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Society Position Statement/White Paper

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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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.

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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.

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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.

1. 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 [13,17] [18,19]. 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 multipatterned 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.

2. Methods

2.1. 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.

2.2. 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.

2.3. 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.

3. 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

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