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***DICER1*-pleuropulmonary blastoma familial tumor predisposition syndrome: a unique constellation of neoplastic conditions**

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Abstract

Germline mutations in *DICER1* are associated with increased risk for a wide variety of neoplastic conditions, including pleuropulmonary blastoma (PPB), cystic nephroma, nasal chondromesenchymal hamartoma, ovarian Sertoli-Leydig cell tumors, botryoid embryonal rhabdomyosarcoma of the uterine cervix, ciliary body medulloepithelioma, pineoblastoma, pituitary blastoma and nodular thyroid hyperplasia or thyroid carcinoma. These tumors may be seen in isolation or in constellation with other characteristic tumor types in individuals or family members. Here we describe the medical history of a child with a heterozygous, loss of function germline *DICER1* mutation and multiple tumors associated with the syndrome. Although germline mutations in *DICER1* are rare, tumors of these types will be seen by practicing pathologists and should prompt consideration of an underlying *DICER1* mutation.

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Keywords

DICER1; pleuropulmonary blastoma; Sertoli-Leydig; thyroid carcinoma; nasal chondromesenchymal hamartoma

CASE REPORT

A 5-year-old girl presented with fevers and a clinical diagnosis of atypical pneumonia. Chest radiograph and computerized tomography (CT) demonstrated a large mass in the right hemithorax with pneumothorax. A right lower lobectomy was performed. Pathologic examination showed a cystic and solid malignant neoplasm. The cysts were lined by bland cuboidal cells. Beneath the epithelium there were focal areas with spindled and stellate cells and nodules of cartilage. The solid tumor was composed of blastema and spindle cell sarcomatous areas (Fig. 1) with large anaplastic cells. Focal areas of rhabdomyosarcomatous differentiation were also seen. The pathologic diagnosis was Type II pleuropulmonary blastoma (PPB). She received six months of chemotherapy with vincristine/adriamycin/cyclophosphamide, vincristine/dactinomycin/cyclophosphamide alternating with cisplatin/doxorubicin and did well. At 8 years of age, approximately 30 months post diagnosis of PPB, she presented with multiple palpable thyroid nodules. Tissue from the thyroidectomy showed multiple follicles lined by follicular cells with optically clear nuclei, brisk mitotic activity and rare, abortive papillary invaginations representing a follicular variant of papillary carcinoma (Fig. 2).

The patient did well until 13 years of age when she presented with lower abdominal pain. Physical examination showed a left inguinal hernia. Ultrasound examination showed multiple peritoneal cysts affecting the right and left round ligaments. The hernia was repaired and cysts were removed. Microscopically these peritoneal cysts were multilocular and lined by bland mesothelial cells; some of the septa were thin and delicate whereas others held a collagenous stroma with mixed chronic inflammation and hemosiderin (Fig. 3). Some septa had hemorrhagic necrosis consistent with torsion. No primitive cells or cambium layers were seen. No cartilage nodules were seen. Six months later the patient presented with nasal congestion and recurrent pelvic pain. An endoscopic examination of her nose showed multiple sinonasal polyps in the left and right sinuses. Endoscopic sinus surgery was performed. Histologic examination of the polyps showed complex arrangements of small and large glandular structures, some of which were cystically dilated. The polyps were lined by respiratory-type epithelium. There was a variably present subepithelial layer of mesenchymal cells within which arose primitive, maturing cartilage nodules as features of the nasal chondromesenchymal hamartoma (NCMH) (Fig.4). During the evaluation for recurrent pelvic pain, the patient was noted to have a new solid and cystic left ovarian mass measuring 17 × 10 × 10 cm. During surgery, bilateral inguinal hernias and additional peritoneal cysts were also identified. Pathologic examination of the ovary showed a Sertoli-Leydig cell tumor (SLCT) with extensive heterologous elements; heterologous mucinous glandular structures were accompanied by elements of Sertoli-Leydig tumor (Fig. 5) Immunohistochemistry showed the mucinous glandular structures were positive for calretinin and weak positivity for cytokeratin 7 and negative staining with cytokeratin 20.

Inhibin showed positivity in the Sertoli-Leydig cells. The peritoneal cysts had the same morphologic features of the cysts removed 7 months prior. Two years from SLCT diagnosis the patient is alive. Results of *DICER1* germline and tumor sequencing are shown in Figure 6. Family history is notable for a distant relative, also diagnosed with PPB.

MATERIALS AND METHODS

The patient was enrolled in the International PPB Registry, the International Ovarian and Testicular Stromal Tumor (OTST) Registry and the PPB genetic study, all approved by the Children's Hospitals and Clinics of Minnesota, Children's National Medical Center and the Washington University institutional human research protection offices. The patient's mother gave permission for inclusion in this case report. DNA was extracted from peripheral blood leukocytes and snap frozen thyroid carcinoma using standard procedures. Full *DICER1* coding region sequencing was performed as previously described.¹ For the PPB, NCMH and SLCT, DNA was extracted from four 10-micron scrolls prepared from respective tumor blocks using protocols described previously.² For the NCMH, laser microdissection was performed on an H&E stained 10 micron paraffin section and epithelial cells and mesenchymal cells (with and without cartilage) were procured for separate sequencing (Molecular Machines and Industries, Haslett, MI). Polymerase chain reaction (PCR) primer pairs to amplify the two common somatic missense regions were designed (Pair 1, RNase IIIb hotspot 1 - Forward: 5'-tggggatcagttgctatgtg-3'; Reverse: 5'-CGGGTCTTCATAAAGGTGCT-3') and (Pair 2, RNase IIIb hotspot 2 - Forward: 5'-tggactgcctgtaaaagtgg-3'; Reverse: 5'-ATGTAAATGGCACCAGCAAG-3'). PCR was performed in a final volume of 25ul containing 20ng DNA, 5pmol of each primer, 2ul dNTP, 2.5uL of 10XPCR Buffer, and 0.5U ExTaq (Takara Bio). The cycling profile included an initial step at 94°C for 5 minutes followed by 33 cycles, which consisted of a denaturation step at 94°C for 35 seconds, annealing step at 58.5°C for 35 seconds, and an elongation step at 72°C for 55 seconds; and a final extension step at 72°C for 10 minutes. The PCR products were cleaned by Shrimp Alkaline Phosphatase and Exonuclease I (USB Corporation). The sequencing reactions were performed using BigDye Sequencing Kit (version 3.1) according to the ABI protocol (Applied Biosystems). Data was collected from Applied Biosystems 3130xl Genetic Analyzer. Sequencing Analysis (version 5.2 Applied Biosystems) was used for data analysis. Variants were annotated using HUGO nomenclature for *DICER1* transcript NM_177438.2.

CASE DISCUSSION

This case illustrates several of the tumor types that may occur in the setting of germline *DICER1* mutation, in this case in the same individual. Additional tumor types commonly associated with *DICER1* mutations and/or PPB include: cystic nephroma (CN), ciliary body medulloepithelioma (CBME), botryoid embryonal rhabdomyosarcoma (ERMS) of the cervix, thyroid nodular hyperplasia and thyroid cancer, pineoblastoma and pituitary blastoma.²⁻¹⁹ Wilms tumor, neuroblastoma and medulloblastoma have also been seen in children with *DICER1* mutations but the prevalence of *DICER1* mutations in these latter three tumor types appears to be low.^{4,19-22}

DICER1 is an important gene with a primary role in the biogenesis of microRNAs (miRNAs) (reviewed in 22). miRNAs are a relatively recently discovered class of small RNA molecules that play a major role in controlling gene expression at the messenger RNA (mRNA) level. miRNA genes are transcribed from genomic DNA and after initial RNA processing in the nucleus are transported into the cytoplasm as a hairpin structure. This structure is recognized by the RNase III enzyme DICER1 which cleaves the 5' and 3' ends of the hairpin RNA into two separate, single strands of small RNA (mature miRNAs). Mature miRNAs recognize target specific sequences in individual mRNA molecules, most commonly in the 3' untranslated region (3'UTR). Binding of a miRNA to its corresponding mRNA(s) leads to either a block in translation or targeted destruction. In effect, these miRNAs suppress gene expression by preventing mRNAs from being translated into proteins. It has been estimated that 30% of the active genes in the human genome are regulated by miRNAs. miRNAs appear to be very important in organ development and suppression of neoplasia; turning off key proliferation genes at the exact right moment for organ differentiation to occur.²³ As a corollary, miRNAs are important in keeping many of these "oncofetal" genes that drive proliferation at an appropriate level after development. Deficient miRNA suppression can lead to oncogenic transformation.²⁴⁻²⁶ Complete absence of the DICER1 protein leads to early death in embryogenesis;^{27,28} however, organisms with one copy of *DICER1* are most often phenotypically normal but with increased risk for developing a unique set of organ-based neoplasias in childhood. The penetrance of these various neoplasms in germline *DICER1* mutation carriers appears low (Hill, Doros and Stewart unpublished data). The reasons for this low penetrance are not completely understood, but preliminary work suggests that a second event affecting the normal *DICER1* allele at a specific time in organ growth or development may also be required. In PPB, CN, NCMH, papillary thyroid carcinoma and SLCT we know that the second event is a partial loss of function, somatic missense mutation affecting one of six amino acids in the RNase IIIb domain of DICER1.^{2,8,9,12,15,16} These mutations lead to defective cleavage of the 5' (also known as 5p) mature miRNA off of the precursor miRNA hairpin.^{12,30} 5p miRNAs include many tumor suppressor miRNAs including the let-7, miR34/449 and miR200 families among others.¹² As demonstrated by the somatic mutation in the NCMH in this case report and a previous report from our group on CN,² somatic missense mutations in *DICER1* do not have an obligate conveyance of a malignant phenotype; NCMH and CN typically have a benign course. Additional genetic events such as loss or mutation of *TP53* in PPB may be required for progression.¹²

Loss of function germline *DICER1* mutations are seen in nearly 70% of all children with PPB regardless of family history. Tumor sequencing has shown that 49/51 PPBs tested have somatic missense mutations (i.e. second hits).¹² Some children without germline *DICER1* mutations have two somatic mutations in *DICER1*, one loss of function mutation and one missense mutation affecting 5p miRNA cleavage.¹² About 40% of these children will have multifocal disease and/or a family history at least suggestive of the *DICER*-PPB familial tumor predisposition syndrome.³¹ Prevalence of germline *DICER1* mutations in NCMH, lung cysts, thyroid carcinoma, nodular thyroid hyperplasia, embryonal brain tumors and other ovarian stromal tumors is the subject of a current study.

The pathologist making the diagnosis of one of the rare neoplasms in this syndrome may be the first to suggest the association of a particular tumor type with *DICER1* mutations to the clinical team. The benefit of detecting germline *DICER1* mutations in a child includes early identification of malignant conditions in young family members through screening and educating families and their treating physicians about early signs of disease (e.g. androgenic symptoms in the case of SLCT, thyroid nodules, vaginal spotting for cervical ERMS). Recommendations for screening in individuals with *DICER1* mutations was summarized in a recent review.³²

Pathological features of neoplasms commonly associated with *DICER1* mutations

Pleuropulmonary blastoma—This rare, embryonal tumor of the lung is analogous to other organ-based tumors of early childhood such as Wilms tumor and neuroblastoma. The malignant cellular component of PPB is a high grade sarcoma that derives from an immature lung mesenchymal cell that has the capacity to differentiate into multiple mesenchymal lineages. Classically there are three types of PPB based on the gross pathology; the purely cystic Type I PPB, cystic and solid Type II PPB and solid Type III PPB.³³ These types are highly correlated with age at diagnosis and outcome and are thought to represent the natural history of the disease. Type I PPB presents in the youngest age group (median 9 months) and has the best prognosis.³⁴ It is a multilocular, thin-walled cyst lined by epithelium. The cyst septa contain variably present primitive mesenchymal tumor cells beneath the epithelium. With tumor progression, these mesenchymal tumor cells eventually expand and overgrow the septa to form a grossly visible septal thickening or solid mass (Type II PPB).³⁵ Type II and III PPBs typically occur in children between the ages of 18 months and 6 years (median 36 and 43 months respectively). Type II PPB differs from Type III PPB in that it retains a grossly visible cystic component. The solid components of both Type II and III PPBs (hereafter solid PPB) are best characterized as a high grade, multipatterned sarcoma that includes two or more of the following patterns: 1) embryonal rhabdomyosarcomatous pattern with ovoid, stellate and spindled cells arranged in a myxoid, pale blue background, 2) blastemal pattern with cohesive clusters of primitive rounded cells with minimal cytoplasm; 3) cartilaginous differentiation with fetal type or high grade malignant cartilage nodules; and 4) spindle cell sarcoma. Anaplasia, similar to that seen in Wilms tumor, is present in 70% of Type II PPB and 90% of Type III PPB (Fig. 7). Immunohistochemistry typically shows diffuse vimentin positivity. Desmin is positive in the rhabdomyosarcomatous and blastemal elements. Myogenin and MyoD1 are positive in a proportion of the tumor cells analogous to ERMS. Epithelial markers are typically absent. Pleural fluid cytology is often negative unless there has been tumor rupture.

The *location* in the lung, the *age* of the patient and the reproducibility of the *multi-patterning* should make a diagnosis of solid PPB in a resection specimen straightforward in most cases. Almost all PPBs are lung based. ERMS and Ewing sarcoma tend to originate in the chest wall or soft tissue of the diaphragm rather than the lung parenchyma. Solid PPB is rare *before* 18 months of age. Infants with mostly solid lung tumors are more likely to have fetal lung interstitial tumor³⁶ or congenital peribronchial myofibroblastic tumor. Solid PPB is rare *after* seven years of age. Primitive, monomorphous spindle cell sarcomas in older children and young adults should raise the possibility of a primary pleuropulmonary synovial

sarcoma in a patient without a known prior history of synovial sarcoma.³⁷ PPBs are typically more heterogeneous than synovial sarcomas, but the spindle cell components of synovial sarcoma and PPB are remarkably similar. Staining for cytokeratin and epithelial membrane antigen and/or identifying gene fusions typical of synovial sarcoma should readily differentiate synovial sarcoma from PPB.³⁷ *DICER1* somatic mutation testing may also be of benefit in this circumstance. Finally pulmonary blastoma differs from solid PPB in that it is a biphasic tumor with malignant epithelial elements and mesenchyme and has a wide age range.

Probably the most common diagnostic challenge with solid PPB occurs in the setting of a needle biopsy of a large lung/chest wall or mediastinal mass. Similar to the North American approach to Wilms tumor, the preferred approach to a resectable PPB is primary resection avoiding tumor spill. However, needle biopsies may be entirely appropriate in large tumors deemed unresectable or in unstable patients or when neoadjuvant chemotherapy is considered. The challenge with needle biopsies is that the amount of tissue is typically small and sampling infrequently demonstrates the multiple patterns that distinguish PPB from other neoplasms. A common scenario is a needle core showing only an embryonal rhabdomyosarcomatous pattern. In this case clinical and radiographic clues can be used to suggest the diagnosis of PPB. Is the tumor primarily in the lung? If yes, it is probably PPB. Primary ERMS of the lung is very rare. Is the tumor primarily in chest wall or mediastinum or diaphragm/subdiaphragm? Then it may be an ERMS and not PPB. Somatic mutation testing for *DICER1* on formalin-fixed, paraffin embedded tumor tissue is now available and could be used in this circumstance as a diagnostic marker for PPB. Is it really important to distinguish PPB from ERMS on an initial needle biopsy? The neoadjuvant chemotherapy regimen for ERMS and PPB is similar and the finding of multiple sarcomatous patterns on microscopic review of the subsequent resection specimen would likely resolve the question. If the final diagnosis is PPB, then magnetic resonance imaging (MRI) scans of the brain, genetic counseling and testing for *DICER1* and screening for other *DICER1* disorders in the child or relatives (as directed by mutation results) would be added to the management plan.³²

The preceding discussion describes the classical natural history of the PPB. With an improving understanding of tumor genetics and pathologic review of many more examples, it has become clear that there is an alternate natural history within the Type I PPB category and not all Type I PPB are destined to progress to Type II and III high grade sarcomas.³⁵ Because of this alternate natural history and the “variably present” tumor cells, Type I PPB is the most diagnostically challenging of the types.

Early PPBs appear in late gestation as a multilocular cyst with thin septa lined by alveolar (and sometimes bronchiolar) epithelium. The septa contain evenly dispersed uncommitted polyclonal mesenchymal cells and small capillaries (Fig. 8A,B). These mesenchymal cells have small, regular, ovoid nuclei with dispersed chromatin in a pale myxoid background. Over a period of weeks to months these bland, uncommitted mesenchymal cells in the septa undergo one of several potential fates: 1) cell death and necrosis resulting in dystrophic calcification, hemosiderin and fibrosis; 2) differentiation resulting in small mature/maturing cartilage nodules, nodules of spindle cells or broad bands of spindled fibroblastic cells; or 3)

neoplastic transformation (Fig. 8 and 9). We have seen numerous examples of multilocular lung cysts in older individuals with *DICER1* mutations (up to age 62). Presumably when all of the immature mesenchyme in an early PPB undergoes either necrosis or differentiation, the risk for transformation is negligible.

Currently there are no objective markers of a “transformed” or “malignant” mesenchymal cell in Type I PPB. But these transformed mesenchymal cells have a more “primitive” appearance and show cytologic features of nuclear enlargement, hyperchromatism and mitotic activity. The even spacing of the cells is lost; the cells crowd each other in layers beneath the epithelium (Fig. 9). Rhabdomyoblastic differentiation is sometimes evident on routine H&E stain. In other cases, it can be demonstrated by myogenin and/or MyoD1 nuclear immunopositivity. From cell cultures of Type I PPB mesenchyme we know there is a clonal subpopulation with a somatic missense mutation in *DICER1* on the allele opposite to the one with the germline *DICER1* mutation (i.e. the second hit) (Hill unpublished data). The acquisition of this second hit and outgrowth of this transformed cellular component may be the variable in determining the neoplastic fate of any given Type I PPB.

Nasal chondromesenchymal hamartoma—NCMH presents in infants, children and young adults as a polypoid nasal mass.³⁸ Symptoms vary depending on size and location and can include persistent nasal drainage and nasal obstruction. Infants with NCMH can present with respiratory or feeding difficulties. Bony destruction can be seen. Surgical removal is curative, although local recurrences can be seen. A recent report documents one adult patient with malignant transformation in an NCMH.³⁹ Microscopically NCMH is composed of epithelial cysts lined by respiratory epithelium and nodules of immature or mature cartilage surrounded by spindle cell mesenchyme.³⁸ The rest of the polyp contains a mucoid ground substance containing inflammatory cells, small vessels and fibrosis. Occasional cases without cartilage nodules have been seen in individuals with *DICER1* mutations. The cartilaginous nodules surrounded by a compact, hypercellular zone of immature stromal cells of NCMH can be confused with ERMS. However, the stromal cells of NCMH lack a myogenic phenotype. Other patterns in NCMH may mimic aneurysmal bone cyst or fibrous dysplasia.

Ovarian sex-cord stromal tumors—Ovarian stromal tumors including SLCT, juvenile granulosa cell tumor, and gynandroblastoma have been seen in children and young adults with germline *DICER1* mutations.¹¹ SLCT in patients with germline *DICER1* may be cystic and solid or solid and often contain heterologous epithelial glandular elements as in this case report. A Sertoli-cell component staining with inhibin staining is invariably present in primary tumors. Tumors may also contain sarcomatous components. Occasionally we have seen tumors with both Sertoli-Leydig cell and juvenile granulosa cell features in children and young adults with *DICER1* mutations. Appropriate intraoperative staging, including sampling of peritoneal fluid is critical to determining the need for adjuvant therapy.

Cystic nephroma—CN is the second most common neoplasm we see in children with *DICER1* mutations.¹⁷ The majority of patients with CN are diagnosed in the first three years of life. CN presents as a circumscribed unilateral or occasionally bilateral cystic mass(es) in an otherwise normal kidney. The cysts can sometimes be simpler in architecture compared

with type I PPB and can resemble dilated tubules with plump, hobnail epithelium.¹⁷ In well-developed cystic tumors, delicate septa divide the lesion into variably sized locules much like the Type I PPB. The cyst septa typically contain bland mesenchymal cells in a pale myxoid matrix with a variable amount of inflammatory cells (Fig. 10). The same somatic missense *DICER1* mutations seen in Type I PPB are also seen in CN.² There appears to be a low risk of malignant transformation in CN with a sarcomatous tumor morphology similar to solid-type PPB.² The clinical differential diagnosis includes other cystic renal tumors of childhood including congenital mesoblastic nephroma (solid and cystic), cystic partially differentiated nephroblastoma (CPDN), cystic Wilms tumor and multicystic dysplastic kidney. Another cystic neoplasm of the kidney that appears to be more common in adults is the so-called mixed epithelial and stromal tumor (a.k.a. adult CN).^{40,41} The cysts of these tumors resemble those in *DICER1*-related CN but often contain a cellular stroma resembling ovarian stroma.

Botryoid Embryonal Rhabdomyosarcoma of the Uterine Cervix—Although botryoid ERMS has been seen in the bladder and biliary tract in individuals with PPB syndrome, the majority of ERMS in this syndrome appear to occur in the uterine cervix of adolescent females.⁴² Vaginal spotting is the most common clinical presentation. Examination shows a polyp or multiple polyps at the cervical os. These polyps may undergo torsion and necrosis; passage of vaginal tissue is another common presentation. Because cervical ERMS is a pedunculated polyp presenting at the cervical os, a benign cervical polyp composed in part of endocervical glands and a squamous mucosa is a common clinical impression. Microscopically, botryoid ERMS show a subepithelial layer of primitive cells beneath an intact epithelium (cambium layer), very similar to the Type I PPB (Fig. 11). Deeper in the polyp stroma the cells are better differentiated, often showing tails of eosinophilic cytoplasm with striations. The background is pale and mucoid and contains a variable number of inflammatory cells. Cartilaginous nodules are present in 35-40% of cervical ERMS. These polyps can be deceptively bland and confused with non-neoplastic polypoid lesions of the cervix such as granulation tissue polyps or mesodermal stromal polyps. Mesodermal stromal polyp is composed of enlarged stellate and spindle cells in a pale staining myxoid stroma without any glandular structures. These stromal cells lack the features of rhabdomyoblasts. Mullerian papilloma is a purely epithelial lesion with a complex papillary pattern; this lesion is seen in children. Mullerian adenocarcinoma is a malignant polypoid lesion of the cervix with a pattern of benign endocervical glands and a spindle cell sarcomatous stroma. Mullerian adenocarcinomas occur in older women and have been confused with ERMS but lack a myogenic phenotype.

Ciliary body medulloepithelioma—CBME (formerly diktyomas or teratoneuromas) are rare intraocular childhood neoplasms linked to *DICER1*-PPB tumor predisposition syndrome.⁷ These tumors present around seven years of age with decreased visual acuity. Although viewed as malignant neoplasms, distant metastases are rare. The basic histologic features are neuroblastic or embryonic-like neural tubules and Homer Wright rosettes accompanied by hyaluronic acid-rich stroma expanding the region of the ciliary body. A teratoid variant may have cartilage or immature skeletal muscle.

Pineoblastoma—Pineoblastoma is a primitive neuroectodermal tumor that arises in the region of the pineal gland. There is resemblance of this neoplasm to medulloblastoma and primitive neuroectodermal tumors elsewhere in the brain. Tumor cells are primitive with high nuclear-cytoplasmic ratio and hyperchromatic nuclei. Both Homer Wright and Flexner-Wintersteiner type rosettes may be seen in these neoplasms (Fig 12). Pineoblastomas should be distinguished from better differentiated pineal parenchymal tumors like pineocytoma and pineal parenchymal tumor of intermediate differentiation. It is uncertain if *DICER1* mutations are associated with better differentiated tumors.

Pituitary blastoma—Pituitary blastoma is a recently described primitive malignant neoplasm of the pituitary gland presenting in infants with symptoms of cortisol excess. These ACTH-producing tumors typically have a mixed pattern of gland and/or rosette-like structures intermixed with small primitive appearing cells with blastemal features and larger secretory cells.

CONCLUSION

The finding of rare tumors including PPB, ovarian stromal tumors especially SLCT, NCMH, CBME and ERMS of the uterine cervix especially at an early age or in constellation with other neoplasms should prompt consideration of germline *DICER1* mutations, a finding which has importance for individual and family screening. When these conditions are seen, the pathologist may wish to consider including this possible association in the pathology report so that attention may be directed to subtleties in the clinical or family history.

The penetrance and expressivity of germline *DICER1* mutations is the focus of ongoing analyses. Pathologists, clinicians and interested families are encouraged to contact the International PPB Registry (www.PPBregistry.org) for PPB and associated diseases, the International OTST Registry (www.OTSTregistry.org) or the National Institutes of Health *DICER1* natural history study (<http://dceg.cancer.gov/research/clinical-studies/dicer1-ppb-study>) for more information about these clinical and research efforts.

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Figure 1. Solid area of Type II PPB, age 5

High power view shows hypercellular areas of primitive cells with hyperchromatic nuclei adjacent to less cellular, looser rhabdomyosarcomatous areas. (H&E; original magnification $\times$ x400)

Figure 2. Well differentiated thyroid carcinoma, age 8

A) Medium power view of dominant thyroid nodule showing tightly packed neoplastic follicles with a thin capsule. Other nodules showed similar morphology but were not encapsulated. B) Higher power view shows crowded nuclei with optical clearing and brisk mitotic activity. Rare abortive papillae and nuclear pseudoinclusions were seen. (H&E; original magnification x200 A, x400 B).

Figure 3. Round ligament cysts, age 13

A and B) Multilocular cysts from the peritoneal surface of the round ligament show septa with variable thicknesses and collagenous stroma. The septa are lined by flattened or cuboidal mesothelial cells. The cystic architecture is reminiscent of Type I PPB (H&E; original magnification x200 A, B).

Figure 4. Nasal chondromesenchymal hamartoma, age 13

A) Low power view of one of the polyps arising from the sinus mucosa. Multiple cysts lined by respiratory epithelium are present. B and C) Low power views of multiple irregular cartilaginous nodules in the subepithelial tissue. D) High power view shows a subepithelial nodule of cartilage surrounded by a thin layer of spindle cells. (H&E; original magnification x100 A, x200 B,C, x400 D).

Figure 5. Sertoli-Leydig cell tumor of the ovary, age 13

A) The majority of the tumor was composed of bland intestinal-type glandular epithelium with a variably cellular background stroma. B) Sertoli-cell areas and areas with primitive cells were seen focally. (H&E; original magnification x200 A, x400 B).

Figure 6. Results of germline and tumor-specific *DICER1* sequencing

The loss of function germline mutation at the canonical splice site at the boundary of the eighth exon-intron was found in peripheral blood leukocyte DNA and in each of the tumor samples. Black arrows point to the positions of *DICER1* germline and somatic mutations in 4 different types of tumor by Sanger sequencing

- germline mutation c.1376+1G>A at boundary of the 8th exon-intron
- somatic mutation c.5428G>T(exon25) in PPB
- somatic mutation c.5438 A>T(exon25) in thyroid carcinoma
- somatic mutation c.5439G>T(exon25) in NCMH
- somatic mutation c.5439G>T(exon 25) in ovary SLCT

Figure 7. Classic sarcomatous patterns in solid PPB

A) Embryonal rhabdomyosarcomatous pattern is often a predominant pattern in solid PPB. This medium power photomicrograph shows a range of myogenic differentiation with bottom center showing poorly differentiated cells, upper right showing variably-shaped nuclei in a myxoid background and the left half of the photomicrograph showing differentiating rhabdomyoblasts with tails of eosinophilic cytoplasm and some visible striations. B) The blastemal pattern in PPB is characterized by relatively cohesive primitive cells with hyperchromatic nuclei and minimal cytoplasm. PPB blastema is often positive for desmin and associates with embryonal rhabdomyosarcomatous patterns. C) Cartilaginous differentiation in PPB can take the form of hypercellular but differentiated cartilage (left) or less well developed cartilaginous nodules arising from spindle cell mesenchyme resembling fetal cartilage (right). D) Spindle cell sarcomatous patterns may also be present. E) Anaplasia, similar to that seen in Wilms tumor, is a common feature of solid PPB. F) *TP53* mutations and/or allelic loss are common in solid PPB. (H&E A-E, Anti-p53 immunostain F; original magnification x200 A,B,D, x100 C, x400 E,F).

Figure 8. Early Type I and non-progressive PPBs

A) Early Type I PPB in a one month-old infant shows cystic expansion of airspaces. The septa are lined by flattened or cuboidal alveolar type 1 and 2 cells. The septa contain evenly dispersed bland uncommitted cells and small capillaries in a pale matrix. B) Higher power

view of same tumor. C) Low power view of this lesion from a nine month-old infant shows the typical multilocular cystic architecture with intersecting septa. The septa are collagenous and hypocellular. D) This Type I PPB shows a field with three septa showing different patterns: coagulative necrosis (upper third), paucicellular collagenous matrix (middle third), and subepithelial primitive cell proliferation (bottom third). E) Higher power views of coagulative necrosis. F) Dystrophic calcification and hemosiderin following coagulative necrosis. G and H) Paucicellular, collagenous septa with evidence of past necrosis and hemorrhage. I and J) Subepithelial mesenchyme showing differentiation toward cartilage and fibroblasts respectively (H&E; original magnification x200 A,D,G,H; x400 B,E,F,I,J; x100 C).

Figure 9. Type I PPBs with primitive cell components

A) Subepithelial layer of primitive cells with hyperchromatic nuclei and inconspicuous cytoplasm. Mitotic activity is present. B) A thicker layer of primitive mesenchymal cells beneath an alveolar lining (cambium layer). Compare the worrisome cytologic features of these cells and cellular density with those in Fig. 8B, 8I and 8J. Eosinophils are common in Type I PPBs that present with pneumothorax. C) Well developed cambium layer beneath the bronchial epithelium. Note the overt skeletal muscle differentiation in deep stroma. D) A different pattern of cambium layer with a less cellular, myxoid stroma. (H&E; original magnification x400 A,B; x100 C x200D).

Figure 10. Cystic nephroma

A) Lower power view showing cystic expansion of an apparent tubule. Septa in this example are collagenous with scattered inflammatory cells. B) High power view of a cyst wall from a different patient shows a lining of plump epithelial cells. The subepithelial zone is myxoid and contains bland mesenchymal cells and scattered inflammatory cells. (H&E; original magnification x100 A, x400 B).

Figure 11. ERMS of the uterine cervix in a child with *DICER1* mutation

A) Medium power view of a cervical polyp from a 14 year-old female who presented with tissue passed from the vagina. The polyp is lined by mucinous endocervical cells. The subepithelial zone (cambium layer) is more densely cellular than the deep stroma. B) A high power view shows primitive cells with hyperchromatic, irregular nuclei and mitotic activity. Desmin and myogenin stained approximately 30-40% of these cells. (H&E; original magnification x200 A, x400 B).

Figure 12. Pineoblastoma in a 30 month old girl with *DICER1* mutation

A biopsy of a pineal region tumor from a two year-old female showed a primitive malignant neoplasm composed of cells with hyperchromatic nuclei and no overt differentiation by H&E stains. Synaptophysin and chromogranin were positive indicating a neurogenic phenotype. A large complex cyst in the left lung was noted on the scout radiograph of the MRI scan. Subsequent genetic testing showed a germline *DICER1* mutation. (H&E, original magnification $\times 400$).