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## **Pituitary blastoma: a pathognomonic feature of germ-line *DICER1* mutations**

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This paper is dedicated to Bernd W. Scheithauer, MD, with whom several of the co-authors have trained and/or collaborated. Bernd was a world-renowned neuropathologist with special expertise in pituitary disease. With his colleagues, Bernd described pituitary blastoma (PitB) in 2008 and he was an early participant in the work reported here. Just before his untimely death, he was thrilled to learn that several additional PitB cases had been accessioned and more so that *DICER1* mutations explain PitB and its association with other childhood blastomas in this familial tumor predisposition syndrome.

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**Abstract**

Individuals harboring germ-line *DICER1* mutations are predisposed to a rare cancer syndrome, the *DICER1* Syndrome or pleuropulmonary blastoma-familial tumor and dysplasia syndrome [online Mendelian inheritance in man (OMIM) #601200]. In addition, specific somatic mutations in the

DICER1 RNase III catalytic domain have been identified in several *DICER1*-associated tumor types. Pituitary blastoma (PitB) was identified as a distinct entity in 2008, and is a very rare, potentially lethal early childhood tumor of the pituitary gland. Since the discovery by our team of an inherited mutation in *DICER1* in a child with PitB in 2011, we have identified 12 additional PitB cases. We aimed to determine the contribution of germ-line and somatic *DICER1* mutations to PitB. We hypothesized that PitB is a pathognomonic feature of a germ-line *DICER1* mutation and that each PitB will harbor a second somatic mutation in *DICER1*. Lymphocyte or saliva DNA samples ascertained from ten infants with PitB were screened and nine were found to harbor a heterozygous germ-line *DICER1* mutation. We identified additional *DICER1* mutations in nine of ten tested PitB tumor samples, eight of which were confirmed to be somatic in origin. Seven of these mutations occurred within the RNase IIIb catalytic domain, a domain essential to the generation of 5p miRNAs from the 5' arm of miRNA-precursors. Germ-line *DICER1* mutations are a major contributor to PitB. Second somatic *DICER1* "hits" occurring within the RNase IIIb domain also appear to be critical in PitB pathogenesis.

## Keywords

*DICER1*; Pituitary blastoma; miRNA; Pediatric tumors

## Introduction

Scheithauer et al. [24] described the first case of PitB occurring in a 13-month-old female Korean child presenting with Cushing's syndrome and diabetes insipidus (case 1 in this report). A sellar and suprasellar mass measuring 3.5 cm was identified on magnetic resonance imaging (MRI). Histopathologically, the excised tumor was distinct from other pediatric adenohypophyseal tumors, exhibiting primitive Rathke-type epithelium arranged in rosettes and glandular structures, small folliculo-stellate (FS) cells and a limited range of fully differentiated secretory cells. Ultrastructurally, the tumorous pituitary resembled that of 10–12 week embryonic-stage pituitary gland [24]. The name "blastoma" was chosen by Scheithauer to reflect the embryonic-primordial appearance and neonatal presentation of these tumors.

In 2012, Scheithauer and colleagues discussed six PitB cases [23] including the 2008 case [24], one recently reported PitB [28], one newly accessioned case, and three cases previously described as pituitary adenomas [15, 16, 20]. In addition, based on the description in the reports, it was considered that three further cases probably represented PitB [13, 14, 17]. The clinical presentations and tumor histopathology of each PitB were strikingly similar, but the main predisposing genetic factors were not known.

The *DICER1* syndrome, or pleuropulmonary blastoma (PPB)-familial tumor and dysplasia syndrome [online Mendelian inheritance in man (OMIM) #601200], is caused by heterozygous germ-line mutations in the *DICER1* gene, which encodes DICER1, a small RNA processing endoribonuclease that cleaves precursor microRNAs (miRNA) into mature miRNAs which, in turn, post-transcriptionally regulate messenger RNA expression [11]. The main manifestations of the *DICER1* syndrome include PPB, cystic nephroma (CN),

Sertoli–Leydig cell tumors (SLCT), multinodular goiter (MNG) and other rare childhood sarcomas and dysplasias [2, 5, 21, 25]. The majority of these known manifestations of the syndrome are fetal, pediatric or adolescent in onset.

In 2011, we identified an inherited *DICER1* mutation in an infant with PitB who had a family history strongly suggestive of *DICER1* syndrome (Supplementary Figure S1: case 3) [28]. We subsequently sought to determine the frequency of germ-line *DICER1* mutations in other children with PitB, as well as to test PitB tumors for the presence of second somatic mutations in *DICER1*.

## Materials and methods

The study was approved by the Institutional Review Board of the Faculty of Medicine of McGill University, Montreal, Quebec, Canada, no. A12-M117-11A. Participants were recruited to the study in compliance with the second edition of the Canadian Tri-Council Policy Statement of Ethical Conduct of Research involving Humans and, because of the ages of the participants, eligible relatives signed a consent form in accordance with the above-mentioned IRB protocol. All potential PitB tumors were reviewed by our central reference pathologists, Dr. Eva Horvath (EH) and Dr. Kalman Kovacs (KK).

### Sample acquisition

We ascertained nine published cases (cases 1–5 and 7–10) and four unpublished cases (cases 6 and 11–13) (Table 1). The cases presented in this manuscript are numbered in the order in which they were ascertained by our group. As detailed in Fig. 1 and Supplementary Table S1, we collected PitB and non-tumor tissue samples as follows: formalin-fixed paraffin-embedded (FFPE) tumor for ten cases, fresh frozen tumor for two cases and FFPE normal tissue for three cases. We obtained extracted tumor DNA from one case and were unable to obtain tumor tissue from two cases. No germ-line DNA samples were attainable from two cases. We obtained DNA extracted from blood lymphocytes from five cases. Genomic DNA was extracted from 3 to 5 ml of blood collected in EDTA blood collection tubes from one case using the Genra Puregene Blood kit (Qiagen, California, USA) following the manufacturer's instructions. The Genra Puregene Blood kit was also used to extract genomic DNA from saliva samples collected from two cases using the Oragen DNA OG-250 DNA collection kit (DNA Genotek, Ottawa, Canada) (Supplementary Table S1). Therefore, of the thirteen cases, we had the ability to determine the origin of the identified mutations for ten cases as both germ-line and somatic samples were available (case 2, cases 4–6 and cases 8–13); for case 1, we had a tumor sample only; for case 3, only germ-line gDNA was available; and for case 7, we were unable to acquire a germ-line and/or somatic DNA sample (Fig. 1 and Supplementary Table S1).

### Molecular screening of *DICER1*

In seven cases, screening for germ-line *DICER1* mutations was conducted on DNA extracted from blood or saliva by PCR and sequencing of the region of interest [7, 22, 30]. In one case where no germ-line *DICER1* mutation was identified by conventional sequencing, we screened for large deletions or duplications using our multiplex ligation-

based probe amplification assay [22]. For four cases, germ-line *DICER1* mutations were screened for using the Fluidigm access array system and next-generation sequencing (Supplementary Table S1). Fluidigm access array system involves an array-based PCR amplification of a specific region of interest (target enrichment). For our purpose, the exons of *DICER1*, all exon–intron boundaries and the 3'UTR were selectively targeted. Parallel amplification of 48 samples was carried out using custom design primers (Supplementary Table S2), designed using Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/>) and to which CS1 and CS2 tails were added. Samples were barcoded during the targeted enrichment to allow for multiplexed sequencing and amplicons were tagged with adaptor sequences during the PCR amplification reaction. Next-generation sequencing was carried out using the Illumina MiSeq (McGill University and Genome Quebec Innovation Centre (MUGQIC)) and the Integrative Genomics Viewer software (IGV version 2.3; <http://www.broadinstitute.org/igv/>) was used to analyze the dataset. All variants identified were confirmed with Sanger sequencing.

Somatic “hotspot” mutations were identified by PCR amplification of gDNA derived from each PitB [29, 30] followed by Sanger sequencing (MUGQIC). Loss of heterozygosity (LOH) analysis in tumor samples was performed by amplifying tumor gDNA by PCR concurrently with the patient's germ-line gDNA, using primers specific to the region of interest. The ~150–200 base-pair PCR products were analyzed by direct Sanger sequencing and the relative intensity of the peaks at the position of the germ-line *DICER1* mutation and/or single-nucleotide polymorphisms (SNPs) within the 3'UTR of the gene were compared between germ-line and tumor gDNA to determine whether LOH occurred (Supplementary Table S3). For one case, genotyping of the short tandem repeat (STR) markers, D14S265 and D14S1054, was performed using  $\gamma$ -P33 as previously described [27] to ascertain LOH in the absence of coding variants that could be interrogated using Sanger sequencing.

### Pathological review

Suspected PitB cases were evaluated by EH and KK using light microscopy, immunohistochemistry, and transmission electron microscopy. For the histologic investigation, FFPE tissue was used. The electron microscopic study was performed on glutaraldehyde fixed and osmicated material.

### Immunohistochemistry

Immunohistochemical staining for adrenocorticotrophic hormone (ACTH) and growth hormone (GH) was performed using the streptavidin–biotin–peroxidase complex method, using antibodies directed against ACTH and GH. Details of the methods including source and dilution of antibodies have been described previously [8, 10]. Immunostaining of Ki-67 and p53 was performed on formalin-fixed, paraffin-embedded sections, 7  $\mu$ m in thickness, that were de-paraffinized using xylol and rehydrated in serial aqueous dilutions of alcohol. Immunohistochemistry was performed after heat induced epitope antigen retrieval using citraconic acid at pH 7.4 and 45 min at 100 °C. Monoclonal mouse anti-human Ki-67 antigen antibody (Dako clone MIB-1) was used at a dilution of 1:100 and monoclonal mouse anti-human p53 antibody (Dako clone DO-7) was used at a dilution of 1:50. For both



antibodies, immunostaining was performed using a DAKO Auto-stainer Link 48 and with DAKO EnVision™ Flex visualization system.

## Results

We investigated 13 cases of PitB, the features of which are summarized in Table 1. Twelve of the 13 cases were reviewed by EH and KK, and the remaining case was included based on convincing published evidence [12, 20, 26]. Four cases were newly ascertained; nine have been previously reported between 1979 and 2012. Eight of the infants were females and five were males. Ages at notice of first symptom ranged from 7 to 24 months (median 8 months) and the ages at pathological diagnosis ranged from 7 to 24 months (median 9 months). Of the 13 infants, five died of disease within 0–26 months of the first surgery (median 8 months) and eight remain alive, with survival time from first surgery to last follow-up ranging from 21 months to 17.4 years (median 5.9 years). Two children with PitB had a personal medical history of other diseases associated with a *DICER1* mutation; a further three children had a family history of diseases suggesting *DICER1* syndrome; and in eight children, PitB was the only notable disease (Table 1). Family pedigrees are depicted in Supplementary Figures S1 to S6.

Cushing's syndrome and/or ophthalmoplegia were the most frequent presenting symptoms of PitB. Elevated blood ACTH levels were demonstrated in seven of the eight patients for which data are available (Table 1). Figure 2 presents the diagnostic MR images and mutation traces from case 12.

Morphologically, all cases showed the classic features of PitB as previously described by Scheithauer and colleagues [23, 24] (Fig. 3). This included a combination of Rathketype epithelial rosettes/glands, small primitive appearing cells and secretory cells, the latter of which were synaptophysin and chromogranin immunoreactive and always expressed ACTH in at least a subset of cells. Some cases also included a GH secreting subset (Fig. 3b, c-I, c-II), whereas ultrastructural features of FS cells were also commonly identified in a subset of tumor cells. However, differences in the proliferative and mitotic activity were evident in some cases. Case 12 was noted to have marked nuclear labelling for Ki-67 (Fig. 4a) and thus had a high proliferative fraction. The tumors of case 3, case 5 [23] and case 11 were similarly markedly proliferative. In contrast, the Ki-67 nuclear labelling in case 2 was estimated at 1.63 % (Fig. 4b), which is low despite the large tumor size detected at presentation. Nuclear expression of p53 protein was present in the cells that form the rosettes, but was scant elsewhere (Fig. 4c, d).

*DICER1* mutation data from each case are summarized in Table 1 and Fig. 5 with additional details including samples analyzed and mutation traces presented in Fig. 1, Supplementary Table S1 and Supplementary Table S4. We were able to obtain germ-line DNA from eleven children with PitB (ten of which were successfully screened for *DICER1* mutations) and PitB tumor tissue from a different set of 11 cases. In one case reported in 1979, we were unable to extract reliable gDNA from FFPE to allow for molecular testing (Supplementary Table S1). Of the ten cases analyzed, nine children with PitB harbored a germline *DICER1* mutation, among which, one germ-line mutation was predicted to affect the critical RNase



IIIb catalytic site of *DICER1*. Nine of ten PitB tumor tissue specimens analyzed had *DICER1* mutations, eight of which are confirmed to be somatic in origin where germ-line gDNA was available. Of the nine *DICER1* mutations identified in the tumor samples, seven are predicted to affect the RNase IIIb catalytic site of *DICER1* (Fig. 5 and Supplementary Table S1), consistent with the vast majority of other reported somatic mutations for *DICER1*-associated diseases (i.e., that they occur within exons encoding the RNase IIIb site). The remaining two somatic mutations identified were LOH of the wild-type allele within the tumor. Overall, we demonstrated *DICER1* mutations in 11 of 12 PitB cases for which we had germ-line and/or tumor sample(s): both germ-line and somatic mutations were present in seven of 12 PitB cases; whereas a germ-line mutation only was found in two cases; a somatic mutation only in one case; one case harbored an RNase IIIb missense mutation in the tumor sample, but was not confirmed to be somatic in origin; and in one case, genetic screening of FFPE gDNA was not successful (Fig. 5 and Supplementary Table S4).

## Discussion

The results presented here expand the spectrum of the *DICER1* syndrome to include PitB as a highly characteristic phenotype with a high likelihood of germ-line and/or somatic *DICER1* mutations. This tumor may now be considered a rare but pathognomonic manifestation of a germ-line *DICER1* mutation. The penetrance appears to be well below 1 %. The clinical phenotype is noteworthy in that the primary manifestation is the onset of Cushing's syndrome during infancy, an exceedingly rare endocrinopathy in this age group. Even in the absence of a formal genetic diagnosis, this clinical presentation should motivate the clinician to look for other *DICER1*-related diseases in the patient and family and to consider PitB a strong diagnostic possibility.

The inclusion of PitB in the blastoma category was based on several factors delineated by Scheithauer [24]. The term “blastoma” implies that the tumor has the appearance of pituitary embryonic tissue and exhibits malignant potential, but questions have been raised as to whether PitB is indeed clinically malignant. Many of the PitBs in our study behave aggressively and were fatal in approximately 40 % of cases (Table 1). Death of these infants could be due to local tumor effects causing increased intracranial pressure and damage to surrounding tissues or due to excess serum cortisol causing severe, often lethal, Cushing's disease. One child had an aggressive recurrence with apparent intraventricular metastases, but histologic confirmation of the CNS disease was not possible. As noted in the results, cell proliferation, as measured by Ki-67 labelling index, within the PitBs studied was variable with four cases exhibiting high proliferative activity (case 3, 5 [23], 11 and 12) and one showing minimal proliferation (case 2) (Fig. 4). Three of the four children with PitBs showing high proliferative activity had recurrences and all four remain alive. These limited data suggest that the Ki-67 proliferation index has uncertain predictive value for PitB, although it should be studied further. The implications of p53 expression in the cells forming the rosettes similarly require further investigation. Perhaps both indolent and more active PitBs exist and as yet, we have only a limited understanding of the biologic behavior of this dysontogenic lesion.

There is also speculation surrounding the cell of origin of PitB. Histological studies suggest perturbation of pituitary stem cells could underlie the pathogenesis of these tumors and the recent advances in the identification of cancer stem cells in other lesions [3, 4] further substantiates this argument. The identification of *DICER1* mutations in our cohort of PitBs (predicted to alter the miRNA profiles of the tumors) taken together with emerging data showing that miRNAs play a role in regulating stem cell markers [19] and the presence of FS “stem cells” within PitB [23] provides a possible explanation of pituitary dysontogenesis leading to PitB that could be explored.

Eight of the nine germ-line mutations identified are loss-of-function mutations that are predicted to inactivate one allele of *DICER1*, suggesting that germ-line mutation of *DICER1* is a key predisposing genetic event. Five of the germ-line *DICER1* mutations we identified are known to be inherited and two are de novo mutations (Fig. 2f and Supplementary Figure S1 to S6). Seven of the nine *DICER1* mutations identified within the tumor samples (eight of which are confirmed to be somatic in origin) were localized within the sequence encoding the protein's RNase IIIb domain, thereby affecting highly conserved amino acid residues (Asp1709, Gly1809, Glu1813) (Fig. 5). Mutations affecting the metal ion-binding amino acid residues of *DICER1*, Glu1705, Asp1709, Asp1810 and Glu1813, have been shown to reduce the processing of mature 5p miRNA strands, shifting the mature miRNA expression within these tumors towards 3p-derived miRNAs, a shift in expression thought to contribute to tumorigenesis [1, 6]. The somatic mutation identified in case 13 was LOH of one allele evident at two STR markers, D14S1054 and D14S265 (Supplementary Figure S7). No germ-line mutation was found within the coding region of *DICER1* in this patient, but we suspect that a non-coding mutation that deleteriously affects the expression of the transcript from the other allele may be present. In five cases where two *DICER1* mutations were not identified, the genetic analysis of *DICER1* in an exon-to-exon approach remains incomplete either due to difficulty amplifying DNA or because we did not have tissue available to us to complete the analysis. We predict that all cases will have two *DICER1* mutations, hypothesizing that a second somatic “hit” (most often affecting an RNase IIIb metal ion-binding residue of *DICER1*) in the embryonic pituitary is required in addition to a loss-of-function germ-line *DICER1* mutation to initiate development of an embryonic-appearing blastomatous tumor.

The germ-line and somatic *DICER1* mutations identified in case 12 require further discussion. The de novo mutation, c.5125G>C [p.(Asp1709His)], is the third reported *DICER1* mutation in lymphocyte gDNA affecting a metal ion-binding residue within an RNase III domain [9]. Approximately, 75 previously reported germ-line *DICER1* mutations are distributed throughout the gene. In contrast, almost all reported somatic mutations in *DICER1* syndrome diseases affect the metal-binding residues. The other two reports of de novo missense mutations directly affecting a metal ion binding site within an RNase III domain were determined by the researchers to be mosaic mutations and were associated with overgrowth, bilateral Wilms tumor and bilateral lung cysts in both of their patients. There was no evidence of overgrowth in our case, but the clinical presentation in this child was particularly severe. In addition to the PitB, the infant has extensive multi-focal bilateral lung cysts (likely PPB Type I or Type Ir) and bilateral renal cystic masses (likely CN) which

have not been biopsied (Fig. 2a, b). Furthermore, with only lymphocyte gDNA and PitB tumor tissue available to us, we were unable to determine whether the child is a mosaic for the observed p.(Asp1709His) mutation. The somatic “hit” in case 12 was identified to be loss of the wild-type allele within the tumor (Fig. 2e), which together with the germ-line mutation, would result in the absence of expression of any normal *DICER1* within the affected cell lineages in the developing pituitary gland. We speculate that a germ-line *DICER1* mutation affecting one of the metal ion-binding sites may result in especially severe clinical disease as in our case 12 and the two children previously reported [9]. In case 1, we identified an RNase IIIb mutation (c.5437G>A; p.[Glu1813Lys]) in FFPE tumor gDNA. In contrast to case 12, LOH was not evident within the tumor. Without a germ-line gDNA sample available, we are unable to confirm whether the identified mutation in case 1 is somatic in origin. Although consistent with somatic mutations most often identified within *DICER1*-related tumors, based on the severe clinical presentation of case 1 (Table 1), perhaps this RNase IIIb *DICER1* mutation is in fact a germ-line mutation. As with case 12, the clinical presentation of case 7 was particularly severe (Table 1), but no tissue was available for molecular analysis.

The specific downstream miRNA perturbations of the germ-line and somatic *DICER1* mutations identified and the mechanisms of tumorigenesis remain to be explored, but may be related to a relative excess of 3p-derived miRNAs as postulated [1, 6] and as recently reported in a case of PPB [18]. Further studies on this rare pediatric tumor should focus on mRNA, miRNA and gDNA profiling and will include explorations of the mechanistic implications of *DICER1* mutations found in PitB. We encourage submission of any suspected PitB case for expert pathological review and intensive molecular study.

## Conclusion

The results of this study suggest that a germ-line *DICER1* mutation is the major and possibly sole predisposing genetic contributor to development of a PitB. The acquisition of a somatic mutation in *DICER1* in the RNase IIIb domain also appears to be a critical second “hit” in PitB pathogenesis. The particularly, complex clinical situation surrounding children with PitB also requires communication between clinicians, neuropathologists and pediatric oncologists to help determine the most effective management of these infants.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

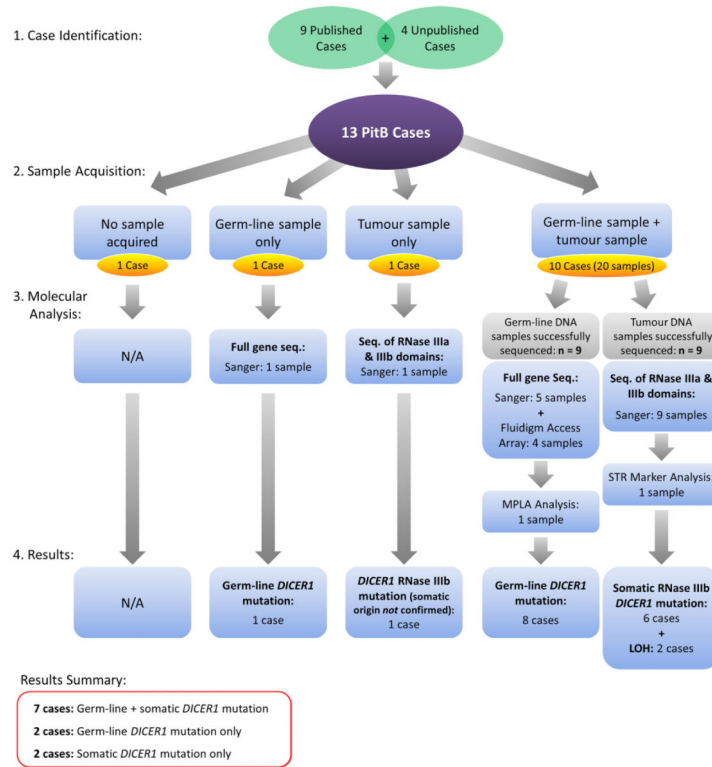
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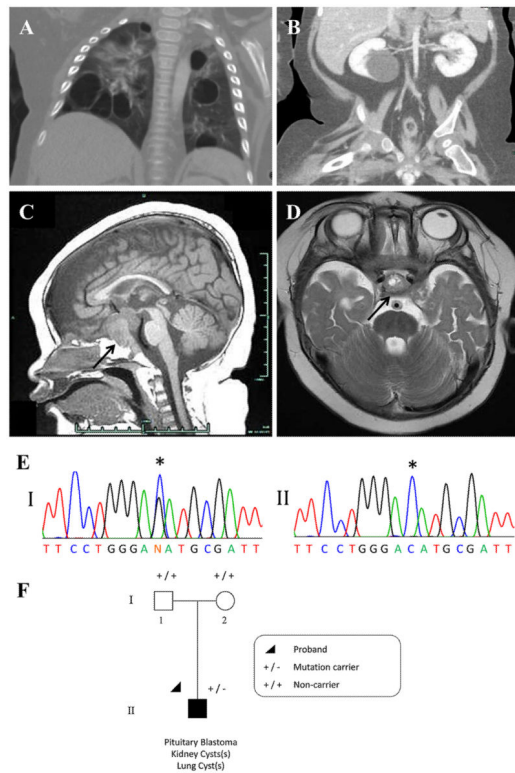
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**Fig. 1.** Flowchart summarizing case identification, sample acquisition, molecular analysis and results of the study

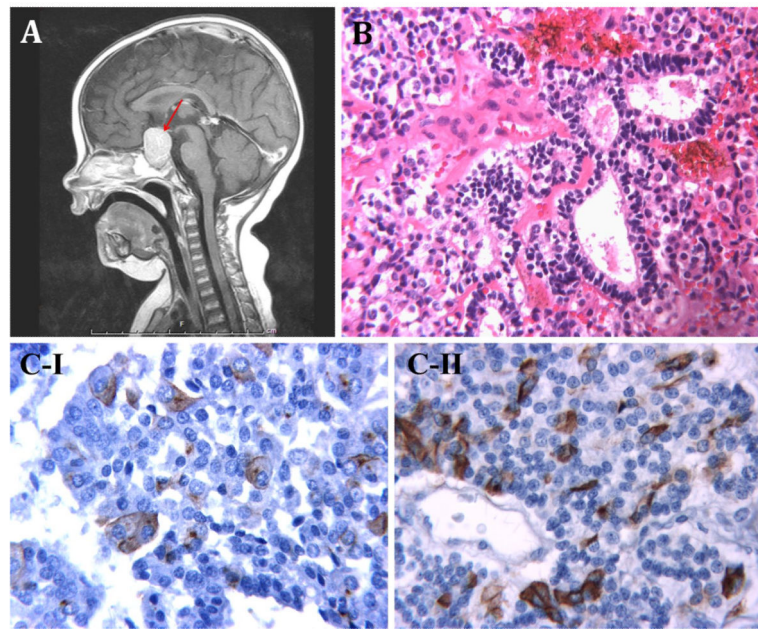




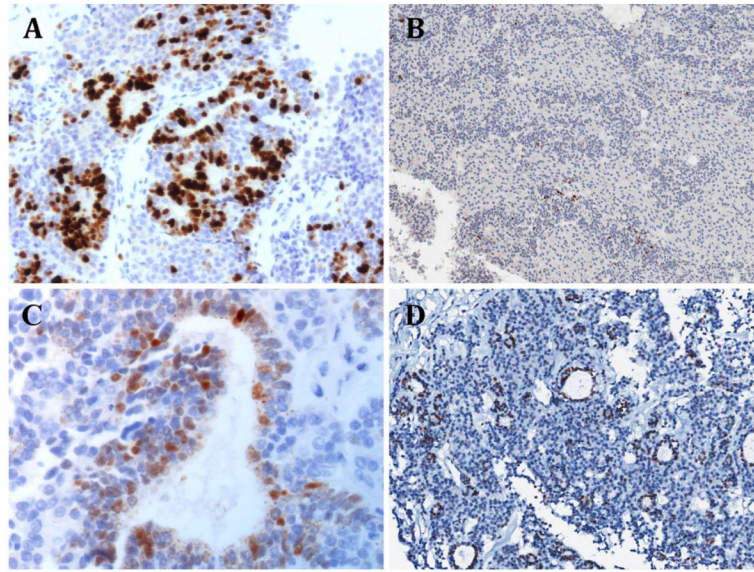
**Fig. 2.**

Case 12: **a** chest CT following IV contrast: multiple bilateral thin-walled air filled cysts evident within all lobes of the lungs; likely PPB Type I or Ir (not biopsied). **b** Coronal CT image of abdomen and pelvis. Liquid-filled cyst in lower pole of right kidney is typical of CN (not biopsied). Other CT images (not shown) also revealed a smaller cyst (likely CN, not biopsied) in the left kidney. **c** T1 midline sagittal MR image. **d** T2-weighted axial MR image just superior to the pituitary. In **c** and **d**, the pituitary tumor is indicated with *arrows*. **e Panel I** a mono-allelic germ-line *DICER1* mutation, c.5125G>C [p.(Asp1709His)]. **Panel II** clear loss of heterozygosity at the position of the germ-line *DICER1* mutation within the tumor (wild-type allele lost). **f** The proband (individual II-1) was diagnosed with PitB at the age of 8 months and was found to carry the de novo c.5125G>C germ-line *DICER1* mutation

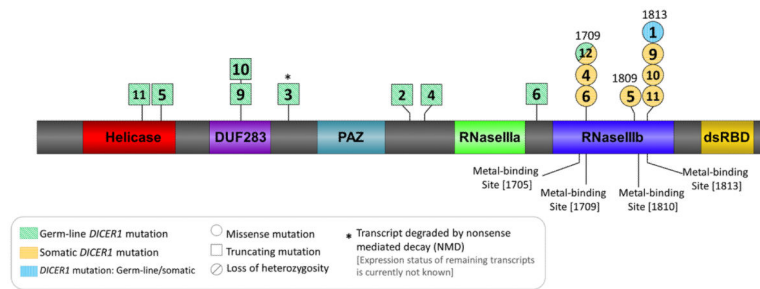




**Fig. 3.**  
**a** case 13, T1-weighted post-contrast midline sagittal MR image showing pituitary region mass (*red arrow*). **b** case 4, hematoxylin and eosin (H&E) staining  $\times 250$ : three enlarged follicles lined by stem cells. **c** Immunohistochemical staining *I* case 10, Growth hormone (GH) immunostaining  $\times 400$ : enlarged GH/alpha subunit cells immunopositive for GH. *II* case 10, ACTH immunostaining  $\times 400$ : small vessel surrounded by stem cells. Some cells display ACTH immunoreactivity



**Fig. 4.** Immunohistochemical staining **a** case 12, Ki-67 labelling  $\times 200$ : there is marked labelling for Ki-67, indicating a high proliferative fraction that is limited to the rosette-like epithelial structures. **b** Case 2, Ki-67 labelling  $\times 100$ : Ki-67 labelling index estimated at 1.63 %, indicating low proliferative activity. **c** Case 12, p53 immunostaining  $\times 400$ . **d** Case 2, p53 immunostaining  $\times 100$ . For **c** and **d**, p53 expression is present in cells forming the rosette structures, but is scant elsewhere

**Fig. 5.**

Graphic representation of the *DICER1* protein structure (NP\_001258211.1) indicating the approximate positions of the germ-line (*green diagonal stripes*) and somatic (*orange horizontal stripes*) *DICER1* mutations observed in the 13 cases being reported. Mutation shaded with *blue vertical stripes* represent mutations that were identified within tumour gDNA, but are not confirmed to be somatic in origin. Case number indicated at the position of each mutation: case 1: somatic *DICER1* amino acid change: p.(Glu1813Lys). Case 2: germ-line *DICER1* amino acid change: p.(Asn1093\*). Case 3: germ-line *DICER1* amino acid change: p.(Tyr793\*), (NMD of mutant). Case 4: germ-line *DICER1* amino acid change: p.(Ser1179Thrfs\*12); Somatic *DICER1* amino acid change: p.(Asp1709Thr). Case 5: germ-line *DICER1* amino acid change: p.(Arg509\*); somatic *DICER1* amino acid change: p.(Gly1809Trp). Case 6: germ-line *DICER1* amino acid change: p.(Asp1437Metfs\*16); somatic *DICER1* amino acid change: p.(Asp1709Asn). Case 9: germ-line *DICER1* amino acid change: p.(Arg676\*); somatic *DICER1* amino acid change: p.(Glu1813Asp). Case 10: germ-line *DICER1* amino acid change: p.(Arg676\*); somatic *DICER1* amino acid change: p.(Glu1813Val). Case 11: germ-line *DICER1* amino acid change: p.(Lys429Alafs\*47); somatic *DICER1* amino acid change: p.(Glu1813Lys). Case 12: germ-line *DICER1* amino acid change: p.(Asp1709His); somatic *DICER1* change: loss of heterozygosity

Table 1

## Literature and case summary

Case #	Sex	1st Sx; age (months)	Age at Dx of PitB (months)	Endocrine function at presentation	Clinical outcome (time from 1st surgery to last FU appointment)	Evidence of <i>DICER1</i> syndrome?	Germ-line <i>DICER1</i> mutation	Somatic <i>DICER1</i> mutation	References
Case 1	F	Cushing's, DI; 13	13	↑ ACTH; nl: PRL, GH; ↓: TSH, LH, FSH	Deceased 1.5 months post-surgery	None	Not available	c.5437 G>A <sup>a</sup>	Scheithauer et al. [24]
Case 2	F	Strabismus, ↓ visual acuity, short stature; 24	24	↑ ACTH; nl: "other markers"	Alive (7 years)	None	c.3277_3280delAACT	No RNase IIIa or IIIb mutation; No LOH	Minet al. [16]; Present case 3 in Scheithauer et al. [23]
Case 3	M	Strabismus, proptosis, hypothyroidism; 9	9	↓ TSH; nl: bone age and growth; ↑ ACTH at 11 months	Alive (3.11 years)	Family: PPB, CN, RC, OS/CT	c.2379T>G (NMD of mutant)	Not available	Present case 1 in Scheithauer et al. [23], Wildt-Runge et al. [28]
Case 4	F	↑ Weight, fatigue; 6	23	↑ ACTH	Alive (7.5 years)	Family: thyroid nodule and hypothyroidism	c.3535_3538delTCTT	c.5125G>T	Moriarty et al. [17]
Case 5	F	Ophthalmoplegia, ptosis; 13	13	nl: TSH, PRL, somatomedin C. (ACTH not measured)	Alive (4.8 years)	None	c.1525C>T (de novo)	c.5425G>T	Present case 2 in Scheithauer et al. [23]
Case 6	M	Strabismus; 5	7	nl: Free T4, TSH; ↑ IGF2, IGFBP blood AFP	Deceased 8 months post-surgery	None	c.4309_4312delGACT	c.5125G>A	Not previously published
Case 7	M	Cushing's; 6	11	↑ Cortisol; nl: ACTH, TSH, GH, PRL; ↓ bone age	Deceased 26 months post-surgery—died of tumor	Bilateral lung cysts; bilateral RC	Not Available	Not Available	Levy et al. [12], Pullins et al. [20], Sumner et al. [26]
Case 8	M	↑ Weight, ↓ height (4); Cushing's (6)	8	↑: ACTH, cortisol; nl: LH, FSH; ↓: TSH, PRL	Deceased at 0 months post-surgery (died post-op)	None	DNA quality too poor	DNA quality too poor	Miller et al. [15]
Case 9	F	↑ Weight, fatigue; 5	7	↑: ACTH, TSH; nl: PRL, GH, HCG	Alive (17.4 years)	None	c.2026C>T	c.5439G>T	List et al. [13]

Case #	Sex	1st Sx; age (months)	Age at Dx of PHB (months)	Endocrine function at presentation	Clinical outcome (time from 1st surgery to last FU appointment)	Evidence of <i>DICER1</i> syndrome?	Germ-line <i>DICER1</i> mutation	Somatic <i>DICER1</i> mutation	References
Case 10	F	DI, ↓ weight, short stature; 12	12	↑ Cortisol; ↓ bone age	Deceased 18 months post-surgery	None	c.2026C>T	c.5438A>T	Maeder et al. [14]
Case 11	F	Hydrocephalus; 7	7	Not known <sup>b</sup>	Alive (23 months)	Family: OSLECT, Thyroid nodule	c.1284delGA	c.5437 G>A	Not previously published
Case 12	M	Cushing's; 8	8	↑: ACTH	Alive (21 months)	Multi-focal bilateral lung cysts & RC	c.5125G>C(de novo)	LOH	Not previously published
Case 13	F	Strabismus, ↑ ICP, "plump" (Cushing's not suspected); 9	9	↑: Cortisol; ↓ TSH; nl: PRL, GH, FT4	Alive (13.4 years)	None	Negative	LOH	Not previously published

*ACTH* adrenocorticotrophic hormone; *AFP* alpha-fetoprotein; *CN* cystic nephroma; *CT* computerized tomography; *Cushing's* Cushing's syndrome with cushingoid facies, truncal obesity, hyper-trichosis with or without acne vulgaris, hypertension and short stature; *DI* diabetes insipidus; *Dx* diagnosis; *F* female; *FSH* follicle stimulating hormone; *F-U* follow-up; *GH* growth hormone; *HCG* human chorionic gonadotropin; *IGF* insulin-like growth factor; *IGFBP* insulin-like growth factor binding protein; *LH* luteinizing hormone; *LOH* loss of heterozygosity; *M* male; *Mo.* months; *nl* normal; *NMD* nonsense-mediated decay; *OSLECT* ovarian Sertoli-Leydig cell tumor; *post-op* post-operatively; *PPB* pleuropulmonary blastoma; *PRL* prolactin; *RC* renal cysts; *Sx* symptoms; *TSH* thyroid stimulating hormone; ↑ increased; ↓ decreased

<sup>a</sup> Mutation not confirmed to be somatic in origin

<sup>b</sup> Due to precipitous admission and operation, pre-operative endocrine function test results are not available