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DICER1-Related Sertoli-Leydig Cell Tumor and Gynandroblastoma: Clinical and Genetic Findings from the International Ovarian and Testicular Stromal Tumor Registry

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Abstract

Background—Ovarian sex cord-stromal tumors (OSCST) include juvenile granulosa cell tumors (JGCT), Sertoli-Leydig cell tumor (SLCT) and gynandroblastoma (GAB) among others. These ovarian sex cord-stromal tumors as well as other tumors including pleuropulmonary blastoma (PPB) may be associated with *DICER1* mutations. We sought to describe the clinical and genetic findings from the first 107 individuals enrolled in the International Ovarian and Testicular Stromal Tumor Registry.

Methods—Medical and family history were obtained for individuals consecutively enrolled in the International Ovarian and Testicular Stromal Tumor Registry. Pathology was centrally reviewed. *DICER1* sequencing was performed on blood and tumor tissue.

Results—Of the 107 participants, 49 had SLCT, 25 had JGCT and 5 had GAB. Nearly all (36/37) SLCTs and 4/4 GAB tested had a *DICER1* mutation in an RNase IIIb domain hotspot; approximately half of these individuals had a predisposing germline *DICER1* mutation. Metachronous SLCTs were seen in 3 individuals with germline *DICER1* mutations. Other *DICER1*-associated conditions were seen in 19% of patients with SLCT or GAB. Three children of women with SLCT were diagnosed with PPB based on genetic testing and clinical screening during the course of this study. All were diagnosed with PPB in its earliest and most curable form (Type I), were treated with surgery alone, and are alive without evidence of disease.

Conclusions—Recognition of the distinct genetic basis for a group of these tumors improves precise classification in difficult cases and promotes mutation-based screening and early detection.

Keywords

DICER1; Sertoli-Leydig cell tumor; gynandroblastoma; sex cord-stromal tumor; ovary

BACKGROUND

Ovarian sex cord-stromal tumors (OSCSTs) account for approximately 10% of all primary ovarian neoplasms during childhood and adolescence.[1] Certain morphologic types of OSCSTs, especially juvenile granulosa cell tumors (JGCTs) and Sertoli-Leydig cell tumors (SLCTs), present primarily in the first two decades of life.[2] In the original large series on JGCTs, over 40% of tumors were diagnosed in girls 10 years of age or less; some were seen in infants.[3] By contrast, SLCTs tend to occur in adolescents and young adult women.[4] Gynandroblastomas (GAB), now classified as sex cord stromal tumors of mixed forms, are composed of both JGCT and SLCT morphologic components and may be diagnosed at any age.[5] Adult granulosa cell tumors are most common in older women but may rarely occur in adolescents and are characterized by somatic *FOXL2* mutations.[6]

The International Ovarian and Testicular Stromal Tumor (OTST) Registry was established in December 2011 to develop a more complete understanding of the clinicopathologic features and genetic basis of this heterogeneous and understudied group of neoplasms. An association between SLCT and other conditions such as thyroid nodules or embryonal

rhabdomyosarcoma has been recognized in the literature since the early 1970s.[4,7] The International Pleuropulmonary Blastoma (PPB) Registry has enrolled children with PPB since its inception in 1988 and noted several probands and relatives with SLCT.[8,9] The linkage between *DICER1* and familial PPB was first reported in 2009, and since that time, many additional studies have documented the association between SLCT and *DICER1* mutations.[8,10–16]

DICER1 encodes an RNaseIII endonuclease which cleaves precursor microRNAs into active miRNA. Mutations in *DICER1* cause aberrant cleavage of mature 5p miRNAs resulting in altered expression of mRNAs with an accompanying risk for various types of neoplasms.[13,1718,19] Individuals with germline mutations in *DICER1* are also at increased risk for several benign and malignant tumors including PPB, cystic nephroma and renal sarcoma, Wilms' tumor, nodular thyroid hyperplasia and thyroid cancer, pineoblastoma and pituitary blastoma.[20–24,25–30]

PPB is the most lethal manifestation of the *DICER1* tumor predisposition syndrome and is primarily seen in infants and young children.[9] The latter tumor progresses from a multiloculated cyst (Type I) to a mixed cystic and solid (Type II) and solid (Type III) high grade multi-patterned primitive sarcoma which fills the hemithorax.[31] With pathologic progression, the survival rate diminishes from 91% in Type I to 74% in Type II to only 53% in Type III.[20] Testing and imaging surveillance of family members with *DICER1*-related disorders may allow detection of PPB in its earliest and most curable form. Likewise, most OSCST may be treated with surgery alone when found as International Federation of Gynecological Oncology (FIGO) stage Ia [T1aN0M0], thus also highlighting the importance of early detection.[2]

The aims of this study are to characterize the clinical and genetic characteristics of sequentially enrolled individuals with SLCT, JGCT and GAB. We determined the frequency of the *DICER1* mutations, and evaluated the impact of predisposing mutations on clinical presentation, outcome, familial surveillance and directed intervention.

METHODS

Study subjects

Individuals in this report were enrolled in the International OTST Registry from December 2011 to March 2016. This study was approved by the Institutional Review Board at Children's Minnesota and Children's National Medical Center. Written informed consent was provided by the patient if 18 years of age or older or by the parent or guardian of each child under 18 years of age. Eligible diagnoses include any OSCST diagnosed at any age with the exception of adult granulosa cell tumors, which were eligible only if diagnosed before age 31, or if co-occurring with a personal or family history of *DICER1*-related conditions. Participants interested in receiving results of germline *DICER1* testing underwent genetic counseling. Individuals also completed family history questionnaires. Pedigrees were reviewed when available. Age at diagnosis/recurrence was classified as the age of the patient at diagnostic surgery/surgery for recurrence. Follow-up data was requested annually. Medical records including operative and pathology reports and treatment data were

collected. All available pathology material was centrally reviewed (LPD, DAH, RHY) and classified according to the World Health Organization (WHO) classification.[4] Central pathology review was separated from genetic testing results. If tissue was not available for central pathology review, the original diagnosis was accepted. Tumors were staged according to the International Federation of Gynecological Oncology (FIGO)/TNM system for ovarian cancer.[1] Patients with neoplasms originally classified as OSCST but found to be other entities (e.g. germ cell tumors) on central review were excluded from this analysis.

Molecular analyses

DICER1 gene sequencing was performed on blood and/or saliva and tumor tissue using either Sanger sequencing or a next generation sequencing assay designed to detect base substitutions and small insertions/deletions in both coding and intron-exon junction flanking regions.[9,18,25] *DICER1* deletion testing was performed on germline DNA on a subset of individuals. *DICER1* mutations identified in blood or saliva at 38–62% variant allele frequency were considered germline. Mutations that were present in multiple tissue types/sites but at a lower variant allele frequency than typical heterozygous were classified as mosaic. Both mosaic and germline mutations were grouped as “predisposing” for statistical analysis with the assumption they were present during embryogenesis. A more detailed description of the methods is provided in Supplemental Methods.

Statistical Analyses

The Kaplan-Meier test was used to examine both time to death and time to recurrence of primary OSCST.[32] Time to additional events such as additional *DICER1*-related conditions (including metachronous ovarian tumors) was calculated separately. Tests of equality of survival distributions were calculated using Breslow and Tarone-Ware. Mood’s Median Test was used to measure differences in median age, stage and differentiation.[33] A Chi square was used to test for differences in the age distribution for individuals with and without predisposing *DICER1* mutations. Analyses were completed using SPSS V23.

RESULTS

Between December 2011 and March 2016, 107 individuals with OSCST had enrolled (Table 1). Pathologic materials from 92% (98/107) of tumors were centrally reviewed. For nine cases (5 JGCT and 4 SLCT) in which no pathologic material was available, the local pathology diagnosis was used. Central review pathologists concurred with the local diagnosis in 80 of 98 cases (82%) (Supplemental Table 1). Thirty nine of 49 (82%) individuals with stage Ia disease were treated with surgery alone; however, three of four individuals (75%) with poorly differentiated stage Ia SLCT also received adjuvant chemotherapy. Chemotherapy was generally given to individuals with JGCT or SLCT if greater than stage Ia with the exception of intraoperatively ruptured JGCT which was generally treated with surgery alone (58.3%, n=14/24). The most common chemotherapy regimen was cisplatin, etoposide and bleomycin (PEB or BEP; SLCT=12, JGCT=4, sex cord stromal tumor with annual tubules (SCTAT)=1 and sex cord stromal tumor (SCST) NOS=2). Two patients with SLCT received only cisplatin and etoposide. One patient with JGCT and one patient with SLCT received carboplatin, etoposide and bleomycin. Additional

individuals received carboplatin and paclitaxel (SLCT=3 and Sertoli cell=1). Four additional individuals (SLCT=2, GAB=1 and AGCT=1) received chemotherapy but the regimen details were unavailable. SLCT, GAB, and JGCT accounted for 79 of 107 cases (74%) Since the focus of this report is *DICER1*, individuals with SLCT, GAB, and JGCT are the focus of further detailed analyses.

Sertoli-Leydig cell tumors

Almost 50% of enrolled individuals had SLCT (n=49; 46%) with a median age at diagnosis of 17 years (range, 2–61 years) (Table 1). Hormonal symptoms were common (Supplemental Table 2). The majority of SLCTs were intermediately or poorly differentiated (Table 2). A retiform pattern was seen in 8/45 centrally reviewed tumors. Nearly half had heterologous histologic elements defined as non-Sertoli-Leydig components, most often mucinous glandular epithelium. Ten tumors had sarcomatous features. A representative example is shown in Supplemental Fig. 1E. About 50% of individuals had stage Ia. All individuals with stage Ia SLCT are presently free of tumor after a median follow-up of 19 months (range, 0–359 months). Overall, 8/49 (16.3%) individuals with SLCT had a recurrence and 4 of these with recurrent disease (50%) died of tumor progression at a median follow-up interval of 35.5 months (range, 8–74 months).

DICER1 RNase IIIb hotspot mutations were identified in 36/37 (97%) Sertoli-Leydig cell tumors sequenced (Supplemental Table 3). In these 36 patients with *DICER1*-mutated SLCTs, 22 individuals had heterozygous germline loss of function (LOF) mutations; and 3 individuals were mosaic for a *DICER1* LOF or RNase IIIb mutation. Eleven individuals had *DICER1* mutations limited to the tumor (Supplemental Table 3). Only one individual with intermediately differentiated SLCT lacked *DICER1* mutations after complete tumor and germline testing; pathologic review did not reveal anything unique about that tumor (Supplemental Figure 1). Of the three patients with well differentiated SLCT histology, two of three were not tested for *DICER1* and the third was negative for a germline *DICER1* mutation.

Gynandroblastoma

Five patients with GAB were enrolled with a median age at diagnosis of 16 years (range, 14–32 years). The SLCT component of each evaluable GAB showed intermediate differentiation. Most were stage Ia (n=4). All five individuals are alive without evidence of disease (Table 3).

Four GABs had tumor tissue available for testing and each contained a *DICER1* RNase IIIb hotspot mutation. Three of these individuals had germline LOF mutations. The fourth patient, whose tumor had an RNase IIIb mutation with an allele frequency of 62%, did not have any detectable germline *DICER1* mutations by sequencing or duplication/deletion analysis.

Juvenile granulosa cell tumors

Twenty-five individuals with JGCT were enrolled. The median age at presentation for JGCT was 9 years (range, 1 month-28 years). Most had hormonal symptoms. Seven individuals

had stage Ia; another tumor was stage Ib [T1bN0M0]. All the stage Ia/Ib patients were treated with surgery alone, and are free of disease after a median follow-up of 9 months (range, 1–77 months). Of 11 patients with tumor stage Ic [T1cN0M0] or greater, 3 (27%) died of disease at a median follow-up time of 17 months (range, 10–44 months) (Table 4). Germline DNA was analyzed in 19 individuals with JGCT; none was positive for a germline *DICER1* mutation. For two individuals with JGCT, tumor tissue was sequenced and no *DICER1* mutation was found.

DICER1 mutation analysis in other tumor types

Germline *DICER1* sequencing was performed for 24 individuals with other types of OSCST (Table 1). No *DICER1* mutations were identified in groups other than SLCT and GAB. One individual with a *DICER1* mutation had a history of undifferentiated ovarian sarcoma and later developed SLCT.

Prognostic Analyses of SLCT and GAB groups

Individuals with predisposing (germline or mosaic) *DICER1* mutations presented at younger median age than those with tumor-limited mutations (16 years, range 4–61 years) vs. (21 years, range 2–61 years) (p =0.151) (Tables 5 & 6). Eighty-two percent vs. 55% of patients with predisposing mutations were younger than 21 years of age at diagnosis (p=0.022). Overall and recurrence free survival in patients with SLCT and GAB was significantly better when disease was limited to the ovary (stage Ia). Poor differentiation was a negative prognostic factor for recurrence free survival (Table 6; Supplemental Fig. 3). Overall and recurrence-free survival was also significantly better for patients with predisposing *DICER1* mutations compared to patients with tumor-limited *DICER1* mutations (Supplemental Figures 2 and 3).

Other *DICER1* syndrome conditions in women with SLCT and GAB

Additional *DICER1* related conditions were frequently seen in our study cohort of SLCT and GAB (Table 5). Three individuals with predisposing *DICER1* mutations developed metachronous SLCT within a range of 5 to 14 years from original SLCT diagnosis. All three metachronous tumors were stage Ia and treated with surgical resection only. Eighteen women (33%) with SLCT or GAB self-reported thyroid nodules and 6 individuals (11%) reported well-differentiated thyroid carcinoma. Thyroid nodules and thyroid carcinoma were not reported in individuals without predisposing *DICER1* mutations in this cohort.

Impact on family surveillance and early diagnosis of PPB

Three infants of women with SLCT were diagnosed with PPB based on genetic testing and clinical screening during the course of this study. These infants were diagnosed with Type I PPB at ages 3, 4 and 7 months of age, were treated with surgery alone and are alive without evidence of disease at a follow-up of 33, 21 and 7 months.

DISCUSSION

This study reports the clinical outcomes, management, and *DICER1* sequencing results in a group of centrally reviewed OSCST. We found that SLCT and GAB are nearly always

DICER1-related tumors. This study confirms and extends previous reports showing *DICER1* mutations in association with SLCT. Slade et al. showed germline *DICER1* mutations in 4/6 SLCTs. Witkowski et al. found somatic mutations in the RNase IIIb domain in 8/15 SLCTs and Heravi-Moussavi et al reported somatic *DICER1* mutations in the RNase IIIb domain of 29% of nonepithelial ovarian tumors including 26 of 43 SLCTs. This study includes both germline and tumor testing along with central review, the latter of which removed some non-SLCT tumors from the study population (see Supplemental Table 1). The combination of these factors may be responsible for a stronger association between SLCT and *DICER1* than was noted in previous reports.[11,15,34–36] None of the individuals with a centrally reviewed diagnosis of JGCT, steroid cell tumor, sex cord-stromal tumor with annual tubules or Sertoli cell tumor had germline *DICER1* mutations, although one individual with a germline *DICER1* mutation developed undifferentiated ovarian sarcoma and later SLCT.[37]

Based on the importance of identifying *DICER1* pathogenic variants in an individual, as well as the moderate rate of discordance (18%) between local and central review (which is typical of rare tumors), we recommend careful and, when feasible, central histopathologic review for all individuals with OSCST tumors with *DICER1* testing for all individuals with SLCT and GAB and consideration of *DICER1* testing in individuals with other OSCST if the medical or family history suggests *DICER1*-tumor predisposition (e.g. thyroid nodules).

In our study, 97% of SLCT are *DICER1*-related which is significantly higher than other series. One potential reason for this difference is that we sequenced both germline and tumor DNA. The vast majority of *DICER1* syndrome cancers including pleuropulmonary blastoma, SLCT and rhabdomyosarcoma have biallelic *DICER1* mutations consisting of a loss of function mutation in one allele and a missense RNase IIIb domain mutation in the other allele. Our previous study of SLCT only had germline testing data available; in that data approximately half of individuals with SLCT had germline *DICER1* mutations, however, tumor tissue was not sequenced. The current study combines germline and tumor testing for *DICER1* and when that testing is combined, nearly all the SLCTs are linked to *DICER1* mutations. Several SLCTs had biallelic DICER1 mutations limited to tumor tissue, which may represent mutations confined to the tumor or very low level mosaicism. A negative germline result does not indicate that the tumor is *DICER1*-unrelated.

Also, in this study, histologic classification included central review which removed some non-SLCT/GAB tumors from the SLCT/GAB cohorts and thus may have contributed to a higher concordance of pathologic to genetic findings. SLCT, GAB and JGCT are uncommon tumors with a broad range of morphologic appearances. Two individuals with *DICER1* mutations were diagnosed with JGCT at the treating institution but after central review reclassified as GAB. For difficult to classify cases, *DICER1* mutation testing of tumor tissue may be useful.

As in previous reports, the outcome for individuals with SLCT was strongly correlated with stage and level of differentiation.[4,38] Individuals with predisposing *DICER1* mutations had significantly better overall and recurrence free survival although additional *DICER1*-related conditions were frequently noted. Metachronous SLCTs were seen in 3 individuals with predisposing *DICER1* mutations up to 14 years after initial diagnosis and associated with a

favorable prognosis. Thus, the finding of a contralateral ovarian mass in an individual with *DICER1* mutation and a previously diagnosed ovarian tumor cannot be assumed to be a recurrence. This consideration has importance for therapeutic planning as a metachronous tumor may be more sensitive to first-line chemotherapy than a true tumor recurrence. Individuals with germline mutations or mosaicism should be followed for the emergence of metachronous disease even after the highest risk period for recurrence has passed.

Identifying a germline *DICER1* mutation or mosaicism in a young woman has considerable implications for the individual and her family.[39] Post treatment surveillance regimens must consider the possibility of underlying tumor predisposition. In addition to the risk for contralateral ovarian tumors, individuals with germline *DICER1* mutations are also at risk for other conditions including nodular thyroid hyperplasia or carcinoma.[24,40] Identification of germline *DICER1* mutations may be especially critical for young children in the family who may be at risk for PPB and other conditions. Early diagnosis improves morbidity and mortality in PPB and may also be relevant for minimizing complications of other *DICER1*-related tumors including cystic nephroma, Wilms tumor, ovarian tumors and certain childhood brain tumors.

In individuals with no detectable germline mutation, there may be a role for tumor specific testing for biallelic mutations in *DICER1*. Conventional treatment algorithms for SLCT empirically use platinum-based chemotherapy similar to the management of epithelial and germ cell tumors; however, a more directed treatment design may be beneficial, particularly in the case of tumors with sarcomatous elements. In contrast to most individuals with germ cell tumors, those with SLCT and GAB lack a reliable blood-based tumor marker. Imaging studies are limited by lack of sensitivity. The nearly universal presence of *DICER1* RNase IIIb mutations could potentially be useful as circulating tumor DNA (ctDNA) biomarkers for monitoring of response to therapy and post treatment surveillance.

Limitations

Tissue for central pathology review was not available for 9/107 individuals. In some cases, although slides were available, sufficient tissue for molecular analysis was not and thus only germline DNA results are available for some individuals. Although this represents the largest series to date, the rarity and heterogeneous clinical presentation of this tumor limits the power of multiple variable analyses.

CONCLUSIONS

Nearly all SLCTs and GAB are *DICER1*-related. No other centrally reviewed OSCST types were found to harbor germline or somatic *DICER1* mutations. These results clearly demonstrate the distinct nature of the pathophysiology of SLCT and GAB when compared to germ cell tumors, epithelial ovarian tumors, and even other OSCST tumors. Recognition of SLCT and GAB as a unique and well-defined molecular group may pave the way for precise diagnosis, rational development of therapies and opportunities to implement mutation-based screening in patients and their young families.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- *DICER1* RNase IIIb mutations were identified in 36/37 SLCT's and 4/4 GABs sequenced.
- Germline or mosaic mutations were found in more than half of those with SLCT.
- Predisposing *DICER1* mutations were associated with higher recurrence free survival.
- *DICER1* testing in women with SLCT facilitated screening of their children for PPB.
- Three children were diagnosed with PPB in its earliest and most curable form.

Table 1

Demographics and outcome for all enrolled individuals (n=107) with ovarian sex cord- stromal tumors.

N=107	SLCT	JGCT	GAB	Sex Cord tumor with annular tubules	Sertoli cell tumor	Steroid cell tumor	AGCT	Sclerosing stromal tumor	Sex Cord tumor, NOS
Patients (n)	49 (46%)	25 (23%)	5 (5%)	5 (5%)	5 (5%)	2 (2%)	10 (9%)	4 (4%)	2 (2%)
Caucasian (%)	37 (76%)	10 (40%)	4 (80%)	4 (80%)	5 (100%)	0	6 (60%)	2 (50%)	1 (50%)
Age @ Diagnosis, Median (years), range	17 (2– 61)	9 (<1– 28)	16 (14– 32)	12 (3– 50)	44 (33– 52)	38 (17– 58)	37 (13– 49)	14 (10– 22)	12 (10– 15)
Median Time from Diagnosis (months), range	32 (0– 624)	15 (0– 415)	11 (3– 117)	23 (0– 47)	33 (18– 68)	11 (1— 20)	46 (3– 203)	35 (1–76)	7.5 (1– 14)
Status									
NED	40 (82%)	21 (84%)	5 (100%)	5 (100%)	5 (100%)	2 (100%)	8 (80%)	4 (100%)	1 (50%)
In TRT	2 (4%)	0	0	0	0	0	0	0	1 (50%)
In Recur TRT	2 (4%)	1 (4%)	0	0	0	0	2 (20%)	0	0
In Additional TRT	1 (2%)	0	0	0	0	0	0	0	0
Death	4 (8%)	3 (12%)	0	0	0	0	0	0	0
Current Age, Median (years), range	26 (4–68)	14 (2–54)	23 (16–35)	20 (7– 53)	48 (36–57)	40 (18–62)	40 (17–61)	18 (13–30)	15 (11–19)
Germline *DICER1* mutation (n=89)	25/41	0/19	3/5	0/5	0/5	0/1	0/9	0/3	0/1
Tumor *DICER1* mutation (n=48)	36/37	0/2	4/4	0/0	0/1	0/1	0/1	0/0	0/0

SLCT= Sertoli-Leydig cell tumor, JGCT= juvenile granulosa cell tumor, GAB= gynandroblastoma, AGCT= adult granulosa cell tumor, NOS= not otherwise specified. NED= no evidence of disease, TRT = treatment, Recur= recurrence

Table 2

Stage, level of differentiation, treatment and outcome for 49 individuals with SLCT.

Stage	Differentiation	Adjuvant Chemo	Surgery Only	NED	Metachronous	Relapse	DOD
IA	Well (n=2)	0	2	2	0	0	0
[T1aN0M0] (n=27)	Intermediate (n=20)	3	17	20	2	0	0
	Poor (n=4)	3	1	4	1	0	0
	Unknown (n=1)	1	0	1	0	0	0
IC [T1cN0M0] (n=6)	Intermediate (n=1)	0	1	0	0	1	1
	Poor (n=5)	2	3	2	0	3	1
IC1 [T1C1N0M0] (n=6)	Intermediate (n=2)	2	0	2	0	0	0
	Poor (n=3)	1	2	2	0	1	1
	Unknown (n=1)	1	0	1	0	0	0
>IC [T1cN0M0] (n=4)	Intermediate (n=2)	2	0	2	0	0	0
	Poor (n=2)	2	0	0	0	1	0
Unknown (n=6)	Well (n=1)	0	1	1	0	0	0
	Intermediate (n=1)	0	1	1	0	0	0
	Unknown (n=4)	1	3	2	0	2	1
All stages (n=49)	Well (n=3)	0	3	3	0	0	0
	Intermediate (n=26)	7	19	25	2	1	1
	Poor (n=14)	8	6	8	1	5	2
	Unknown (n=6)	3	3	4	0	2	1

FIGO 1a: limited to one ovary, capsule intact, negative washings; 1c1: surgical spill. SLCT= Sertoli-Leydig cell tumor, NED= no evidence of disease, DOD= died of disease, Chemo= chemotherapy, NOS= not otherwise specified

Table 3

Stage, treatment and outcome characteristics of five individuals with gynandroblastoma.

Stage	Differentiation	Adjuvant Chemo	Surgery Only	NED	Relapse	DOD
IA [T1aN0M0] (n=4)	Intermediate	1	3	4	0	0
IIC [T2cN0M0] (n=1)	Intermediate	1	0	1	0	0
All stages (n=5)	Intermediate	2	3	5	0	0

FIGO 1a: limited to one ovary, capsule intact, negative washings; IIb Chemo= chemotherapy, NED= no evidence of disease, DOD =died of disease

Table 4

Stage, treatment and outcome characteristics of 25 individuals with JGCT.

Stage	Adjuvant Chemo	Surgery Only	NED	Relapse	DOD
IA [T1aN0M0] (n=7)	0	7	7	0	0
IB [T1bN0M0] (n=1)	0	1	1	0	0
IC [T1CN0M0] (n=4)	1	3	2	2	1
IC2[T1C2N0M0] (n=4)	2	2	4	0	0
>Ic [T1CN0M0] (n=3)	2	1	1	2	2
Unknown (n=6)	0	6	6	1	0
All Stages (n=25)	5	20	21	5	3

Abbreviations: JGCT= juvenile granulosa cell tumor, NED= no evidence of disease, DOD= died of disease, Chemo= chemotherapy

Table 5

Clinical and pathologic characteristics by predisposing *DICER1* mutation category

	Germline Loss of Function	Mosaic		Tumor-limited mutations	Germline & Tumor Negative	Incompletely Tested*
		Loss of function	RNase IIIb hotspot			
# of Patients (n=54)	25	2	1	12	1	13
Age @Diagnosis (year), Median (range)	16 (4–61)	21 (4–27)	5	21 (2–61)	37	23 (4–54)
Diagnosis						
SLCT (n=49)	22#	2	1	11	1	12
GAB (n=5)	3	0	0	1	0	1
Additional disease foci						
PPB	2	0	1	0	0	0
Cystic Nephroma	2	0	1	0	0	0
Thyroid Nodules	14	2	1	0	0	1
Thyroid Cancer	4	2	0	0	0	0
ERMS	1	0	0	0	0	0
NCMH	1	0	0	0	0	0
Intestinal polyps	1	0	0	0	0	0
Metachronous SLCT	3	0	0	0	0	0
Outcome (months) Diagnosis to Follow up						
# Patients NED	24	2	0	9	1	9
# Patients AWD	0	0	1	3	0	2
# Patients Recur	2	0	0	5	0	1
# Patients alive (Median, months)	24 (61)	2 (22)	1 (82)	10 (12)	1 (2)	12 (12)
# Patients deceased (Median, months)	1& (57)	0	0	2 (14)	0	1 (8)

= 1 patient also developed an undifferentiated ovarian sarcoma, NOS

& = *DICER1* mutation by inference, patient not directly tested but patient's sister tested positive for *DICER1* following patient's death

* = 6 germline negative, tumor not tested

Abbreviations: SLCT= Sertoli-Leydig cell tumor, GAB= gynandroblastoma, PPB= pleuropulmonary blastoma, NED= no evidence of disease, AWD= alive with disease, Recur= recurrence, ERMS= embryonal rhabdomyosarcoma, NCMH= nasal chondromesenchymal hamartoma

Table 6

Sertoli-Leydig cell tumor and Gynandroblastoma: Prognostic factors

	DICER1		Stage		Differentiation	
	Predisposing mutation	**Tumor- specific mutation**	**Ia/T1aN0M0**	**>Ia /T1a- N0-M0**	**Poorly**	**Well & Intermediate**
Overall Survival %	96.4%	81.8%	100%	82.4%	85.7%	97.1%
p-value	0.020*		0.038*		0.262	
Recurrence Free Survival %	92.9%	72.7%	100%	70.6%	71.4%	97.1%
p-value	0.050*		0.007*		0.039*	
DICER1 (predisposing vs. tumor-specific)	----------		55%	76%	75%	55%
p- value			0.252		0.263	
Age Median, years (Range)	16 (4–61)	21 (2–61)	16 (2–40)	16 (4–61)	19 (4–61)	16 (2–40)
p-value	0.151		0.860		0.490	