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REVIEW

An update on the central nervous system manifestations of DICER1 syndrome

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Abstract

DICER1 syndrome is a rare tumor predisposition syndrome with manifestations that predominantly affect children and young adults. The syndrome is typically caused by heterozygous germline loss-of-function *DICER1* alterations accompanied on the other allele by somatic missense mutations occurring at one of a few mutation hotspots within the sequence encoding the RNase IIIb domain. *DICER1* encodes a member of the microRNA biogenesis machinery. The syndrome spectrum is highly pleiotropic and features a unique constellation of benign and malignant neoplastic and dysplastic lesions. Pleuropulmonary blastoma (PPB), the most common primary lung cancer in children, is the hallmark tumor of the syndrome. Other manifestations include ovarian Sertoli-Leydig cell tumor, cystic nephroma arising in childhood, multinodular goiter, thyroid carcinoma, anaplastic sarcoma of the kidney, embryonal rhabdomyosarcoma, and nasal chondromesenchymal hamartoma, in addition to other rare entities. Several central nervous system (CNS) manifestations have also been defined, including metastases of PPB to the cerebrum, pituitary blastoma, pineoblastoma, ciliary body medulloepithelioma, and most recently primary *DICER1*-associated CNS sarcomas and ETMR-like infantile cerebellar embryonal tumor. Macrocephaly is a recently reported non-neoplastic, haploinsufficient phenotype. In this manuscript, we review the CNS manifestations of DICER1 syndrome.

Keywords *DICER1* · Brain tumor · Pineoblastoma · Pituitary blastoma · CNS sarcoma · *DICER1*-associated CNS sarcoma · Ciliary body medulloepithelioma · ETMR

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Introduction to DICER1 syndrome

DICER1 syndrome is a rare tumor predisposition syndrome with manifestations that predominantly affect children and young adults (Online Mendelian Inheritance in Man #601200). First evidence of its existence was reported by Priest and colleagues in 1996 [48]. Here, they noted the familial clustering of the rare sarcomatous lung tumor of childhood, pleuropulmonary blastoma (PPB), either alone, or with other rare neoplastic and dysplastic conditions. Most frequently, the syndrome is inherited in an autosomal dominant manner and is caused by genetic alterations of *DICER1* [26]. The *DICER1* gene product is an RNase III endoribonuclease that functions canonically in the microRNA (miRNA) biogenesis pathway where it cleaves miRNAs from their hairpin-shaped precursors. The resulting mature ~ 22 nucleotide-long miRNAs function to negatively regulate the expression of protein-coding genes at the post-transcriptional level [21, 24]. The identification of *DICER1* as the gene causing DICER1 syndrome came in 2009 following a linkage study performed by Hill and colleagues

who identified heterozygous germline *DICER1* pathogenic variants in 11 of 11 tested families with PPB [26]. Since then, studies have revealed the DICER1 syndrome phenotype to be pleiotropic and comprise a unique constellation of benign and malignant conditions: in addition to PPB, the most common primary lung cancer in children [17, 18, 47], the syndrome predominantly features ovarian Sertoli-Leydig cell tumor, embryonal rhabdomyosarcoma primarily of the uterine cervix, multinodular goiter, thyroid carcinoma, cystic nephroma arising during childhood, anaplastic sarcoma of the kidney, nasal chondromesenchymal hamartoma, and other rare entities (Table 1) [21]. DICER1 syndrome also features a spectrum of central nervous system (CNS) manifestations which are the focus of this review.

DICER1 syndrome genetics

Most individuals with DICER1 syndrome are heterozygous for a germline *DICER1* loss-of-function pathogenic variant. Germline deletions involving one or more exons of *DICER1* [3, 4, 57], or the entire *DICER1* locus have also been documented [10, 11, 25, 75]. While approximately 87% of heterozygous germline *DICER1* pathogenic variants occurring in children with PPB are inherited in an autosomal dominant manner, approximately 13% arise de novo [4]. Approximately 10% of DICER1 syndrome cases are the result of somatic mosaicism for a pathogenic *DICER1* variant [4, 16, 32]. DICER1 syndrome-related tumors very typically harbor an additional somatically acquired missense mutation in exon 24 or 25 encoding the RNase IIIb cleavage domain, involving one of the following codons: p.E1705, p.D1709, p.G1809, p.D1810, or p.E1813 [21]. DICER1 syndrome thus fulfils Knudson's two-hit hypothesis, but in contrast to the classic model, barring a few exceptions, the event on the second allele does not fully abrogate DICER1 function [21]. Instead, hotspot mutations in the RNase IIIb domain have been shown to interfere with DICER1's ability to process miRNAs from the 5′ strand of hairpin-shaped precursor-miRNA molecules resulting in altered 5p to 3p miRNA expression ratios [52, 53, 64, 76]. 5p miRNA levels are globally and consistently reduced in *DICER1*-mutated tumors [52, 53, 64, 76]. Hypothesized mechanisms of *DICER1*-related tumorigenesis include the loss of tumor suppressor miRNAs of 5p origin (e.g. members of the let-7 family of miRNAs), leading to upregulation of their target oncogenes. Given that DICER1 syndrome tumors are frequently blastomatous in nature, it could be postulated that the drastic but

Table 1 Summary of DICER1 syndrome manifestations

CNS manifestations	Abbreviation	Approximate age range of susceptibility
Metastasis and embolism of PPB to CNS	–	Up to 36 months following PPB Dx
Pituitary blastoma	PitB	0–24 months
Pineoblastoma	PinB	2–25 years
Ciliary body medulloepithelioma	CBME	3–10 years
ETMR-like infantile cerebellar tumor	–	Not determined
Primary *DICER1*-associated CNS sarcoma	CNS sarcoma	4–12 years*
Macrocephaly	–	–
Prototypical non-CNS manifestations	**Abbreviation**	**Approximate age range of susceptibility**
Pleuropulmonary blastoma (Types I, Ir, II, and III)	PPB	0–72 months
Occult lung cysts	–	Discovered at any age
Cystic nephroma	CN	0–48 months
Ovarian Sertoli-Leydig cell tumor	SLCT	5–45 years
Multinodular goiter/differentiated thyroid cancer	MNG/DTC	5–40 years
ERMS of the uterine cervix	cERMS	4–45 years
Nasal chondromesenchymal hamartoma	NCMH	6–18 years
DICER1-associated anaplastic sarcoma of kidney	D1ASK	2–20 years
Other selected non-CNS manifestations		
Juvenile hamartomatous intestinal polyps, bladder ERMS, ovarian ERMS		
Other selected non-CNS manifestations		
Juvenile hamartomatous intestinal polyps, bladder ERMS, ovarian ERMS		

CNS central nervous system, *Dx* diagnosis, *ETMR* Embryonal Tumor with Multilayered Rosettes, *ERMS* embryonal rhabdomyosarcoma

*Indicated age range is derived from cases with biallelic *DICER1* alterations, one of which is germline in origin; Age range for CNS sarcomas bearing biallelic somatic *DICER1* mutations is 0–20 years

distinct miRNA dysregulation results in the downregulation of cellular differentiation pathways accompanied by upregulation of proliferation pathways, leading to tumors exhibiting immature histological appearances. Additional mechanistic studies are required to examine the specific downstream effects of miRNA perturbation and their role in DICER1 syndrome tumorigenesis.

A small percentage (~ 10%) of patients are found to have a mosaic *DICER1* mutation [4]: those patients with mosaic distributions of RNase IIIb hotspot mutations develop a greater number of disease foci at significantly younger ages than their non-RNase IIIb-mosaic counterparts [4, 16]. In contrast, those with mosaic loss-of-function variants tend to have one or two foci of disease. Regardless of the type of mosaic variant, a second mutation is present which may include loss of heterozygosity (LOH) [4].

A subset of tumors has been found to harbor biallelic *DICER1* alterations limited to the tumor (i.e. biallelic somatic mutations). These patients have disease involving only a single organ and are not considered syndromic or at risk of developing other DICER1 syndrome lesions [4, 6, 9, 12] although consideration should be given to possible unrecognized mosaicism of the identified loss-of-function mutation [6].

Because *DICER1* alterations in both benign and malignant syndrome-related conditions appear to be identical (i.e. one loss-of-function variant coupled with an RNase IIIb hotspot mutation), the mechanisms underlying neoplastic potential of certain DICER1 syndrome-related tumors are not yet known but are hypothesized to be due to the presence of additional oncogenic alterations in other genes. *TP53* is frequently inactivated in *DICER1*-mutated PPB [52, 64] and anaplastic sarcoma of the kidney [78]. *NRAS* and *BRAF* are also mutated in some PPBs [52]. In contrast, screening for known oncogenic driver mutations of papillary thyroid carcinoma in *DICER1*-related thyroid cancers did not reveal any such alterations [74, 77], indicating the need for further study.

The penetrance of germline *DICER1* pathogenic variants for clinical phenotypes in non-probands has been calculated to be ~ 5% by age 10 years, increasing to ~ 20% by age 50 years [70]. Heterozygotes typically develop one or two phenotypes of which MNG and occult small lung cysts are the most frequent [4]. Penetrance is significantly lower for other phenotypes including the CNS manifestations of DICER1 syndrome.

CNS manifestations

CNS manifestations of DICER1 syndrome often present a diagnostic challenge. Metastasis of PPB to the CNS is relatively frequent [41, 46]; while PPB Type I is a multi-cystic neoplasm that is not usually aggressive, PPB Types II and III, which are cystic and cystic/solid, respectively, and which contain sarcomatous areas, can metastasize to the CNS. Primary tumors include pituitary blastoma [14, 58], pineoblastoma [13, 56], ciliary body medulloepithelioma (an ocular tumor of neuroepithelial origin) [8, 42], and the two recently recognized conditions: primary *DICER1*-associated CNS sarcoma exhibiting rhabdomyoblastic differentiation [10, 34] and ETMR-like infantile cerebellar embryonal tumor [73] (Table 1). Several other CNS tumors have been reported in the context of DICER1 syndrome and/or *DICER1* alterations but lack definitive evidence of genetic association. These include medulloblastoma [48], intracranial medulloepithelioma [7], anaplastic meningeal sarcoma [13], and glioblastoma multiforme [1, 49]. Macrocephaly has recently been documented in *DICER1* heterozygotes [30]. Below, we review the histology and molecular characteristics of CNS conditions established to be included in DICER1 syndrome:

Metastases and embolism of PPB to the CNS

Metastasis of thoracic PPB to the cerebrum is the most frequent CNS event encountered in DICER1 syndrome patients [41, 46]. The CNS is also the most frequent site of distant PPB metastasis. In a series of 235 cases of PPB Types II ($n = 124$), II–III ($n = 21$), and III ($n = 90$), 26 CNS metastatic events were documented, thus occurring in 11% of patients with "advanced" PPB in the study [41]; 4/26 cases involved local chest relapse together with CNS metastasis and 22/26 were isolated CNS events without concomitant relapse of chest disease [41]. No cases of metastatic PPB Type I have been described. Importantly, the CNS seems to be a "sanctuary site" for PPB with CNS metastases occurring up to 36 months after PPB diagnosis and characteristically without concurrent chest disease [41, 46]. The International PPB Registry currently recommends surveillance head magnetic resonance scans of PPB Types II and III patients be performed every 3 months until 36 months following PPB diagnosis (https://www.ppbregistry.org/health-professionals/basic-facts-about-ppb/metastasis/). The most frequent site of PPB CNS metastasis is the cerebrum. PPB cerebral metastases can be fulminant—exemplified by symptomatic bi-frontal disease developing 6 weeks following a magnetic resonance scan considered normal at the time of the scan and in retrospect [46]. Meningeal PPB metastases and metastases to the spinal cord are documented but rare [46]. Because PPB can extend into the thoracic great vessels, such as Wilms tumor, PPB tumor embolism to the CNS following chest surgery has been observed causing either dry or hemorrhagic infarction with both early and late tumor growth at the infarction site [45, 46]. An example of the latter was described by Tan Kendrick and colleagues in the case of a 3-year-old girl, who, one day after surgical excision of PPB Type II, was found to have a large embolic cerebral

infarction. A year later, a 5 cm hemorrhagic tumor was discovered at the site of the infarct [71, 72].

The histological appearance of metastatic PPB is similar to the primary lung lesion but may show a predominance of rhabdomyoblastic or spindle cell elements [46]. Metastatic PPB may be histopathologically indistinguishable from the recently described primary *DICER1*-associated CNS sarcoma (discussed below); distinction can be achieved by assessing prior or current personal medical history of PPB of the lung, combined with genetic testing of both the chest and CNS lesions: the presence of a different RNase IIIb hotspot mutation in each of the chest and CNS lesions would be indicative of them being separate events; whereas the presence of the same RNase IIIb hotspot mutation in both tumors would be suggestive (although not definitive) of the CNS lesion being metastatic PPB. The presence of multiple cerebral lesions in a known PPB patient also suggests metastasis [46].

Pituitary blastoma

Pituitary blastoma is an exceedingly rare tumor of the anterior pituitary that was described by Scheithauer in 2008 and 2012 [61, 62]. To date, 16 cases have been described [14, 23, 29, 58]. Pituitary blastomas occur in children aged 2 years and younger. Presenting symptoms include Cushing syndrome (a rare endocrinopathy in infants), ophthalmoplegia, and/or diabetes insipidus. Blood adrenocorticotrophic hormone (ACTH) is typically elevated, and the levels of other pituitary hormones can be variably affected. On radiographic imaging, pituitary blastoma appears as a hypophyseal and suprahypophyseal cystic/solid or solid mass [14, 58, 61, 62] (Fig. 1a).

The histology, which resembles that of the embryonic stage pituitary gland, is complex and is composed of small blastema-like cells, glandular structures resembling Rathke epithelium, adenohypophysial folliculostellate and secretory cells (Fig. 1b). Mitotic activity can vary from case to case, and areas of necrosis can be present [61]. Most cases have varying degrees of ACTH and growth hormone immunoreactivity which is an unusual combination of hormonal secretions in pituitary tumors. Immunohistochemical staining for other pituitary hormones (prolactin, follicle-stimulating hormone, luteinizing hormone, thyrotropin, and alpha subunit) are typically negative [14, 61]. The immunoprofile of pituitary blastoma includes keratins, galectin-3, annexin-1, scant GFAP and S100 expression in folliculostellate cells, and synaptophysin and chromogranin expression in secretory cells. p53 and p27 are positive in a significant proportion of the small, immature cells, and Ki67 expressivity varies widely and does not appear to be a good prognostic indicator [14]. It is not yet known whether pituitary blastoma is malignant or benign, but its intracranial location makes it life-threatening [14].

Of the 16 cases described to date, 14 have undergone genetic testing and all 14 were determined to have had one or more pathogenic variants in *DICER1* [14, 23, 29, 58]. More specifically, 11/14 (79%) patients were heterozygous for a germline *DICER1* pathogenic variant (10 loss-of-function; 1 RNase IIIb hotspot [ref [14], case 12]). In a 13-case series [14] and three brief reports [23, 29, 58], one patient was found to be *DICER1* wild-type on germline testing, but had a somatic RNase IIIb hotspot mutation; similar somatic hotspot mutations were identified in 9/14 tumors; 2/14 tumors had LOH; one tumor lacked both an RNase IIIb hotspot

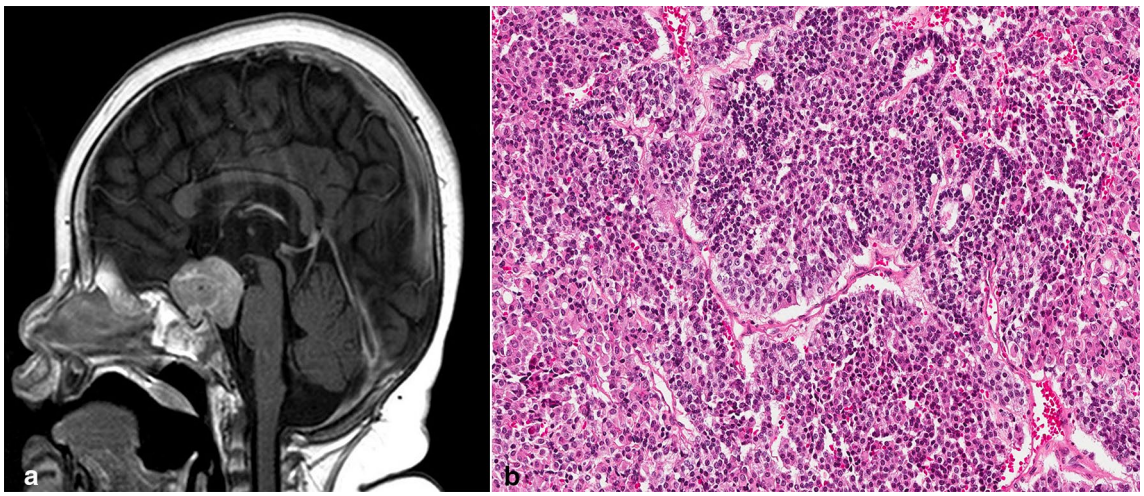


Fig. 1 Pituitary blastoma, a pathognomonic lesion of DICER1 syndrome. **a** Sagittal T1 magnetic resonance image showing a large pituitary tumor and prominent posterior neck adipose tissue, consistent with the Cushingoid appearance of a subset of pituitary blastoma patients (Image courtesy of Megan Moriarty-Kelsey, MD). **b** Hematoxylin and eosin (H&E)-stained section demonstrating Rathke-type epithelium, pituitary secretory-type cells, and occasional blastemal elements

mutation and LOH and two tumors were not sequenced [14, 29]. Thus, pituitary blastoma appears to be pathognomonic for *DICER1* syndrome.

Pineoblastoma

Pineoblastoma is a rare primitive neuroectodermal tumor arising in the pineal gland, classified as a WHO grade IV tumor [20]. Pineoblastoma represent less than 0.5% of all intracranial tumors and approximately 35% of pineal parenchymal tumors. Pineoblastomas are most frequent in the first two decades of life with a median age at diagnosis of approximately 13 years [20, 40]. Given the anatomic location, presenting symptoms include vomiting, headaches, and altered mental status [8]. Diagnostic imaging usually reveals a large mass in the pineal region with possible extension in the surrounding structures (Fig. 2a) [20].

The histology of pineoblastoma is indistinguishable from most other embryonal tumors of the central nervous system. It is a hypercellular neoplasm with sheet-like growth pattern, high nuclear-to-cytoplasmic ratio, hyperchromasia, molding, and occasional rosette formation (Fig. 2b). Mitoses are frequent, and necrosis can be present. Immunohistochemical staining for neuronal and neuroendocrine markers (synaptophysn, chromogranin) are positive, and neurofilament protein immunoreactivity is usually only focal. Pineoblastomas lack genetic alterations typical of medulloblastoma [43], which may assist with informing diagnosis in cases in which the site of origin is uncertain. This is exemplified by a case reported by Kline and by Raleigh in which the diagnosis of a medulloblastoma in an 18-month-old boy was revised to pineoblastoma following genetic testing: two *DICER1* alterations were identified in the lesion (loss-of-function and RNase IIIb hotspot mutations) and medulloblastoma-specific genetic changes were absent [33, 54]. This re-classification occurred because *DICER1* mutations have not been reported in a medulloblastoma, so their presence in a putative medulloblastoma suggests reconsideration of a tumor's classification. Also, *SMARCB1* and *SMARCA4* remain intact in pineoblastoma, distinguishing them from atypical teratoid/rhabdoid tumors.

Only a few genes have been implicated in the pathogenesis of pineoblastomas. Pineoblastomas occur as so-called "trilateral retinoblastoma" in the setting of germline *RB1* mutations which are likely an important predisposing factor [31]; the incidence of somatic *RB1* mutations in pineoblastoma remains unknown. The potential association between *DICER1* and pineoblastoma was investigated when the tumor occurred with other DICER1 syndrome phenotypes [55, 56]. To date, 8 pineoblastoma patients have been found to be heterozygous for germline *DICER1* pathogenic alterations. Divergent from the typical tumor-specific somatic missense RNase IIIb hotspot mutations, loss of heterozygosity of the wild-type *DICER1* allele appears to be the usual somatic event in *DICER1*-related pineoblastomas [13, 37, 56]. With that said, somatic RNase IIIb hotspot missense mutations have now been identified in 2 cases [33, 37, 54]. Although not widely implemented in clinical practice, immunohistochemical staining can be used to screen pineoblastomas for *DICER1* alterations; loss of DICER1 protein expression was observed in a small number of pineoblastomas bearing biallelic *DICER1* inactivating alterations [13] (Fig. 2c). Two separate studies which utilized whole-exome

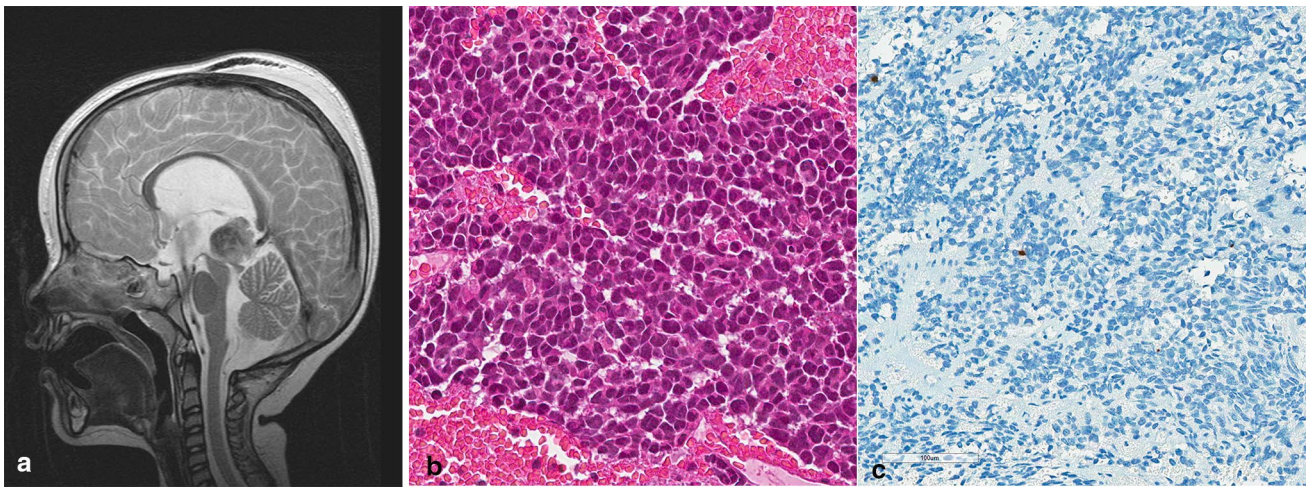


Fig. 2 Pineoblastoma in the setting of DICER1 syndrome. **a** Sagittal T2-weighted magnetic resonance image from a 2-year-old boy showing a mass arising from the pineal gland (Image courtesy of R. Paul Guillerman). The child also had a lung cyst. **b** H&E section showing pineoblastoma exhibiting primitive cells with high nuclear-to-cytoplasmic ratio, hyperchromasia and cellular overlap. **c** The absence of DICER1 expression in a *DICER1*-related pineoblastoma on immunohistochemical staining (Abcam, Anti-DICER1 antibody ab14601)

and/or whole-genome sequencing determined that pineoblastomas are characterized by mutually exclusive, biallelic events in either *DICER1* or *DROSHA* in a subset of cases [37, 69]: a total of 5 tumors lacking *DICER1* alterations were each found to have homozygous deletions of *DROSHA*. *DROSHA* encodes a protein that functions upstream of DICER1 in the miRNA biogenesis pathway. These findings indicate that perturbation of miRNA processing plays a fundamental role in pineoblastoma development. Lee and colleagues further determined that genetic testing may be useful in distinguishing between pineoblastoma and pineal parenchymal tumors of intermediate differentiation on the basis that the latter, which are WHO grade II or III tumors, rather than having microRNA biogenesis defects, bear recurrent small in-frame insertions in the *KBTBD4* gene that are absent in pineoblastoma [37].

Ciliary body medulloepithelioma

Ciliary body medulloepithelioma (CBME) is a rare embryonal ocular tumor that arises from the primitive medullary epithelium of the optic cup [44, 65]. Although rare, it is the second most common tumor of the eye in pediatric patients, after retinoblastoma. CBME is usually diagnosed in the first two decades of life and the mean age at diagnosis is 5 years [5]. Presenting symptoms include changes in visual acuity and headaches. The ophthalmologic exam shows abnormal vessels, leukocoria, and a mass originating in the ciliary body. Enucleation is frequently required but can be avoided in rare cases [5, 50]. In a systematic ophthalmologic examination of *DICER1* heterozygotes, two CBMEs were detected incidentally [27]. Other ocular abnormalities observed in the study include decreased visual acuity, retinitis pigmentosa, retinal degeneration, cataracts, optic nerve anomalies, drusen, and epiretinal membranes, although further study is required to substantiate their association with the syndrome [27].

Histologically, CBME is classified as benign or malignant and non-teratoid or teratoid (the latter indicating the presence of heterologous elements) [67]. The tumor is composed of sheets and cords of poorly differentiated neuroepithelial cells that resemble embryonic ciliary epithelium and retina (Fig. 3a). Teratoid medulloepithelioma contains areas of hyaline cartilage, rhabdomyoblasts, or glial and neuronal tissue (Fig. 3b) [60]. Areas of pigmentation are frequent. The criteria to classify CBME as benign versus malignant are not well defined. Although many CBMEs demonstrate malignant features, such as increased mitotic rate and areas with primitive appearance resembling retinoblastoma (Fig. 3c), metastasis and local invasive behavior are not common. The only recognized prognostic indicator is extraocular extension [5, 44, 66].

Approximately 23 CBMEs, either confirmed or suspected *DICER1*-related, have been documented. Fifteen of 23 cases had one or more alterations in *DICER1*: 7/15 occurred in *DICER1* heterozygotes [10, 15, 22, 27, 68]; 1/15 in an RNase IIIb mosaic patient [16]; and another 7/15 were found to bear RNase IIIb hotspot mutations (comprehensive germline *DICER1* testing was not performed in the latter 7 cases) [19, 59]. The remaining 8/23 CBMEs were noted

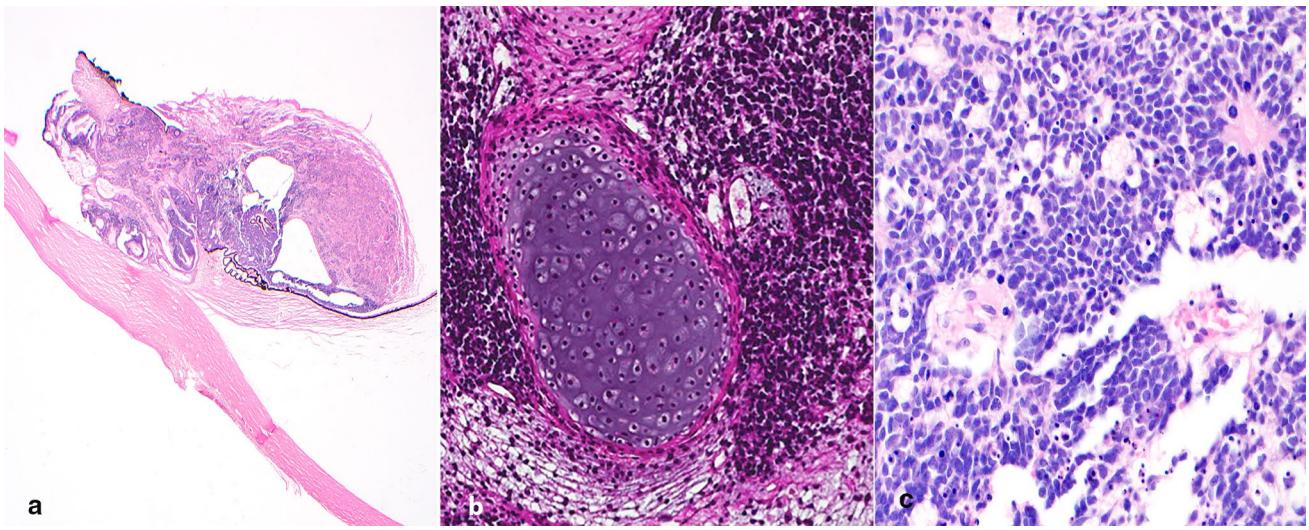


Fig. 3 Ciliary body medulloepithelioma. **a** Low-power view of a globe with a meduloepithelioma arising in the ciliary body. **b** A *DICER1*-associated teratoid ciliary body medulloepithelioma with primitive cells admixed with cartilage and immature mesenchyme. **c** Malignant ciliary body medulloepithelioma composed of primitive cells that grow in sheets and occasionally form true rosettes; numerous mitoses and apoptotic figures are present. Figure 3a, c are courtesy of Dr. Melike Pekmezci, MD, Neuropathologist and Ophtalmic Pathologist at UCSF Medical Center

in patients with personal medical histories remarkable for DICER1 syndrome-related lesions (PPB, lung cysts, or thyroid adenoma), but without molecular confirmation [28, 35, 36, 45], including a 2-year-old child with both CBME and pineoblastoma [39]. Given the rarity of CBME in DICER1 syndrome patients, standardized screening for CBME is not currently recommended, except in cases in which vision and other ocular abnormalities are revealed by a regular ophthalmologic examination [63].

Other *DICER1*-associated CNS embryonal tumors

Two interesting cerebellar embryonal tumors with features resembling embryonal tumor with multilayered rosettes (ETMR) were recently described by Uro-Coste and colleagues in girls aged 11 and 8 months, respectively [73]. The tumors were both LIN28A immunopositive but lacked the chr19q13.41 miRNA cluster (*C19MC*) amplification, which characterizes ETMR. Genetic testing identified biallelic *DICER1* alterations: each of the girls was heterozygous for a germline *DICER1* pathogenic variant and each tumor harbored a second somatic RNase IIIb hotspot mutation [73]. Other embryonal tumors known to be associated with DICER1 syndrome (e.g. pineoblastoma and pituitary blastoma) were excluded from the differential diagnosis due to the tumors' cerebellar location. The two tumors clustered separately from primary *DICER1*-associated CNS sarcoma and CBME on methylation profiling but closely to other ETMRs. Uro-Coste and colleagues hypothesize that biallelic alterations of *DICER1* (which alter miRNA expression patterns [51, 53, 64, 76]) might have the same effect as the amplification of the *C19MC* locus in a subset of medulloepitheliomas, ETMR not otherwise specified (NOS), or embryonal tumors NOS, although this requires further investigation.

One of the current authors (SA) encountered a case similar to that of Case 2 described by Uro-Coste and colleagues [73]—we discuss it here for the purpose of illustration and to underline the importance of molecular testing in establishing a diagnosis: A 2 month-old girl presented with signs

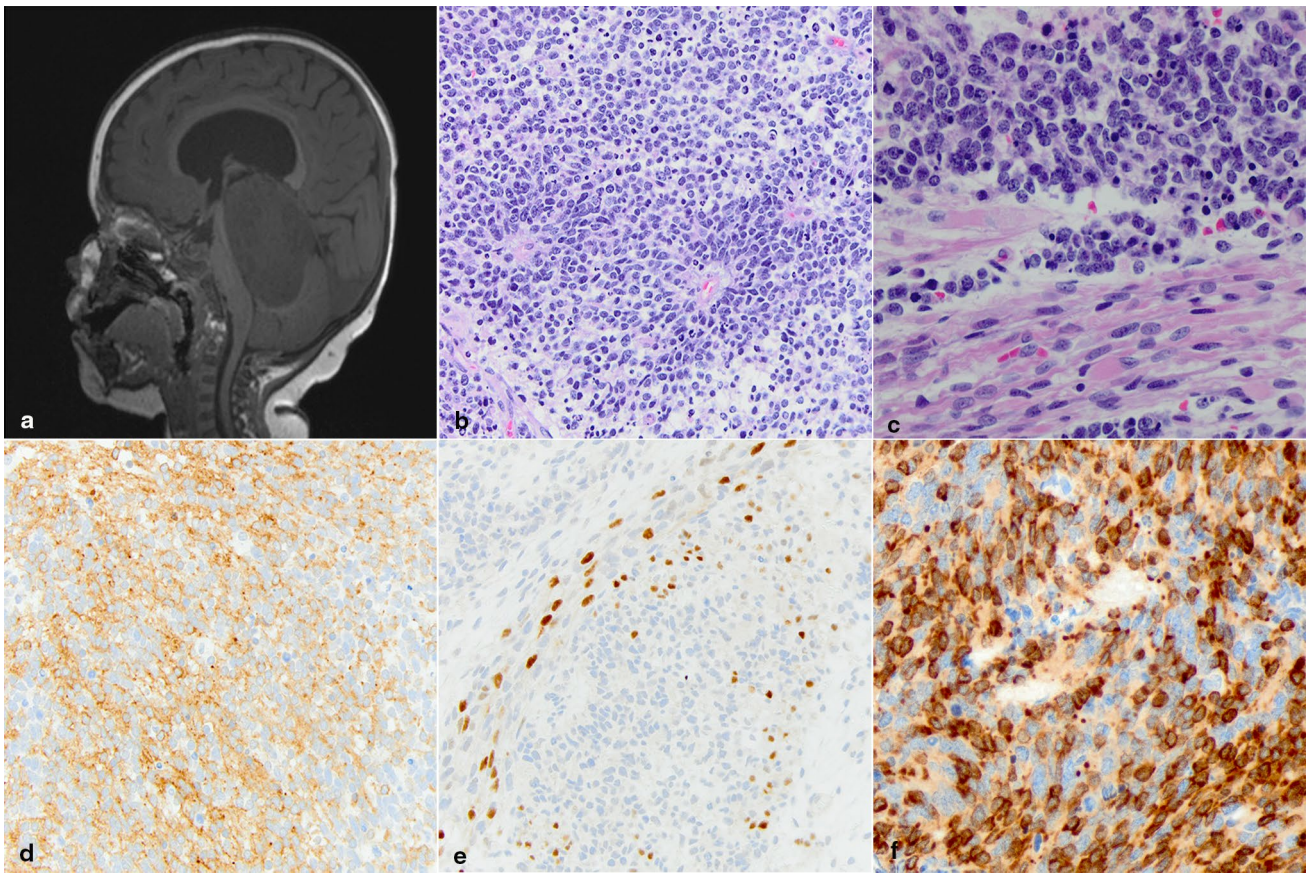
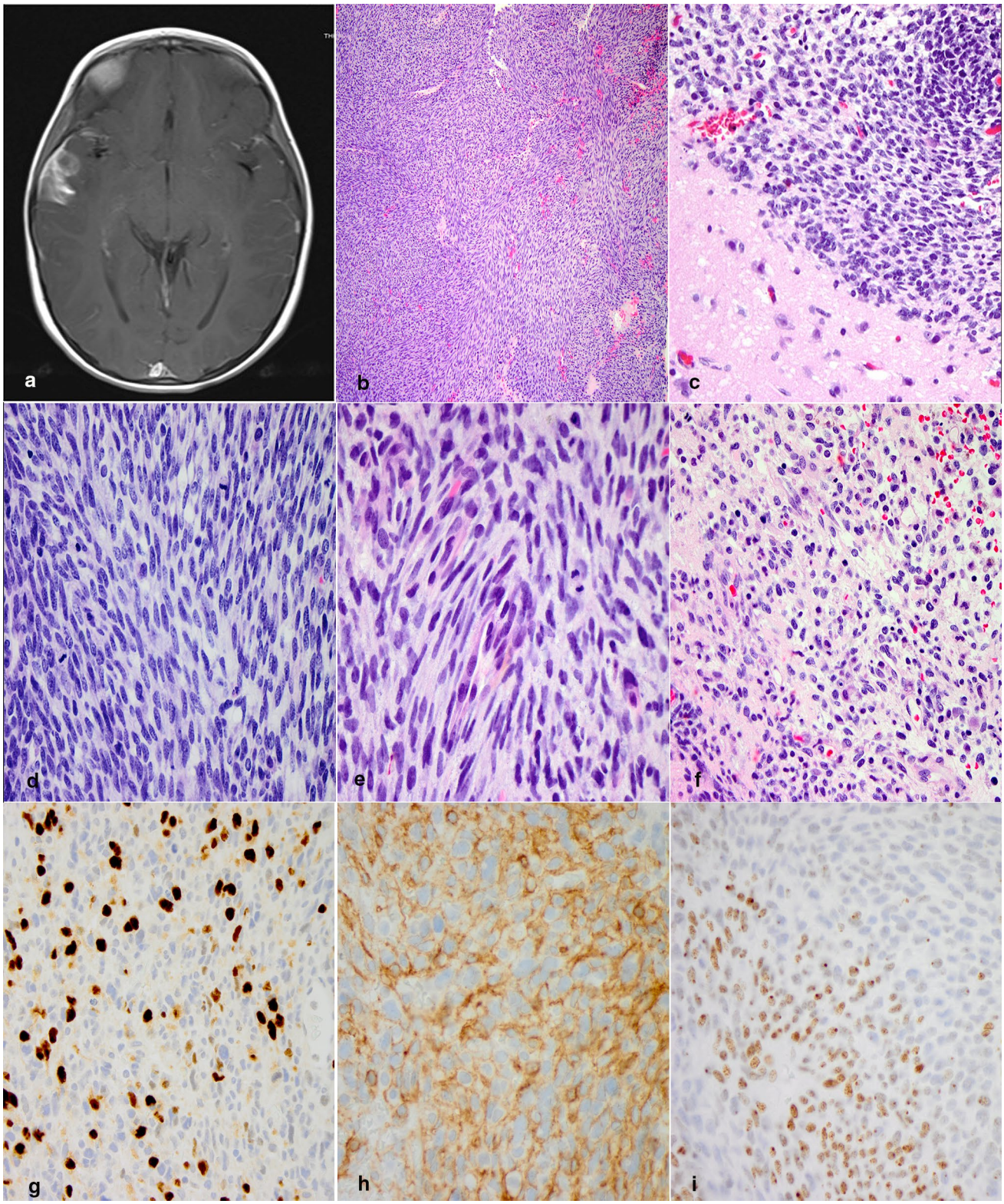


Fig. 4 A case of ETMR-like *DICER1*-associated embryonal tumor. **a** Sagittal T1 magnetic resonance image showing a large third ventricle mass involving the superficial aspect of the vermis and causing hydrocephalus. **b** H&E-stained section showing an embryonal tumor in a background of neuropil; multilayered angiocentric arrangement can be appreciated. **c** Skeletal muscle fibers admixed with the embryonal component of the tumor. **d** Synaptophysin immunostain. **e** Myogenin immunostaining highlights occasional skeletal muscle fibers. **f** Diffuse LIN28 immunoexpression



of increased intracranial pressure and was found to have a large intraventricular tumor infiltrating the thalamus and superficially, the vermis of the cerebellum (Fig. 4a); the exact anatomic origin was difficult to establish on magnetic resonance imaging, but was known not to be the pineal gland which appeared intact and displaced by the tumor. Histologically, the tumor was composed of primitive small blue cells with irregular nuclear membranes and karyomegaly

◂**Fig. 5** Primary *DICER1*-associated CNS sarcoma. **a** Axial T1 magnetic resonance image demonstrating an ill-defined superficial tumor that involves the leptomeninges and cortex of the right temporal lobe. **b** Low-power H&E-stained section showing a predominantly spindle-cell neoplasm with a fascicular pattern of growth. **c** Although most of the tumor was compact, it infiltrated the cortex at the periphery. **d** Spindle-cell morphology increased cellularity and frequent mitoses. **e** Spindle-cells admixed with cells containing eosinophilic cytoplasmic tail in keeping with skeletal muscle differentiation. **f** Less cellular areas with more oval and round cellular contours and occasional rhabdomyoblasts. **g** Myogenin highlights frequent rhabdomyoblasts. **h** Membranous CD99 expression in the majority of tumor cells. **i** H3K27me3 immunostain demonstrating loss of trimethylation in a significant number of tumor cells, particularly in areas that resemble malignant peripheral nerve sheath tumor; Barr bodies are visualized as dot-like immunoexpression, consistent with female gender

in a background of neuropil (Fig. 4b). The tumor was composed of sheets exhibiting cellular overlap, and occasional multilayered rosettes and pseudo-rosettes were seen. Mitoses were abundant and necrosis and apoptotic figures were easily identified. Rhabdomyoblasts and myocytes were seen scattered throughout the tumor (Fig. 4c). A synaptophysin immunostain highlighted the neuropil and the primitive tumor cells (Fig. 4d) and the skeletal muscle fibers stained positive with myogenin (Fig. 4e). The LIN28 immunostaining was extensively positive in the tumor cells (Fig. 4f), raising the possibility of an ETMR. However, array CGH did not reveal somatic *C19MC* amplification. Furthermore, targeted exon sequencing demonstrated two *DICER1* mutations: one truncating and the other a missense affecting a hotspot with the RNase IIIb domain. The radiology finding of uninvolved pineal gland and the immunonegativity for CRX (not shown) ruled out a pineoblastoma; therefore the possibility of a *DICER1*-associated embryonal tumor was discussed in the pathology report, leading to blood-derived DNA testing which showed that the truncating *DICER1* mutation identified above [c.823G > T (p.E275*)] was germline in origin.

These rare cases highlight the importance of molecular characterization of embryonal tumors that do not fit in already well-described categories. ETMR-like infantile cerebellar embryonal tumors appear to be a rare manifestation of DICER1 syndrome, and such a diagnosis should motivate genetic analysis of the patient and tumor and referral for genetic counselling.

Primary *DICER1*-associated CNS sarcoma

Primary mesenchymal tumors of the CNS are rare and can include meningioma and solitary fibrous tumor/hemangiopericytoma, as well as a range of other less common entities. Primary CNS sarcomas pose a diagnostic challenge given their frequent lack of differentiation.

A sarcomatous histologic pattern is frequent in DICER1 syndrome tumors (PPB, embryonal rhabdomyosarcoma (ERMS) of the uterine cervix and other sites, anaplastic sarcoma of the kidney), but primary CNS sarcomas are a rare phenotype. Until recently, the only two cases documented in the literature were a cerebral sarcoma histologically indistinguishable from PPB in a 19-year-old male from a DICER1 syndrome family [48] and a brainstem ERMS in a girl with a germline *DICER1* pathogenic variant [13]. However, characteristic *DICER1* alterations are now being identified in a broader range of CNS sarcomas.

The first case of a *DICER1*-associated cerebral sarcoma was presented at the Diagnostic Slide Session at the 2017 American Association of Neuropathology Annual Meeting [2]: On magnetic resonance imaging, a 3-year-old girl of Peruvian descent with headaches was found to have a right temporal lobe tumor involving the leptomeninges and cortex (Fig. 5a). The tumor was surgically excised and light microscopic examination revealed a hypercellular mesenchymal neoplasm with syncytial and fascicular growth pattern (Fig. 5b, c). Cells with large, hyperchromatic atypical spindled nuclei were observed and mitoses were frequent (Fig. 5d). Some areas had more eosinophilic cytoplasm and rhabdomyoblastic cytology (Fig. 5e), while others were slightly less cellular and composed of round-to-oval cells admixed with rhabdomyoblasts (Fig. 5f), which were highlighted by myogenin immunostaining (Fig. 5g). The differential diagnoses included gliosarcoma, anaplastic meningioma, malignant peripheral nerve sheath tumor/malignant Triton tumor, rhabdoid tumor, synovial sarcoma, rhabdomyosarcoma, and malignant hemangyopericytoma. A resemblance to PPB Type III was appreciated, particularly in areas that were less cellular and had round-to-oval cells, but the child had no chest disease. The tumor cells were extensively immunoreactive for CD99 in a membranous pattern (Fig. 5h). H3K27me3 immunostaining was performed (given the differential diagnosis of malignant peripheral nerve sheath tumor) and showed a mosaic pattern of loss of trimethylation in a significant number of (but not all) tumor cells (Fig. 5i). GFAP, OLIG2, SOX2, SOX10, synaptophysin, CAM5.2, EMA, CD34, TLE1, STAT6, somatostatin receptor 2A, and ALK were negative, and INI1 and BRG1 were retained, ruling out most of the differential diagnoses mentioned above. Genetic testing revealed copy number alterations involving chromosomes 2, 12q (amplification) and 6q (deletion), a *KRAS* c.35G>A (p.G12D) activating mutation, and notably, two somatic *DICER1* mutations: an RNase IIIb hotspot mutation, c.5125G>A (p.D1709N), and a splice variant, c.904-1G>A. In light of the genetic findings, a diagnosis of primary *DICER1*-associated CNS sarcoma was rendered [2].

Shortly after this presentation, three of the current authors (LdK, JRP, and WDF) published the case of a child with a primary CNS sarcoma exhibiting rhabdomyoblastic differentiation and similar histology to the above-mentioned case.

Unlike the previous case, the child had a germline pathogenic variant in *DICER1*—a chromosome 14q32 deletion encompassing *DICER1*. The tumor also harbored a somatic RNase IIIb hotspot mutation on the remaining allele. This child also had an occult lung cyst, cystic nephroma, and a malignant teratoid CBME [10].

Koelsche and colleagues recently published an extensive study in which they identified a group of intracranial sarcomas (previously classified as intracranial malignant tumor, embryonal sarcoma, glioblastoma, gliosarcoma, extra-skeletal mesenchymal chondrosarcoma, primitive neuroectodermal tumor, and CNS sarcoma NOS) that exhibited a distinct methylation profile [34]: A set of 22 CNS sarcomas clustered separately from all other entities included in the methylation study. The ages of diagnosis of the 22 cases ranged from 0 to 76 years with a mean age of 6 years. The tumors exhibited similar histological features to the two sarcoma cases described above. They were composed of highly cellular areas arranged in fascicles and/or had areas of reduced cellularity with oval-to-spindle cells. Although the histological features varied, all had rhabdomyoblasts or rhabdomyoblast-like cells. A high mitotic rate was evident, and foci of necrosis were present. None of the tumors contained cartilage. Smooth muscle actin was present on immunohistochemical staining, and myogenin immunostaining highlighted occasional rhabdomyoblasts. In support of these tumors constituting a distinct entity, one or more *DICER1* alterations were detected in 21 of the 22 tumors: a germline loss-of-function *DICER1* pathogenic variant was identified in two of five patients for whom germline DNA was available and each of their tumors harbored a second somatic RNase IIIb hotspot mutation. A further 4 tumors had an RNase IIIb hotspot mutation coupled with a loss-of-function or missense alteration; 7 tumors had an RNase IIIb hotspot mutation and LOH; and in 8 tumors, only an RNase IIIb hotspot mutation was identified. 12/22 tumors also harbored mutations in *TP53*. Other genes recurrently mutated in the series included *NF1, KRAS, NRAS,* and *FGFR4* [34]. Because the germline status was known for only 5 of 22 patients and because only one *DICER1* mutation was identified in several patients, and taken together with the first report detailed above [2], it is possible that *DICER1* mutations are important in many or even all such cerebral sarcomas, without the implication that an affected patient exhibits DICER1 syndrome. Indeed, the unifying genetic and histologic profiles suggest these tumors represent a new *DICER1*-related entity. In practice, CNS sarcomas with rhabdomyoblastic differentiation should prompt consideration for primary *DICER1*-associated CNS sarcoma, genetic analysis of the tumor, and referral for germline *DICER1* genetic testing and counselling.

Other childhood CNS tumors have been reported in DICER1 syndrome families and/or in the context of *DICER1* alterations, but lack definitive evidence of association with the syndrome: A medulloblastoma was diagnosed in a 4-year-old boy who had had PPB 2 years earlier [48]. Another medulloblastoma occurred in a *DICER1* pathogenic variant heterozygote [68]. However, as discussed above, profiling of the genomic landscapes of medulloblastoma strongly suggests medulloblastoma is not a DICER1 syndrome tumor [43]. An intracranial medulloepithelioma (initially diagnosed as an ependymoma) has been mentioned without molecular documentation in a 7-month-old sister of a PPB patient [7]; an anaplastic meningeal sarcoma was noted in a 3-year-old female relative of a DICER1 syndrome patient, also without molecular findings [13]. Glioblastoma multiforme (GBM) occurred in a young patient ~ 6 years after radiation treatment for metastatic PPB (personal communication) [49], and two further cases of GBM from The Cancer Genome Atlas were found to harbor *DICER1* alterations: one GBM bore an RNase IIIb hotspot mutation with an allele frequency of 96%, and the other harbored a somatic RNase IIIb hotspot mutation in addition to a somatic in-frame deletion [1]. An atypical choroid plexus papilloma occurred in a young child with PPB Type I, and although initially postulated to be a DICER1 syndrome tumor, the choroid plexus lesion was later shown not to be *DICER1*-related following a thorough genetic workup of the case [6, 38].

Non-neoplastic CNS manifestations of DICER1 syndrome

Non-neoplastic manifestations of DICER1 syndrome are few. Developmental delay, overgrowth, and macrocephaly have been documented in two patients with mosaic *DICER1* RNase IIIb hotspot mutations [32]. Macrocephaly is frequent in *DICER1* pathogenic variant heterozygotes compared to family controls. Khan and colleagues performed a systematic study of head circumference and height in 110 participants including 67 *DICER1* heterozygotes and 43 family controls [30]. 42% of *DICER1* heterozygotes were macrocephalic (head circumference above 97th percentile, none of whom had a head circumference below the 3rd percentile) in contrast to 12% of family controls (5% of whom had head circumference below the 3rd percentile). The difference remained significant after adjusting for height [30]. The diverse ocular changes associated with *DICER1* mutation were discussed earlier.

To conclude, several of the more frequent phenotypic expressions of DICER1 syndrome were first documented just over two decades ago. In the years following, new tumoral associations have been continuing to come to light. In this review, we have discussed the CNS lesions associated with DICER1 syndrome and demonstrated how

detailed germline and tumor-based *DICER1* genetic testing has established their inclusion in the syndrome. The rarity of many of the *DICER1*-associated CNS lesions and their potential morphologic obscurity present diagnostic challenges.

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