epirubicin 3	Relapse (abdominal and pelvic cavities, extensive, DFS, 7), died (9)
15	66	Estrogenic manifestation				LH + BSO	Moderately/poorly differentiated	Right	IC	No	NED (62)
16	72	Estrogenic manifestation			*ATF1*, *FOXL2*	LH + BSO	Moderately/poorly differentiated	Right	IA	TC 2	NED (58)
17	27	Androgenic manifestation				Lap RSO	Moderately/poorly differentiated	Right	IC	No	NED (40)

Abbreviations: BSO, bilateral salpingo-oophorectomy; DFS, disease-free survival; FIGO, International Federation of Gynecology and Obstetrics; Lap, laparoscopy; LH, laparoscopic hysterectomy; LND, lymph node dissection; LSO, left salpingo-oophorectomy; NED, no evidence of disease; OMEN, omentectomy; PEB, platinum, etoposide, and bleomycin; Rcyst, right ovarian cystectomy; RSO, right salpingo-oophorectomy; TAH, transabdominal hysterectomy; TC, taxol and carboplatinum; Tra, transabdominal.

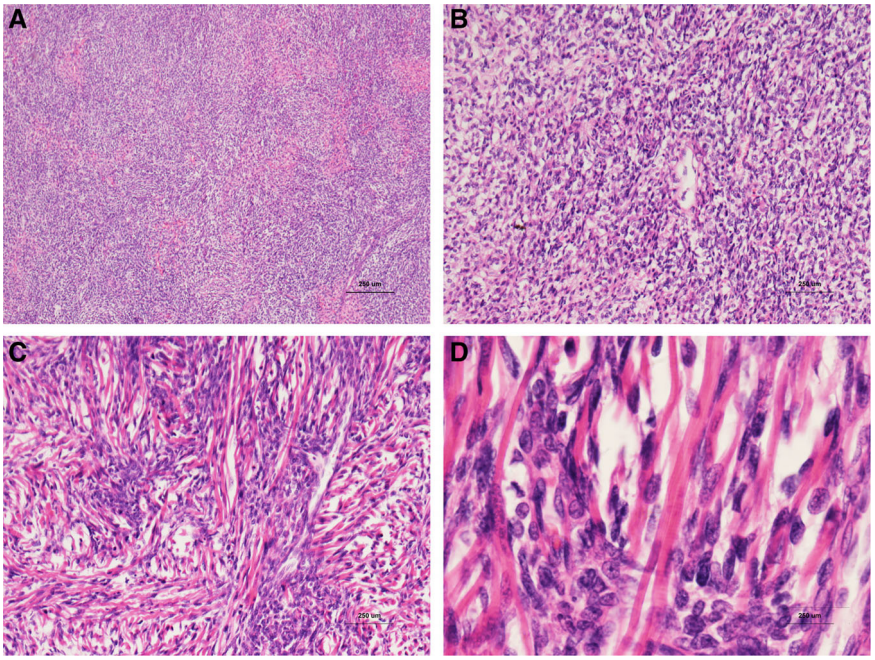


Figure 2. The histologic photos of the poorly differentiated tumors. **(A):** Poorly differentiated Sertoli-Leydig cell tumors (SLCTs; H&E staining, ×40). **(B):** Poorly differentiated SLCTs (H&E staining, ×100). **(C):** SLCTs with rhabdomyosarcoma of heterologous elements (H&E staining, ×100). **(D):** SLCTs with rhabdomyosarcoma of heterologous elements (H&E staining, ×400).

performed in one patient by laparoscopy. Transabdominal hysterectomy, bilateral salpingo-oophorectomy, lymph node dissection, omentectomy, and appendectomy were performed in one patient. In total, six patients received postoperative adjuvant therapy: three had the combination chemotherapy of BEP, whereas two and one received taxol combined with carboplatinum or epirubicin, respectively. The median follow-up time was 49 months (9–81 months). One patient (case 12) died from breast cancer and was considered censored. Three patients had a clinical relapse. In one patient (case 4), the clinical relapse occurred in the contralateral ovary 52 months after the primary treatment; after oophorocystectomy and postoperative chemotherapy, this patient was alive without evidence of disease for 20 months. Another case of relapse (case 9) was observed in the pelvic cavity 38 months after the primary treatment, and the cancer was local; after tumor resection and postoperative chemotherapy, the patient was alive without evidence of disease for 35 months. The last case of relapse (case 14) occurred in the abdominal and pelvic cavities 7 months after the primary treatment; the tumor was extensive, and the patient died from SLCTs 2 months after the relapse.

Potential pathogenic and likely pathogenic germline mutations were identified based on ACMG standards and guidelines. Furthermore, based on the information in the COSMIC database, we selected four tumor-associated mutations in four genes, namely, *DICER1, FOXL2, PALB2*, and *PMS2*. The *DICER1* mutations were found in four patients (cases 1, 2, 4, and 14). *FOXL2* and *PALB2* mutations were found in case 11 and a *PMS2* mutation in case 9. Thus, in the analyzed patients with SLCTs, the germline tumor-associated mutation rate of patients with SLCTs was 6 in 17 (35.3%), and the germline *DICER1* mutation rate was 4 in 17 (23.5%).

Four patients were younger than 18 years at the time of diagnosis; among them, three had germline *DICER1* mutations. Younger patients (<18 years) had a significantly higher *DICER1* mutation rate (3/4, 75.0%) than older patients (1/13, 7.7%; $p = .022$). The rate of patients with *DICER1* germline mutations in patients with heterologous elements in their tumors was significantly higher than that in patients without (2/2, 100.0% vs. 2/15, 13.3%; $p = .044$). The rates of patients with germline *DICER1* mutations in the patients with endocrine changes, unilateral tumors in the left ovary, or moderately differentiated homogeneous tumor were not significantly different from those in patients without these characteristics (3/14, 21.4% vs. 1/3, 33.3%; $p > .999$; 3/5, 60.0% vs. 1/12, 8.3%; $p = .053$; 1/7, 14.3% vs. 3/10, 30.0%; $p = .603$, respectively).

In addition, the patients with *DICER1* germline mutations were more likely to have tumors with heterologous elements at the time of the initial diagnosis (2/4, 50.0% vs. 0/13, 0.0%; $p = .044$).

Among the six patients with germline tumor-associated mutations, three exhibited clinical relapse, and one died of the disease, whereas none of the eleven patients without such mutations had cancer progression or died. The DFS analysis indicated that patients with germline tumor-associated mutations had a significantly poorer prognosis compared with those without them ($p = .007$; Fig. 3A). Among the 4 and 13 patients with and without germline *DICER1* mutations, 2 and 1 exhibited clinical relapse, and 1 and 0 died from the disease, respectively. As can be seen from Figure 3B, patients with germline *DICER1* mutations were more likely to exhibit clinical relapse, although the tendency was not statistically significant ($p = .069$; Fig. 3B).

In terms of somatic mutations, firstly, subclone evolution analyses of bilateral ovarian tumors were performed.

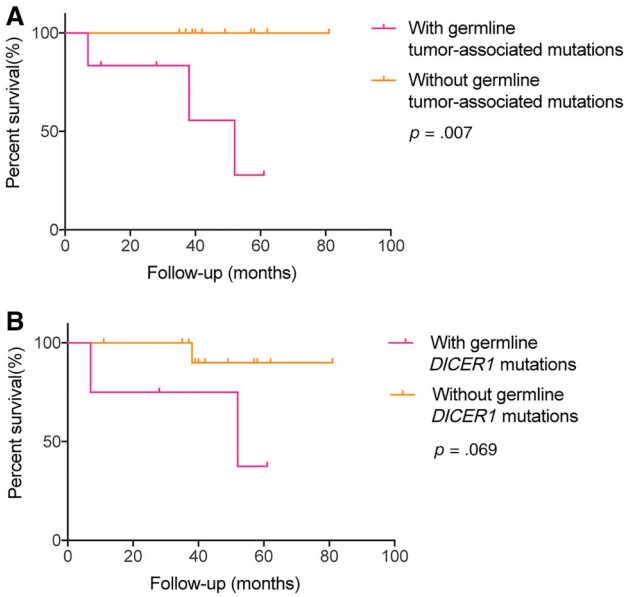
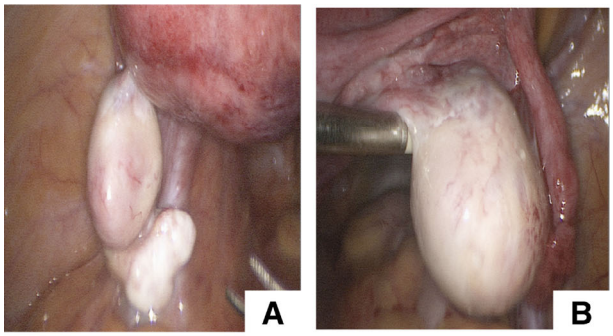


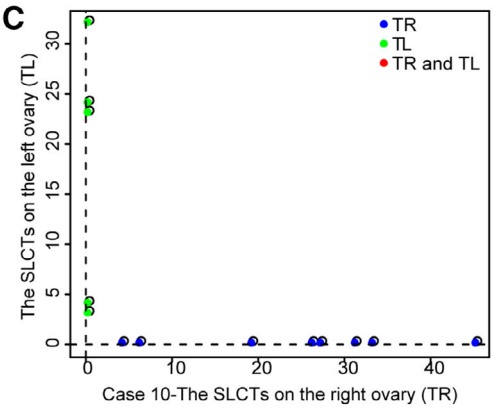
Figure 3. The curve of disease-free survival.

One patient (case 10), with FIGO stage IB, showed tumors limited to both ovaries. During surgery, the capsules of the bilateral tumors were intact (Fig. 4 A, B) and no malignant cells were found in ascites or peritoneal washings. Based on the criterion that the mutation VAF should be no less than 3%, nonsynonymous mutations in the exon and mutations in the splicing region were detected. As was shown in Figure 4, the clones of the bilateral ovarian tumors were found to be specific, and no evolutionary relationship was found (Fig. 4C), indicating that the bilateral ovarian SLCTs were primary and not metastatic.

In one patient (case 4), the apparent clinical relapse occurred in the contralateral ovary 52 months after the primary treatment without involving other sites. From the germline mutation perspective, this patient was found to have a germline *DICER1* mutation, which supported the diagnosis of metachronous contralateral ovarian SLCTs because of the report by Schultz et al. in 2017 of metachronous SLCTs in some individuals with *DICER1* germline mutations [29]. Moreover, from the somatic mutation perspective, the clones of the bilateral ovarian tumors were found to be specific, and no evolutionary relationship was found, indicating that the tumor of the apparent clinical relapse was actually primary (Fig. 4D).

The bilateral ovarian tumors from case 10 and case 4 were identified to be primary tumors; thus, 19 tumor samples were included in the analyses of subsequent somatic mutations. Firstly, we selected the top 11 high-rate somatic protein–altering mutations and evaluated the somatic mutation landscape (Fig. 5). Somatic mutations were most commonly found in *CDC27* (10/19, 52.6%), *DICER1* (4/19, 21.1%), *MUC22* (4/19, 21.1%), *MUC2* (2/19, 10.5%), *MUC17* (2/19, 10.5%), *RAD50* (2/19, 10.5%), *SON* (2/19, 10.5%), *ZNF708* (2/19, 10.5%), *CACNA1E* (2/19, 10.5%), *KIF1B* (2/19, 10.5%), and *PTH2* (2/19, 10.5%). Furthermore, according to COSMIC, oncogenic driver mutations were found in *DICER1* (4/19, 21.1%), *ATF1* (1/19, 5.3%), *BCL11A* (1/19, 5.3%), *FGFR1* (1/19, 5.3%), *FOXL2* (1/19, 5.3%), *MUC16* (1/19, 5.3%), *MUC4* (1/19, 5.3%), *PABPC1* (1/19, 5.3%), *PRDM16* (1/19, 5.3%), and *QKI* (1/19,





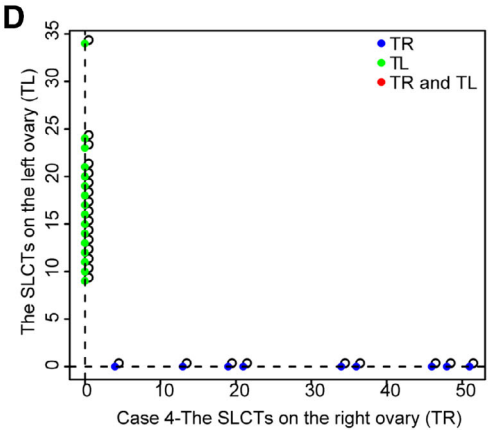


Figure 4. The subclone evolution analysis of tumors. **(A):** The intraoperative view of SLCTs on the left ovary. **(B):** The intraoperative view of SLCTs on the right ovary. **(C):** Two-dimensional analysis of bilateral ovarian tumors; no overlapped mutation was detected in case 10. **(D):** Two-dimensional analysis of bilateral ovarian tumors; no overlapped mutation was etected in case 4.
Abbreviations: SLCT, Sertoli-Leydig cell tumor; TL, the tumor on the left ovary; TR, the tumor on the right ovary.

5.3%). The expression of these genes was significantly enriched in three Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways associated with cancer, namely, aldosterone synthesis and secretion (*PLCB1*, *ATF1*, *CAMK1D*, and *PDE2A*; $p = .004$), breast cancer (*JAG2*, *NOTCH4*, *DLL4*, *FGFR1*; $p = .017$), and P13K-Akt signaling (*ITGB4*, *LAMA1*, *OSM*, *ITGB7*, *RELN*, *FGFR1*; $p = .030$).

Through the analysis of CCFs, *CDC27* and *DICER1* mutations were found to be the top two high-possibility mutations correlated with the tumorigenesis of SLCTs. *CDC27*

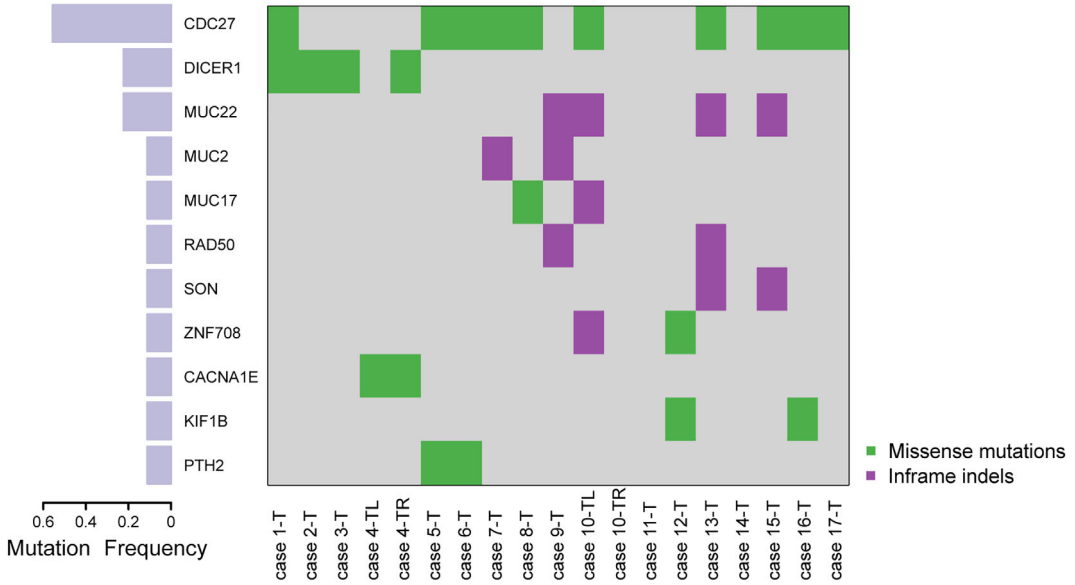


Figure 5. Heat map showing somatic mutation profiles of Sertoli-Leydig cell tumors. **Middle:** Mutated genes are ranked based on the mutation frequency. **Left:** The frequency of each mutated gene in all samples.

was verified to be a pseudogene by the Sanger sequencing. Therefore, CCFs analyses demonstrated that *DICER1* mutations were correlated with the tumorigenesis of SLCTs.

The identified genes with somatic mutations were analyzed using the OncoKB database with the aim to reveal the genes with alterations that can be targeted by drugs. One patient carried such a mutation in the *FGFR1* gene (case 5; 1/17, 5.8%), which could be susceptible to treatment with drugs, namely, AZD4547 [43], BGJ398 [44], erdafitinib [45, 46], and Debio1347 [47–49].

In terms of *DICER1* somatic mutations, among the 19 tumor samples, 4 (21.1%) had somatic *DICER1* mutations: c.5125G > A:p.D1709N, c.5425G > A:p.G1809R, c.5125G > A:p.D1709N, and c.5428G > C:p.D1810H, all of which had been reported in the previous study on DICER1 syndrome [50]. Among 15 other tumor samples, which did not have variations in the *DICER1* gene, 1 tumor sample (case 8) had a mutation in the *NOTCH4* gene, which is in the same KEGG pathway as *DICER1*.

Among the five tumor samples from the four patients younger than 18 years, four had somatic *DICER1* mutations (4/5, 80.0%). The rate of tumors with *DICER1* somatic mutations in these tumors from the younger patients was significantly higher than that from the older patients (4/5, 80.0% vs. 0/14, 0.0%; $p = .001$). The rates of tumors with somatic *DICER1* mutations in tumors from patients with endocrine changes (4/16, 25.0% vs. 0/3, 0.0%; $p > .999$), tumors on the left ovary (2/6, 33.3% vs. 2/13, 15.4%; $p = .557$), poorly differentiated tumors (0/4, 0.0% vs. 4/15, 26.7%; $p = .530$), or tumors with heterologous elements (0/2, 0.0% vs. 4/17, 23.5%; $p > .999$) were not significantly different from those in tumors from patients without these features.

Discussion

SLCTs are very rare, and consequently, few studies on this cancer type have been published. To comprehensively analyze this disease at the genetic level, we evaluated the results of studies involving at least 10 patients, which were published from January 2012 to August 2019 and reported in Embase, PubMed, and Web of Science. As a result, eight studies were selected (Table 2) [26–33]; all of them investigated SLCTs by specifically targeting the *DICER1* gene. To the best of our knowledge, our study is the first to employ WES to analyze the SLCTs genetic landscape.

In addition, this is also the first study to examine the rate of *DICER1* mutations in SLCTs in the population of Chinese patients with SLCTs and to report a relatively higher rate of both somatic and germline *DICER1* mutations in younger patients (<18 years).

De Kock et al. reported that well-differentiated SLCTs are different neoplasms from moderately higher than that observed in our study differentiated and poorly differentiated subtypes with a different pathogenesis, from the perspective of both morphologic parameters and *DICER1* mutations [32]. Therefore, well-differentiated SLCTs were excluded, and all our included patients had moderately or poorly differentiated SLCTs. Previous studies found that the rates of somatic *DICER1* mutations in moderately or poorly differentiated SLCTs were reported as 33.3% [31], 48.6% [26], 60.0% [27], 66.7% [30], 97.3% [29], and even up to 100.0% [32], that is, higher than that observed in our study (21.1%). Studies on the rate of germline *DICER1* mutations in SLCTs also revealed higher mutation frequencies (40.0%–69.0%) [28, 29, 32] compared with that observed here (23.5%). The reason for the discrepancy may be that the proportion of younger patients in our study was relatively lower than those in previous studies, which, considering that both somatic and germline *DICER1* mutation rates were relatively higher in the younger population, may account for the decreased overall rate.

Regarding the tumorigenic mechanism of *DICER1* mutations, some investigators support the two-hit tumor suppression model [51], whereas others suggest that haploinsufficiency may

Table 2. The genetic landscape of Sertoli-Leydig cell tumors in previous studies

First author	Year published	Country	Sequencing method	Germline mutations	Somatic mutations
Karnezis [26]	2019	Canada Germany	Hotspots of *DICER1* RNase IIIb domain mutations	NA	Well-differentiated, 0/5 (0.0%) Moderately/poorly differentiated, 18/37 (48.6%) (moderately, 17/31; poorly, 1/6)
De Kock [32]	2017	Canada	*DICER1* gene	Well-differentiated, 0/2 (0.0%) Moderately/poorly differentiated, 18/26 (69%)	Well-differentiated, 0/4 (0.0%) Moderately/poorly differentiated, 30/30 (100.0%)
Kato [27]	2017	Japan	Hotspots of *DICER1* RNase IIIb domain mutations	NA	Moderately/poorly differentiated, 6/10 (60%)
Schultz [29]	2017	U.S.	*DICER1* gene	Well-differentiated, 0/1 (0.0%) Moderately/poorly differentiated or unknown, 25/40 (62.5%)	Moderately/poorly differentiated or unknown, 36/37 (97.3%) Moderately/poorly differentiated, 22/23 (95.7%)
Goulvent [31]	2016	France	Exon 24 and 25 of *DICER1* gene	NA	Well-differentiated, 0/1 (0.0%) Intermediately/poorly differentiated, 6/18 (33.3%)
Conlon [30]	2015	U.S.	Exon 24 and 25 of *DICER1* gene	NA	Well-differentiated, 0/2 (0.0%) Intermediately/poorly differentiated, 20/30 (66.7%)
Witkowski [33]	2013	Australia England Germany Canada	*DICER1* RNase IIIa and IIIb domain mutations	NA	8/15 (53.3%)
Heravi-Moussavi [28]	2012	U.S. Canada	*DICER1* RNase IIIb domain mutations	4/10 (40.0%)	26/43 (60%)

Abbreviation: NA, not available.

also promote the occurrence of *DICER1*-associated tumors [52, 53]. In the current study, three patients had both germline and somatic *DICER1* mutations, and two patients had either germline or somatic *DICER1* mutations; a single hypothesis does not interpret this phenomenon. Therefore, we think that the role of *DICER1* in SLCTs may be similar to that of *BRCA1/2* in ovarian cancer, that is, tumorigenic effects associated with somatic biallelic inactivation, whereas haploinsufficiency may also promote the tumor formation and progression [54, 55].

There are four possible mechanisms of bilateral ovarian tumors: metachronous tumors, synchronous tumors, metastasis, and recurrence [56]. In terms of clinical manifestation, a contralateral tumor not occurring outside of the ovary, for example, in the pelvis, is considered metachronous and not a relapse [7]. The likelihood of metachronous tumor formation is an important consideration for therapeutic planning because, first, a metachronous tumor may be more sensitive to first-line treatment and may have better prognosis than a recurrent tumor, and second, such patients should be followed for the emergence of metachronous tumors even after the longest period estimated for the recurrence risk has passed [29]. Identifying whether the tumor is primary or not is a major challenge for the pathologist. Furthermore, the abovementioned metachronous SLCTs diagnosed by clinical manifestation actually may be metastatic or recurrent tumors [57]. In recent years, numerous studies have evaluated pairs of tumor specimens to examine the similarity of the somatic mutation characteristics of the tumors and to test for clonal relatedness [58]. Previous studies defined the metachronous tumor of SLCTs from the clinical manifestation perspective [7, 29]. This is the first study to identify the metachronous SLCTs from the whole exon perspective.

In addition, in this study, the bilateral ovarian tumors of one patient (case 10) may be synchronous SLCTs or metachronous SLCTs with a relatively short time interval between the occurrences of the bilateral ovarian tumors. However, no germline or somatic *DICER1* mutations were found for this patient. In previous studies, the metachronous SLCTs always occurred in patients with *DICER1* germline mutations [25, 29, 32], and few studies were available about the synchronous bilateral SLCTs. This is the first finding that the occurrence of bilateral ovarian primary SLCTs is not limited only to patients with *DICER1* germline mutations.

DICER1 germline mutations cause a hereditary cancer predisposition syndrome [59]. Testing for *DICER1* germline mutations can contribute to the early diagnosis of relevant cancers: first, the malignant PPB types II and III arise from the precursor PPB type I, and early diagnosis and treatment

can improve prognosis; second, prompt diagnosis of cystic nephroma can result in partial rather than total nephrectomy; third, prompt diagnosis of SLCTs at an early stage could help avoid chemotherapy and improve survival [60]. Interestingly, in the current study, the rate of germline *DICER1* mutations was significantly higher (75.0%) in the younger patients with SLCTs, for whom germline genetic testing could be more critical.

Moreover, the information regarding *DICER1* germline mutations may have a prognostic value. In this study we found that patients with *DICER1* germline mutations may be more likely to exhibit clinical relapse; one of the underlying reasons may be an increased likelihood of developing metachronous tumors. Another reason could be that heterologous elements, especially the rhabdomyosarcoma elements, are more likely to occur in patients with *DICER1* germline mutations.

Overall, germline mutations in cancer-predisposing genes have been identified in 8.5% of patients with different types of cancers [61]. However, for SLCTs the frequency of these mutations in the relevant genes, such as *DICER1*, *PMS2*, *FOXL2*, and *PALB2*, is higher and can cumulatively reach 35.3%. *PMS2* is a gene involved in DNA mismatch repair and linked to Lynch syndrome, which is described as an inherited predisposition to colorectal, uterine endometrial, and ovarian cancers [62]. *FOXL2* plays a crucial role in the ovary development and female fertility [63], and its mutations are considered potential drivers of the pathogenesis of adult-type granulosa-cell tumors [64]. Furthermore, somatic *FOXL2* mutations have been reported in some patients with SLCTs [26]. *PALB2*, which colocalizes with *BRCA2* gene in nuclear foci, has been recognized as an important component of the cellular machinery involved in homologous recombination–mediated DNA repair, and heterozygous germline mutations of *PALB2* result in an increased susceptibility to breast and pancreatic cancers [65, 66]. However, to date, there are no reports on the associations between germline *PMS2*, *FOXL2*, and *PALB2* mutations with SLCTs. The effect of each identified variation is unclear, and future studies are needed to determine the role of these germline mutations in SLCT development.

One limitation of this study is a small sample size, which was due to the rarity of the disease and expiration of the storage time for FFPE samples.

Conclusion

Our study shows that both germline and somatic *DICER1* mutations are more frequent in younger patients with SLCTs. The germline mutation testing may be clinically important for patients with SLCTs, especially for those younger than 18 years at the time of diagnosis. Moreover, regarding the clinical relapse of SLCTs, differentiating whether it is primary or recurrent is of significance.

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Disclosures

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