LETTER TO THE EDITOR
DICER1 Mutations in an Adolescent with Cervical Embryonal Rhabdomyosarcoma (cERMS)

To the Editor: Embryonal rhabdomyosarcomas of the cervix (cERMS) ("cervical sarcoma botryoides") are rare embryonal tumors, usually diagnosed in childhood or adolescence, with a world literature of uterine/cervical ERMS limited to 115 cases [1]. The association of cERMS with germline *DICER1* mutations was first established in three families by Foulkes in 2011, and subsequently reported by Dehner et al., although the coexistence of cERMS and ovarian Sertoli-Leydig cell tumors (OSLCT) had previously been recognized [2–4]. In 2012, Doros et al. [5] reported germline *DICER1* mutations in 2/52 (3.8%) sporadic ERMS, including 7 uterine/vaginal/pelvic cases.

Characteristic somatic *DICER1* mutations affecting the RNase IIIb domain have been identified in 60% of OSLCT, including tumors with additional germline *DICER1* mutations [6]. These "hotspot" somatic mutations, in codons encoding metal binding sites, have also been reported in individuals with germline *DICER1* mutations and WT and PPB [7,8]. To date, only one previous individual with cERMS has been demonstrated to carry both a germline and somatic *DICER1* mutation [6].

We report an adolescent with cERMS that harbors two *DICER1* mutations. A 13-year old female of French Canadian descent

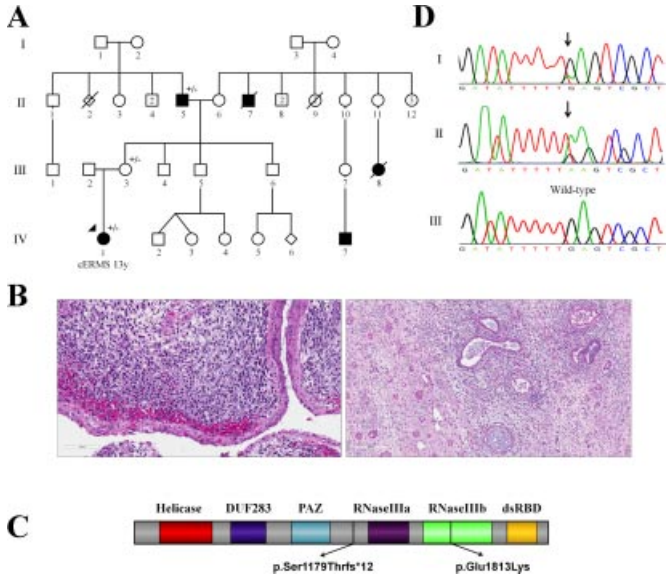

Fig. 1. **A**: Pedigree of the family studied: Individuals positive for a germ-line *DICER1* mutation (indicated with +/−) include the proband (Individual IV-1), diagnosed with embryonal rhabdomyosarcoma of the cervix (cERMS) at 13 years of age, the proband's mother (Individual III-3) and the proband's maternal grandfather (Individual II-5). Individual III-3 and individual III-4 underwent thyroidectomies for thyroid cysts and unknown reasons respectively. In addition, individual II-5 was diagnosed with prostate cancer at the age of 70 years, individual II-7 (deceased) had a cancer of which the primary source remains unknown, individual III-8 was diagnosed with carcinoma of the breast at 45 years of age, and individual IV-7 has leukemia. Furthermore, individuals II-2 died in early infancy ("large babies") and individual III-1 presented with hydrocephalus in childhood. **B**: I: HPS, 20X: The presence of atypia and hyperchromasia of the stroma that has condensed under the surface epithelium ("cambium layer") is evident. II: HPS, 10X: A different highpower field, towards the center of the fragment, showing stromal condensation around glands, a characteristic feature of cERMS, and around a well-circumscribed nodule of mature cartilage (bottom center), which is also well described in cERMS. **C**: Graphic representation of the DICER1 protein structure indicating the approximate positions of the p.Ser1179Thrfs*12 (germ-line) and p.Glu1813Lys (somatic) amino acid changes. **D**: Chromatogram showing the somatic (c.5437G>A) mutation in tumor gDNA (panel I). The expression of the mutation was confirmed with the sequencing of cDNA synthesized from tumor RNA, which too, possesses the c.5437G>A somatic mutation (panel II). The wild-type sequence is illustrated in panel III.

DICER1 is an RNase III enzyme which cleaves microRNA (miRNA) precursors into mature non-coding miRNAs, which regulate mRNA expression. *DICER1* germline mutations are associated with a pleiotropic cancer predisposition syndrome which also includes predisposition to pleuropulmonary blastoma (PPB), cystic nephroma, OSLCT, multinodular goiter, and Wilms tumor (WT) [2].

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presented with abnormal bleeding and vaginal polypoid mass. Family history included reported thyroidectomies in her mother and maternal uncle (Fig. 1A). Pathology of the polyp (Fig. 1B) demonstrated characteristic features of cERMS including condensation of hyperchromatic stromal cells beneath surface epithelium ("cambium layer") as well as around deeper glands. In addition, distinctive stromal cartilage nodules were seen. Chest computed tomography (CT) revealed a 1.9 cm cystic lesion in the right upper lobe consistent with a type Ir PPB. A deleterious germline mutation, c.3535_3538delTCTT, was observed in exon 22 of *DICER1* (Ambry Genetics, Aliso Viejo, CA). This mutation causes a translational frameshift, p.Ser1179Thrfs*12, with a predicted alternate stop codon that truncates the protein subsequent to the PAZ domain, and results in the loss of the RNase IIIa, RNase IIIb and double-stranded RNA-binding (dsRBD) domains (Fig. 1C). We subsequently identified an acquired somatic mutation, c.5437G>A, predicted to result in p.Glu1813Lys at the protein level, in the formalin-fixed paraffin-embedded (FFPE) cERMS (Fig. 1D, Panel I). Sequencing of cDNA synthesized from tumor RNA confirmed the expression of this mutation (Fig. 1D, Panel II). Following surgical resection, and 1-year following completion of COG low risk rhabdomyosarcoma protocol ARST0331 (vincristine, actinomycin D, cyclophosphamide), the patient remains cancer free.

Our case adds to the growing literature suggesting the importance of specific somatic hotspot mutations as key mutational events in tumors with both germline or somatic *DICER1* loss of function mutations [9]. Screening for a germline *DICER1* mutation should be considered in all individuals presenting with cERMS, even in apparently "sporadic" cases, as the low penetrance associated with *DICER1* germline mutations (indicated in our family) may result in an unremarkable family history.

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