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Testicular Sertoli cell tumor and potentially testicular Leydig cell tumor are features of *DICER1* syndrome

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Conflicts of interest

The authors declare no conflict of interest.

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Abstract

DICER1 syndrome is a rare pediatric autosomal dominant inherited disorder predisposing to various benign and malignant tumors. It is caused by a germline pathogenic variant in *DICER1* and the second hit for tumor development is usually a missense hotspot pathogenic variant in the DICER1 ribonuclease IIIb domain. While *DICER1* predisposing variants account for about 60% of ovarian Sertoli-Leydig cell tumors, no *DICER1*-related testicular stromal tumors have been described. Here we report the first two cases of testicular stromal tumors in children carrying a *DICER1* germline pathogenic variant: a case of Sertoli cell tumor and a case of Leydig cell tumor diagnosed at 2 and 12 years of age, respectively. A somatic *DICER1* hotspot pathogenic variant was detected in the Sertoli cell tumor. This report extends the spectrum of *DICER1*-related tumors to include testicular Sertoli cell tumor and potentially testicular Leydig cell tumor. Diagnosis of a testicular Sertoli cell tumor should prompt *DICER1* genetic testing so that patients with a *DICER1* germline pathogenic variant can benefit from established surveillance guidelines. *DICER1* genetic evaluation may be considered for testicular Leydig cell tumor. Our findings suggest that miRNA dysregulation underlies the etiology of some testicular stromal tumors.

Keywords

DICER1; Sertoli cell tumor of the testis; Leydig cell tumor of the testis; testicular stromal tumor

Introduction

DICER1 syndrome is a rare autosomal dominant inherited disorder predisposing to a variety of benign and malignant tumors including pleuropulmonary blastoma (PPB), cystic nephroma, non-epithelial ovarian tumors, multinodular goiter or thyroid cancer, and certain brain tumors such as pineoblastoma and pituitary blastoma. Tumors arise mainly during childhood and adolescence, with a moderate age- and sex-dependent penetrance[1]. It is caused by *DICER1* germline monoallelic pathogenic variant resulting in loss-of-function of the DICER1 protein, a ribonuclease that plays a major role in microRNA maturation. The somatic *DICER1* second hit that leads to tumor development is usually a missense hotspot pathogenic variant in amino acids 1705, 1709, 1809, 1810 or 1813, resulting in partial loss-of-function, disrupting the ribonuclease IIIb activity of the protein[2].

Non-epithelial ovarian tumors have been widely described in the *DICER1* syndrome, mostly ovarian Sertoli-Leydig cell tumors that are associated with a *DICER1* predisposing pathogenic variant in nearly 60% of the cases[3]. However, several studies conducted on testicular germ cell tumors failed to detect any established *DICER1* pathogenic variant, although one tumor did harbor a non-hotspot variant of uncertain significance in codon 1725[4–6]. Other studies identified a germline *DICER1* pathogenic variant in a man with a

history of seminoma and three somatic *DICER1* hotspot variants: one non-seminoma, one yolk-sac tumor and one mixed germ cell tumor[7–9].

Here we report the first two cases of testicular stromal tumors in individuals with germline *DICER1* pathogenic variants, extending the spectrum of *DICER1*-related tumors to testicular Sertoli cell tumors and potentially testicular Leydig tumors.

Case 1

This was a child diagnosed with a testicular Sertoli cell tumor and a PPB at 2 and 4 years, respectively (Figure 1). At 2 years old, the child was referred to the Montpellier University Hospital, France, for a right testicular mass treated by total unilateral orchiectomy. Pathology examination allowed the diagnosis of Sertoli cell tumor of the testis. At 4 years old, the child developed cough, fever, shortness of breath and weakness. A chest X-ray revealed near-complete opacification of left hemithorax associated with mediastinal shift. An enhanced chest computed tomography scan showed a heterogeneous enhancing mass measuring 14×10×8mm and a cystic lesion suggesting diagnosis of type II PPB, which was confirmed by pathology examination. Treatment was delivered according to the European PPB strategy: pre-operative chemotherapy regimen was four courses of IVADo (ifosfamide, vincristine, actinomycin D, doxorubicin). A left thoracotomy was performed with resection of a large cystic-solid tumor measuring 16×9×3cm. The resection margins were free of tumor but residual viable tumor tissue was observed. He completed five courses of VP16 plus carboplatin chemotherapy successfully after surgery. No radiotherapy was performed. The patient remains in remission at his last follow-up 24 months later.

The child and his relatives had a genetic consultation after diagnosis of PPB. *DICER1* genetic testing was performed at the Institut Curie, Paris, after patients signed a consent form for this analysis, or parent(s) signed for their children. *DICER1* gene analysis in the child showed a monoallelic germline *DICER1* c.4206+2dup variant in intron 22 that was likely to impact splicing by prediction tools. Subsequent mRNA analysis confirmed a pathogenic defect: this variant lead to in-frame exon 22 skipping resulting in loss of 52 amino acids in ribonuclease IIIa functional domain and an additional defect consisting in an out-of-frame skipping of exons 22 and 23 resulting in a premature termination codon. This genetic testing combining DNA and mRNA analysis confirmed the diagnosis of a *DICER1* pathogenic variant. *DICER1* gene analysis on Sertoli cell tumor sample identified the *DICER1* c.5428G>T, p.(Asp1810Tyr) hotspot pathogenic variant in the ribonuclease IIIb domain.

Genetic testing in the child's relatives showed the presence of the *DICER1* pathogenic variant in his mother affected with thyroid nodules, his maternal uncle who underwent thyroidectomy for multinodular goiter at the age of 10 years, and his unaffected 8-year old brother. His maternal grandmother diagnosed with ovarian cyst at the age 37 years was negative for the *DICER1* pathogenic variant. No genetic testing was performed on his maternal grandfather who was diagnosed with thyroid cancer at the age 42 years.

Case 2

This patient was recruited to the National Institutes of Health (NIH) Clinical Center for a comprehensive outpatient evaluation as part of the IRB-approved "*DICER1*-related Pleuropulmonary Blastoma Cancer Predisposition Syndrome: A Natural History Study" (National Cancer Institute [NCI] Protocol 11-C-0034; NCT01247597) after identification of the *DICER1* c.2650+1G>T pathogenic variant in his brother. All male subjects evaluated at the Clinical Center were offered a single standard scrotal ultrasound and all imaging was centrally reviewed by a single radiologist. There were 110 males evaluated at the Clinical Center through July 2020, including 61 male *DICER1*-carriers and 49 male controls. If a finding on the initial ultrasound warrants additional follow up, participants are instructed to seek further care through their local medical provider.

This is a now 12-year-old male with a germline *DICER1* c.2650+1G>T pathogenic variant who initially presented at 6 years and 8 months to the NIH Clinical Center for evaluation. His past medical history was unremarkable without any clinically apparent consequences from the germline *DICER1* pathogenic variant, with the exception of macrocephaly. Review of his scrotal ultrasound showed a 2x1mm minimally vascular echogenic mass identified in the mid-upper left testis (Figure 2). The lesion was asymptomatic and not palpable. Approximately six years after his initial evaluation, the patient had a repeat scrotal ultrasound that showed the left testicular lesion was now 6x5x6mm in size, with increased peripheral echogenicity and tiny calcifications. It remained asymptomatic. Given the interval increase in size of the mass, the patient underwent a testis-sparing surgery. Pathology examination allowed the diagnosis of Leydig cell tumor of the testis. The patient remains in complete remission nine months after surgery.

Clinical tumor sequencing with a comprehensive 124-cancer gene panel (Solid Tumor Mutation Panel; Texas Children's Hospital) was performed on DNA (450ng) extracted from multiple slides that contained about 80% tumor cells. Tumor DNA sequencing revealed the known germline *DICER1* c.2650+1G>T pathogenic variant at 45% allele frequency and no pathogenic variant in other genes. No somatic *DICER1* variation was detected in the tumor sample, including at the five known *DICER1* hotspot codons, despite greater than 1250X coverage for those bases.

Several relatives carried the germline *DICER1* c.2650+1G>T pathogenic variant in the family: the brother diagnosed with type I PPB and multinodular goiter at one and three years, respectively, the mother diagnosed with multinodular goiter at 14 years, the maternal grandfather with thyroid cysts at 57 years, a mother's half-sister (by father) with multinodular goiter, and a half-sister's son with thyroid cysts at 17 years. A maternal uncle diagnosed with teratocarcinoma of the testis at 31 years did not carry the *DICER1* variant.

Discussion

Germline *DICER1* pathogenic variants detected in a child diagnosed with Sertoli cell tumor of the testis and PPB and a child diagnosed with Leydig cell tumor of the testis reveal

that testicular Sertoli cell tumors and potentially Leydig cell tumors are part of *DICER1* syndrome tumor spectrum.

The *DICER1* c.4026+2dup variant identified in case 1 is a novel variant affecting splicing, as confirmed by mRNA analysis. This variant was considered as pathogenic according to ACMG-AMP guidelines[10]. There was a cosegregation between this germline variant and numerous *DICER1*-related lesions in the family as it was identified in this child diagnosed with PPB (the hallmark tumor of the *DICER1* syndrome), and his mother and maternal uncle, who were diagnosed with thyroid nodules or multinodular goiter, respectively. Early-onset of multinodular goiter, especially in males, is highly evocative of *DICER1* syndrome[11]. The maternal grandfather diagnosed with a thyroid cancer was likely to harbor the *DICER1* pathogenic variant, which was also suggested by the absence of the variant in the maternal grandmother. The presence of the *DICER1* pathogenic variant in the unaffected 8-year old brother may be explained by the moderate age- and sex-dependent penetrance of *DICER1* pathogenic variant for neoplasms, estimated to be about 5% at 10 years of age[1].

Sertoli cell tumors account for 3% of primary prepubertal testis tumors[14]. Association of testicular cysts with this tumor type is common[15]. Inhibin A has been demonstrated as a sensitive marker for Sertoli cell tumors. These tumors also usually express vimentin. Cytokeratin AE1/AE3 could be expressed and does not exclude the diagnosis. Overall, these pathologic features were consistent with the diagnosis of Sertoli cell tumor for case 1. The child was tested negative for *STK11*, the gene responsible for Peutz-Jeghers syndrome that is associated with skin lesions and several neoplasms including testicular stromal tumors[12]. There was no genetic testing for Carney complex, a rare inherited syndrome that is associated with skin lesions, endocrine tumors and several neoplasms including testicular stromal tumors[13]. However, the patient had no clinical manifestations suggesting Carney complex. The *DICER1* c.5428G>T, p.(Asp1810Tyr) hotspot pathogenic variant detected in Sertoli cell tumor has been previously reported in several *DICER1*-related lesions including ovarian Sertoli-Leydig tumors and multinodular goiter[7,16]. The identification of a *DICER1* hotspot pathogenic variant as a second hit in testicular Sertoli cell tumor is evidence that dysregulation of DICER1 is involved with the pathogenesis of this tumor[17].

The *DICER1* c.2650+1G>T variant identified in case 2 has been previously identified in a patient diagnosed with PPB and reported as pathogenic in ClinVar[17,18]. This variant is predicted to abolish splice donor site, resulting in out-of-frame exon 16 skipping and premature termination codon. There was a cosegregation between this germline variant and *DICER1*-related lesions in the family: the child's brother with type I PPB and multinodular goiter, and several relatives with multinodular goiter or thyroid cysts. The uncle with testicular teratocarcinoma did not carry the *DICER1* variant but this tumor type has not been associated with *DICER1* syndrome. This variant was considered as pathogenic according to ACMG-AMP guidelines[10].

Leydig cell tumors account for 4% of primary prepubertal testis tumors[14]. No other pathogenic variant has been identified in the Leydig cell tumor DNA analysis from a comprehensive 124-cancer gene panel including *STK11* and *PRKAR1A* genes, involved in Peutz-Jeghers syndrome and Carney complex, respectively[12,13]. Case 2 had a comprehensive

screening that showed no skin lesion and no personal history of endocrine disorders which could have suggested these syndromes. The tumor was tested negative for the five *DICER1* hotspot codons and a sixth codon (D1713) that has been recently proposed as a *DICER1* hotspot pathogenic variant location[2]. Rare cases of *DICER1* loss of heterozygosity (LOH) have been described as a second inactivation hit in *DICER1*-related tumors, mainly in pineoblastoma[2]; this is unlikely to have occurred in this tumor given the 45% germline pathogenic variant allele frequency in tumor analysis and the 80% tumor cellularity. An undetected *DICER1* genetic alteration or *DICER1* haploinsufficiency may be the causal factor for case 2 testicular Leydig cell tumor development. This observation of testicular Leydig cell tumor in a *DICER1* germline pathogenic variant carrier could also be a coincidental finding but rarity of these tumors and the well-documented association of *DICER1* pathogenic variants with ovarian Sertoli-Leydig tumors suggest that *DICER1* genetic alteration was the driver event for Leydig cell tumor development. Further studies on testicular Leydig cell tumors are needed to confirm *DICER1* alteration as the causal factor.

A *DICER1* germline pathogenic variant is associated with multiple benign and malignant early-onset lesions and it is therefore recommended to start clinical and imaging surveillance at birth[19]. If testicular Sertoli cell tumor had been known as a *DICER1*-related tumor, the *DICER1* surveillance recommendations may have allowed for this child an earlier diagnosis of PPB, with a better OS and less aggressive chemotherapy[20].

These novel observations have direct clinical relevance and offer insight into tumor pathogenesis. Diagnosis of a variety of tumors, including PPB and ovarian Sertoli-Leydig tumors, are recommended indication criteria for *DICER1* genetic testing[18]. Diagnosis of a testicular Sertoli cell tumor should prompt *DICER1* genetic testing so that patients with a *DICER1* germline pathogenic variant can benefit from established surveillance guidelines and earlier diagnosis of *DICER1*-related tumors. *DICER1* genetic evaluation may be considered for testicular Leydig cell tumor. Our findings suggest that miRNA dysregulation underlies the etiology of some testicular stromal tumors.

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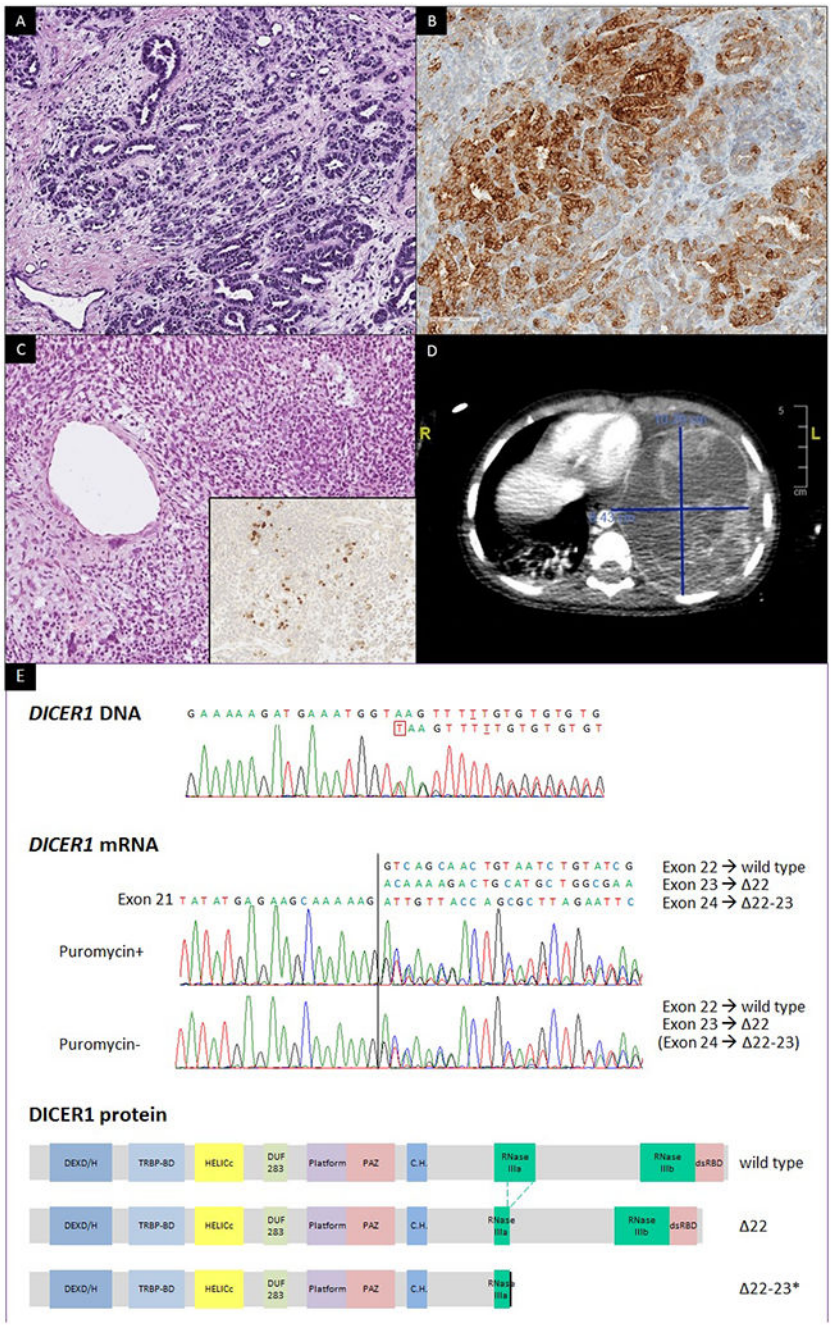

Figure 1.

Case 1, testicular Sertoli cell tumor and pleuropulmonary blastoma images, and *DICER1* genetic analysis. Testicular Sertoli cell tumor pathology: (A) Hematoxylin and Eosin staining (200x magnification), showing well-differentiated component with tubules surrounded by basement membrane and a central lumen. Pathology examination revealed a biphasic proliferation consisting of small undifferentiated stromal hyperchromatic cells and tubular epithelial structures with hyperchromatic cubic lining. On the periphery were images of cystic distention and a thin band of testicular tissue composed of immature

seminiferous tubules; (B) Inhibin reactivity, 200x magnification. Immunohistochemistry (IHC) of the tumor cells was positive for inhibin, vimentin, CD99, WT1 and cytokeratin AE1/AE3, and negative for CEA and cytokeratin 5/6. (C) Pleuropulmonary blastoma (PPB) pathology: Hematoxylin and Eosin staining (200x magnification), showing round and spindle cell undifferentiated proliferation with marked atypia and edematous background. *Bottom right insert*: myogenin reactivity, 200x magnification. IHC of the tumor cells was positive for myogenin, desmin, vimentin and CD99, and negative for CK-AE1/AE3, EMA, synaptophysin, chromogranin, TTFF1, S-100 and SMA. The proliferative index evaluated by an anti-Ki67 antibody was 80%. (D) PPB image: chest computed tomography scan, showing a heterogeneous enhancing mass in the left hemithorax associated with pleural nodules and moderate pleural effusion and a cystic lesion in the left upper lung with air-fluid levels. (E) *DICER1* genetic analysis. *Top*: electropherogram of DNA analysis showing the *DICER1* heterozygous pathogenic variant c.4206+2dup (in a square) and the *DICER1* homozygous common single nucleotide polymorphism c.4206+9G>T, rs1778057 (underlined). *Middle*: electropherograms of mRNA analysis from patient-derived lymphoblastoid cell lines that were grown with (puromycin+) or without puromycin (puromycin-), an inhibitor of nonsense-mediated mRNA decay (NMD). Δ symbol represents exon skipping. Sequences of the three transcripts are described, and Δ22-23 transcript is written in brackets for electropherogram without puromycin as this transcript leading to a premature termination codon undergoes NMD, hence the fluorescence signal of its sequence is close to background noise after culture without puromycin. *Bottom*: schematic representation of predicted effect on DICER1 protein, a loss of 52 amino acids in RNase IIIa domain for Δ22 transcript, and a truncated protein for Δ22-23 transcript with a black line that represents the frameshift with six different amino acids at the end of the truncated protein. * symbol for Δ22-23 protein is used as this truncated protein is expected to be present at a very low level because of NMD that leads to Δ22-23 transcript degradation.

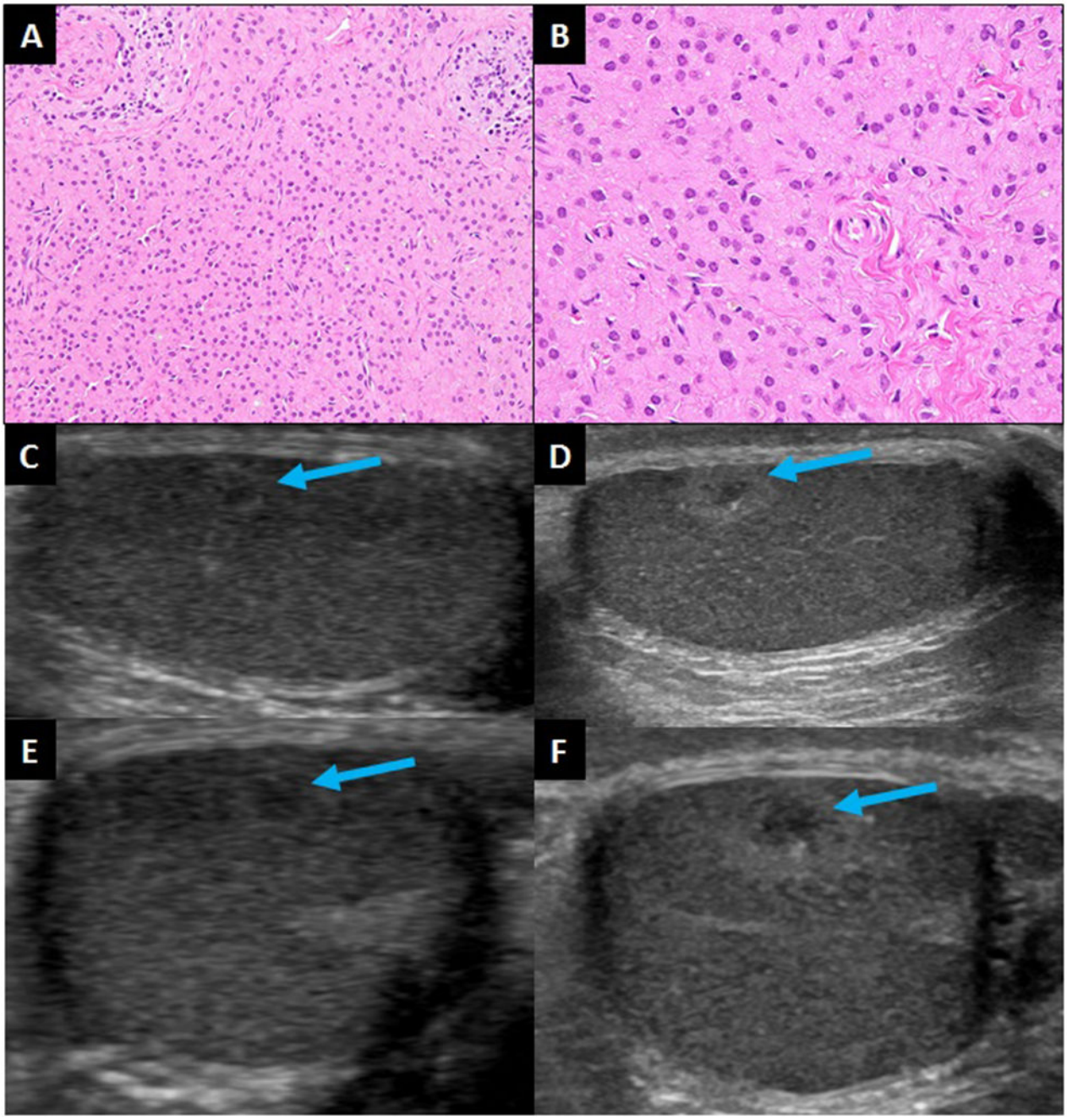

Figure 2.

Case 2, testicular Leydig cell tumor pathology and ultrasound images. *Up*: Hematoxylin and Eosin staining of testicular Leydig cell tumor demonstrating polygonal cells with eosinophilic cytoplasm and oval nuclei without atypia. (A) 20x magnification. (B) 40x magnification. Tumor consisted of sheets of uniform Leydig cells with moderately eosinophilic cytoplasm and no detectable mitotic activity. IHC was positive for MART-1, synaptophysin, inhibin, and calretinin, and negative for WT1, chromogranin and OCT4. *Bottom*: longitudinal section of the testicular mass (arrows) from initial ultrasound (C) and

follow up ultrasound (D). Transverse section of the testicular mass (arrows) from the initial ultrasound (E) and follow up ultrasound (F).