Acta Neuropathol (2014) 128:583–595
DOI 10.1007/s00401-014-1318-7

ORIGINAL PAPER

Germ-line and somatic *DICER1* mutations in pineoblastoma

Leanne de Kock · Nelly Sabbaghian · Harriet Druker · Evan Weber · Nancy Hamel · Suzanne Miller · Catherine S. Choong · Nicholas G. Gottardo · Ursula R. Kees · Surya P. Rednam · Liselotte P. van Hest · Marjolijn C. Jongmans · Shalini Jhangiani · James R. Lupski · Margaret Zacharin · Dorothée Bouron-Dal Soglio · Annie Huang · John R. Priest · Arie Perry · Sabine Mueller · Steffen Albrecht · David Malkin · Richard G. Grundy · William D. Foulkes

Received: 28 May 2014 / Revised: 26 June 2014 / Accepted: 28 June 2014 / Published online: 15 July 2014
© Springer-Verlag Berlin Heidelberg 2014

Abstract Germ-line *RB-1* mutations predispose to pineoblastoma (PinB), but other predisposing genetic factors are not well established. We recently identified a germ-line *DICER1* mutation in a child with a PinB. This was accompanied by loss of heterozygosity (LOH) of the wild-type allele within the tumour. We set out to establish the prevalence of *DICER1* mutations in an opportunistically ascertained series of PinBs. Twenty-one PinB cases were studied: Eighteen cases had not undergone previous testing for *DICER1* mutations; three patients were known carriers of germ-line *DICER1* mutations. The eighteen PinBs were sequenced by Sanger and/or Fluidigm-based next-generation sequencing to identify *DICER1* mutations in blood gDNA and/or tumour gDNA. Testing for somatic *DICER1* mutations was also conducted on one case with a known germ-line *DICER1* mutation. From the eighteen PinBs, we identified four deleterious *DICER1* mutations, three of which were germ line in origin, and one for which a germ line versus somatic origin could not be determined; in all four, the second allele was also inactivated leading to complete loss of DICER1 protein. No somatic *DICER1* RNase IIIb mutations were identified. One PinB arising in a germ-line *DICER1* mutation carrier was found to have LOH. This

Electronic supplementary material The online version of this article (doi:10.1007/s00401-014-1318-7) contains supplementary material, which is available to authorized users.

L. de Kock · W. D. Foulkes
Department of Human Genetics, McGill University, Montreal, QC, Canada

L. de Kock · N. Sabbaghian · W. D. Foulkes (✉)
Department of Medical Genetics, The Lady Davis Institute, Segal Cancer Centre, Jewish General Hospital, 3755 Cote St. Catherine Road, Montreal, QC H3T 1E2, Canada
e-mail: william.foulkes@mcgill.ca

H. Druker
Division of Haematology/Oncology, The Hospital for Sick Children, The University of Toronto, Toronto, ON, Canada

H. Druker
Department of Molecular and Medical Genetics, The University of Toronto, Toronto, ON, Canada

E. Weber · N. Hamel · W. D. Foulkes
Department of Medical Genetics, Research Institute of the McGill University Health Centre, Montreal, QC, Canada

N. Hamel · W. D. Foulkes
Program in Cancer Genetics, Department of Oncology and Human Genetics, McGill University, Montreal, QC, Canada

S. Miller · R. G. Grundy
Children's Brain Tumour Research Center, School of Clinical Sciences, Queen's Medical Centre, University of Nottingham, Nottingham, UK

C. S. Choong
Child and Adolescent Health Service, Subiaco, WA, Australia

C. S. Choong
Department of Endocrinology and Diabetes, Princess Margaret Hospital for Children, Perth, WA, Australia

N. G. Gottardo
Department of Paediatric Oncology/Haematology, Princess Margaret Hospital for Children, Perth, Australia

N. G. Gottardo
Division of Children's Leukaemia and Cancer Research, Telethon Kids Institute, The University of Western Australia, Perth, Australia

U. R. Kees
Division of Children's Leukaemia and Cancer Research, Telethon Kids Institute, The University of Western Australia, Perth, Australia

study suggests that germ-line *DICER1* mutations make a clinically significant contribution to PinB, establishing *DICER1* as an important susceptibility gene for PinB and demonstrates PinB to be a manifestation of a germ-line *DICER1* mutation. The means by which the second allele is inactivated may differ from other *DICER1*-related tumours.

Keywords *DICER1* · miRNA processing · Paediatric brain tumours · Pineal gland · Childhood cancer · Mutation · Pineoblastoma · OMIM #601200

Introduction

Pineoblastoma (PinB) is a rare primitive neuroectodermal tumour (PNET) arising in the pineal gland. PinBs are classified as a WHO grade IV tumour and comprise one-fourth to one-half of pineal parenchymal tumours [12, 40]. The mean age of onset is 12.6 years but with a wide range of 1–39 years [12, 26]. PinBs are uncommon tumours, although there is one familial example reported [23, 30]. Due to the rarity of PinB, little is known about their underlying biology and genetics. Germ-line mutations in the retinoblastoma (Rb) gene *RB-1* can lead to PinB in the so-called "trilateral Rb" [20] and there is about a 1 % incidence of PinB among children with Rb who are treated with current protocols [35, 47]. Children with a family history of Rb, and those treated by external beam radiation therapy (EBRT) have a five- to tenfold higher incidence of PinB compared with those without a family history or not treated with EBRT [4, 27]. A notable reduction in incidence of PinB could be related to a preventive effect of chemotherapeutic treatment of Rb [43], the withholding of EBRT [25], or a combination of the two. However, since the incidence of PinB is ten times higher in bilateral Rb than in unilateral Rb (0.5 % among unilateral Rb; 5–13 % among sporadic bilateral Rb; 5–15 % among familial bilateral Rb) [20, 35], germ-line mutations in *RB-1* are likely to be a major predisposing factor. Despite this, it is not known what proportion of unselected PinBs carry germ-line *RB-1* mutations. Moreover, the importance of other predisposing genetic factors is not established.

Recently, we published a case report of a child with PinB and a germ-line *DICER1* mutation; loss of heterozygosity (LOH) of the wild-type *DICER1* allele was detected within the tumour [38]. Neither of these two events had been previously reported. We subsequently set out to establish (a) the prevalence of germ-line and somatic *DICER1* mutations in PinB and (b) the mechanism by which the somatic hits occur in PinB.

Germ-line mutations in *DICER1* predispose individuals to a distinctive autosomal dominant tumour/dysplasia predisposition syndrome with only moderate penetrance

S. P. Rednam
Department of Pediatrics, Hematology-Oncology, Baylor College of Medicine, Houston, TX, USA

L. P. van Hest
Department of Clinical Genetics, VU University Medical Center, Amsterdam, The Netherlands

M. C. Jongmans
Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands

S. Jhangiani · J. R. Lupski
Human Genome Sequencing Center, Baylor College of Medicine, Houston, TX, USA

J. R. Lupski
Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

J. R. Lupski
Texas Children's Hospital, Houston, TX, USA

M. Zacharin
Department of Endocrinology and Diabetes, Royal Children's Hospital, Parkville, Melbourne, VIC, Australia

D. Bouron-Dal Soglio
Department of Pathology, CHU-Sainte Justine and University of Montreal, Montreal, QC, Canada

A. Huang
Division of Hematology, The Hospital for Sick Children, Toronto, ON, Canada

J. R. Priest
Minneapolis, MN, USA

A. Perry
Departments of Pathology and Neurological Surgery, UCSF Medical Center, San Francisco, CA, USA

S. Mueller
Departments of Neurology, Pediatrics and Neurosurgery, UCSF Medical Center, San Francisco, CA, USA

S. Albrecht
Department of Pathology, Montreal Children's Hospital and McGill University, Montreal, QC, Canada

D. Malkin
Division of Haematology/Oncology, The Hospital for Sick Children, The University of Toronto, Toronto, ON, Canada

D. Malkin
Departments of Pediatrics and Medical Biophysics, The University of Toronto, Toronto, ON, Canada

D. Malkin
Department of Paediatrics, University of Toronto, Toronto, ON, Canada

(OMIM #601200) resulting in quite rare diseases of children and young adults. Included are pleuropulmonary blastoma [7, 33, 42], cystic nephroma [2, 11], Wilms tumour [49] and rare anaplastic sarcoma of kidney [11], multinodular goitre [36] and differentiated thyroid carcinoma [9], ovarian sex cord stromal cell tumours, especially Sertoli–Leydig cell tumours [15, 48], embryonal rhabdomyosarcoma of the uterine cervix [46] and other sites [10], ciliary body medulloepithelioma [31], nasal chondromesenchymal hamartoma [32], pituitary blastoma [8] and pineoblastoma [38].

Methods

Patients and samples

Our study population included 21 PinBs (Table 1; Fig. 1 and Supplementary Figure S1), six of which were clinically referred (cases 8–11, 17 and 18), twelve of which were obtained from a registry or pathology department (cases 1–7 and 12–16), and three of which occurred in known carriers of germ-line *DICER1* mutations (previously unreported) (cases 19–21). Two of the latter individuals (cases 19 and 20) had been screened for *DICER1* mutations due to co-existing conditions that raised suspicion of DICER1 syndrome and in the third case (case 21), the *DICER1* mutation was revealed by exome sequencing. For these three cases, we present brief case histories, pedigrees and somatic mutation analysis to illustrate some features of DICER1-related PinB. All cases of PinB were diagnosed by experienced neuropathologists at the referring institutions using standard criteria (WHO classification) with appropriate ancillary methods, such as immunostains; no patient had been previously diagnosed with a Rb. 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, where indicated, eligible relatives signed a consent form in accordance with the above-mentioned IRB protocol.

Molecular methods

Sanger sequencing and/or Fluidigm access array-based next-generation sequencing, as described previously [8], was used to identify coding *DICER1* mutations and mutations located near the exon–intron boundaries in blood gDNA ($n = 4$), in gDNA from PinB cell lines ($n = 2$) [18, 19], in gDNA extracted from fresh frozen tumours ($n = 10$) and in gDNA we extracted from formalin-fixed paraffin-embedded (FFPE) tumours ($n = 7$). DNA was extracted from FFPE tumour samples using 3–7 tumour tissue sections, 10 μm in thickness, using the QIAamp DNA FFPE Tissue Kit (QIAGEN, Toronto, ON, Canada) according to manufacturer's instructions. DNA from fresh frozen tumour tissue was extracted using the Qiagen AllPrep DNA/RNA Mini Kit (QIAGEN, Toronto, ON, Canada). cDNA was synthesized from tumour RNA using the QuantiTect Reverse Transcription Kit (QIAGEN, Toronto, ON, Canada). The germ-line *DICER1* mutation was identified in one case through whole exome sequencing; the methods have been previously described [17]. The mode of ascertainment of the cases, sample acquisition and molecular analyses is outlined in Fig. 1 and Supplementary Figure S1.

Tumours were screened for somatic *DICER1* RNase III mutations by PCR amplification of gDNA [48, 49] followed by Sanger sequencing [McGill University and Genome Quebec Innovation Centre (MUGQIC)]. We screened for *DICER1* mutations occurring outside of the RNase IIIa and IIIb domains using the Fluidigm access array system and next-generation sequencing. Where no germ-line *DICER1* mutations were identified by conventional sequencing, we screened for large deletions or duplications using a multiplex ligation-based probe amplification (MLPA) assay (Fig. 1 and Supplementary Figure S1) [39].

LOH analysis in tumour samples was performed by PCR amplification of tumour gDNA concurrently with the patient's germ-line gDNA (where available), using primers specific to the region of interest [8]. The 150–250 base-pair PCR products were analysed by direct Sanger sequencing and the relative intensity of the peaks at the position of the germ-line *DICER1* mutation and/or SNPs (single-nucleotide polymorphisms) within the 3′UTR of the gene were assessed for LOH. Genotyping of the short tandem repeat (STR) markers D14A274, D14S1059, D14S1030 and D14S65 was performed by PCR amplification using end-labelling with ^{33}P γ-ATP followed by separation by acrylamide gel electrophoresis as previously described [45] to ascertain LOH in the absence of coding variants that could be interrogated using Sanger sequencing (Supplementary Figure S1).

Immunohistochemistry (IHC)

Immunohistochemical analysis was performed on deparaffinised 4-μm tissue sections incubated with anti-DICER antibody ab14601 (Abcam, Cambridge, MA, USA) as previously reported [36], using a 1:50 dilution. The anti-DICER1 antibody binds to a region within the PAZ domain of the protein. We were able to obtain adequate material to carry out IHC analysis of eight tumours.

Table 1 Pineoblastoma case summary

PinB case	Sex	Age at diagnosis	Vital status	Evidence of *DICER1* syndrome	Germ-line *DICER1* analysis		Somatic *DICER1* analysis		*DICER1* IHC
					Germ-line mutation	MLPA	Somatic *DICER1* mutation	LOH	
1	M	15 mo	Deceased 20 months post-Dx	Not known	Negative[a]	Negative	Negative	No LOH	ND
2	F	13 mo	Deceased 10 months post-Dx	Not known	Negative[a]	Negative	Negative	No LOH	ND
3	F	6 mo	Deceased 10 months post-Dx	Not known	Negative[a]	Negative	Negative	3 × 3′UTR SNPs homozygous	ND
4	M	24 mo	Alive	Not known	Not available	Negative	rs61751177[b]	No LOH	ND
5	M	12 mo	Not known	Not known	Negative[a]	Negative	Negative	No LOH	ND
6	M	11 yrs	Not known	Not known	Negative[a]	Negative	Negative	2 × 3′UTR SNPs homozygous	ND
7	F	11.7 yrs	Alive	Not known	Negative[a]	Negative	Negative	No LOH	ND
8	M	3 yrs	Alive	Bilateral real cysts, Dx 5yrs	c.4754C>G, p.(Ser1585*)	Not relevant	Negative	No LOH	Loss of staining
9	M	2 mo	Deceased 11 months post-Dx	None	Negative	Negative	Negative	No LOH	Staining retained
10	F	3.3 yrs	Alive	Thyroid nodule, Dx 7yrs	c.5103C>A, p.(Tyr1701*)	Not relevant	(Negative, see LOH)	LOH	Loss of staining
11	F	17.1 yrs	Alive	Father: Wilms tumour	c.4633dupT, p.(Ser1545Phefs*7)	Not relevant	(Negative, see LOH)	LOH	Loss of staining
12	M	5 yrs	Not known	Not known	Not available	ND	c.3280_3281delTT, p.(Leu1094Argfs*9); c.3675C>G, p.(Tyr1225*)	Not relevant	Loss of staining
13	F	12 yrs	Not known	Not known	Not available	ND	c.2040+59insT	Not relevant	Staining retained
14	M	3 yrs	Not known	Not known	Not available	ND	c.2040+59insT; rs12018992	Not relevant	Staining retained
15[c]	F	11 mo	N/A	Not known	Negative	Negative	Negative	No LOH	ND
16	M	8 mo	N/A	Not known	Negative	Negative	Negative	No LOH	ND
17	F	2 yrs	Alive	None	Negative	Negative	Negative	No LOH	ND
18	F	19 mo	Alive	None	Negative	ND	Negative	Negative	ND

Table 1 continued

PinB case	Sex	Age at diagnosis	Vital status	Evidence of *DICER1* syndrome	Germ-line *DICER1* analysis		Somatic *DICER1* analysis		*DICER1* IHC
					Germ-line mutation	MLPA	Somatic *DICER1* mutation	LOH	
19	M	24 yrs	Alive	Family history of thyroid abnormalities	c.1498A>T, p.(Lys500*)	Not relevant	(Negative, see LOH)	LOH	Loss of staining
20	F	10 years	Alive	Cervical and vaginal fibroepithelial polyp, SLCT, cERMS, brainstem ERMS	c.4050+1G>A	Not relevant	Not available	Not available	ND
21	M	2 years	Deceased 26 months post-Dx	PPB; family history: multinodular goitre; meningeal sarcoma[d]	c.4407_4410delTTCT, p.(Ser1470Leufs*19)	Not relevant	Not available	Not available	ND

Cases 19–21: previously unpublished, selected PinB cases

Dx diagnosis, *cERMS* cervical embryonal rhabdomyosarcoma, *ERMS* embryonal rhabdomyosarcoma, *F* female, *LOH* loss of heterozygosity, *M* male, *Mo* months, *MLPA* multiplex ligation-based probe amplification assay, *ND* not done, *Post-Dx* after diagnosis, *PPB* pleuropulmonary blastoma, *SLCT* Sertoli–Leydig cell tumour, *SNPs* single-nucleotide polymorphisms, *UTR* untranslated region, *Yrs* years

[a] By default: sequencing studies and MLPA analysis of *DICER1* in the tumour samples revealed no mutations

[b] In case number 4, we identified a DICER1 sequence polymorphism, c.1935G>A (rs61751177), that is not likely to be pathogenic

[c] The same result was found in a cell line established from a metastatic lesion from patient 15

[d] Meningeal sarcoma is not definitively associated with the DICER1 syndrome and the patient is untested (Fig. 4c)

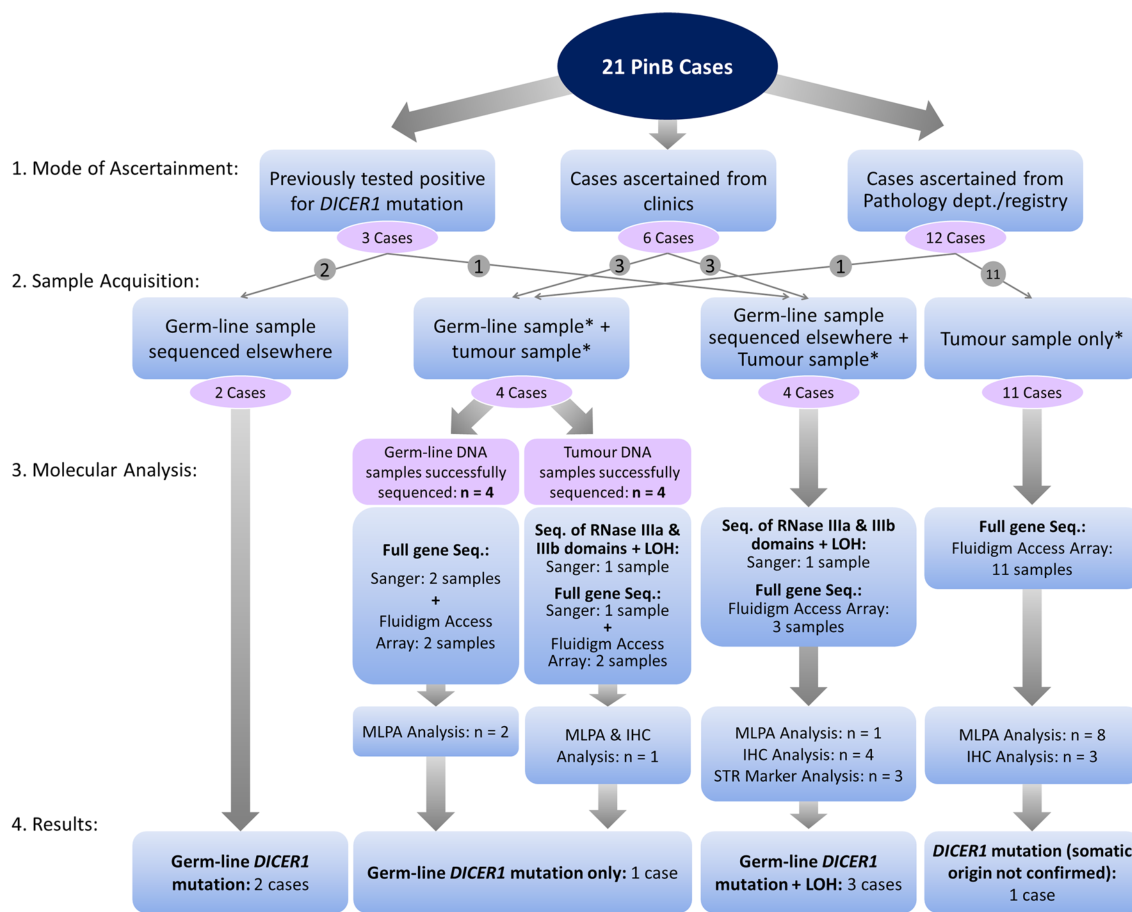

Fig. 1 Flow chart summarizing the mode of ascertainment of cases, sample acquisition, molecular analysis and the results of the study. *Asterisk* indicates samples sequenced by us. Sequencing of gDNA not performed by us was conducted at: referring institution ($n = 2$), Ambry Genetics (Aliso Viejo, CA, USA) ($n = 1$), Prevention Genetics (Marshfield, WI, USA) ($n = 2$), or at Baylor-Hopkins Center for Mendelian Genomics (Houston, TX, USA) ($n = 1$)

Results

The median age at diagnosis of the 21 PinBs was 2 years (range of 2 months to 24 years). Eleven of the patients were male and ten were female, and for the fourteen cases where vital status is known, nine children remain alive and five died of disease 10–26 months post-diagnosis. We identified three unambiguously deleterious germ-line mutations in the eighteen PinBs that had not undergone previous *DICER1* genetic testing (cases 1–18) (Table 2). All three mutations—case 8: c.4754C>G, p.(Ser1585*); case 10: c.5103C>A, p.(Tyr1701*); and case 11: c.4633dupT, p.(Ser1545Phefs*7)—are predicted to prematurely truncate the DICER1 protein and each of the mutations was associated with absence of DICER1 immunostaining attributable to a loss of full-length DICER1 protein within the tumours. To look for LOH within the tumours, we used four STR markers mapping in and around *DICER1* on chromosome 14q. Informative markers showed LOH in cases 10 and 11 (Supplementary Figure S2). In contrast, no LOH was seen in case 9 (Supplementary Figure S2) which is consistent with our other data (Table 1; Fig. 2a), as no DICER1 DNA or protein abnormality was found in this case. For case 8, no somatic mutation was identified within the

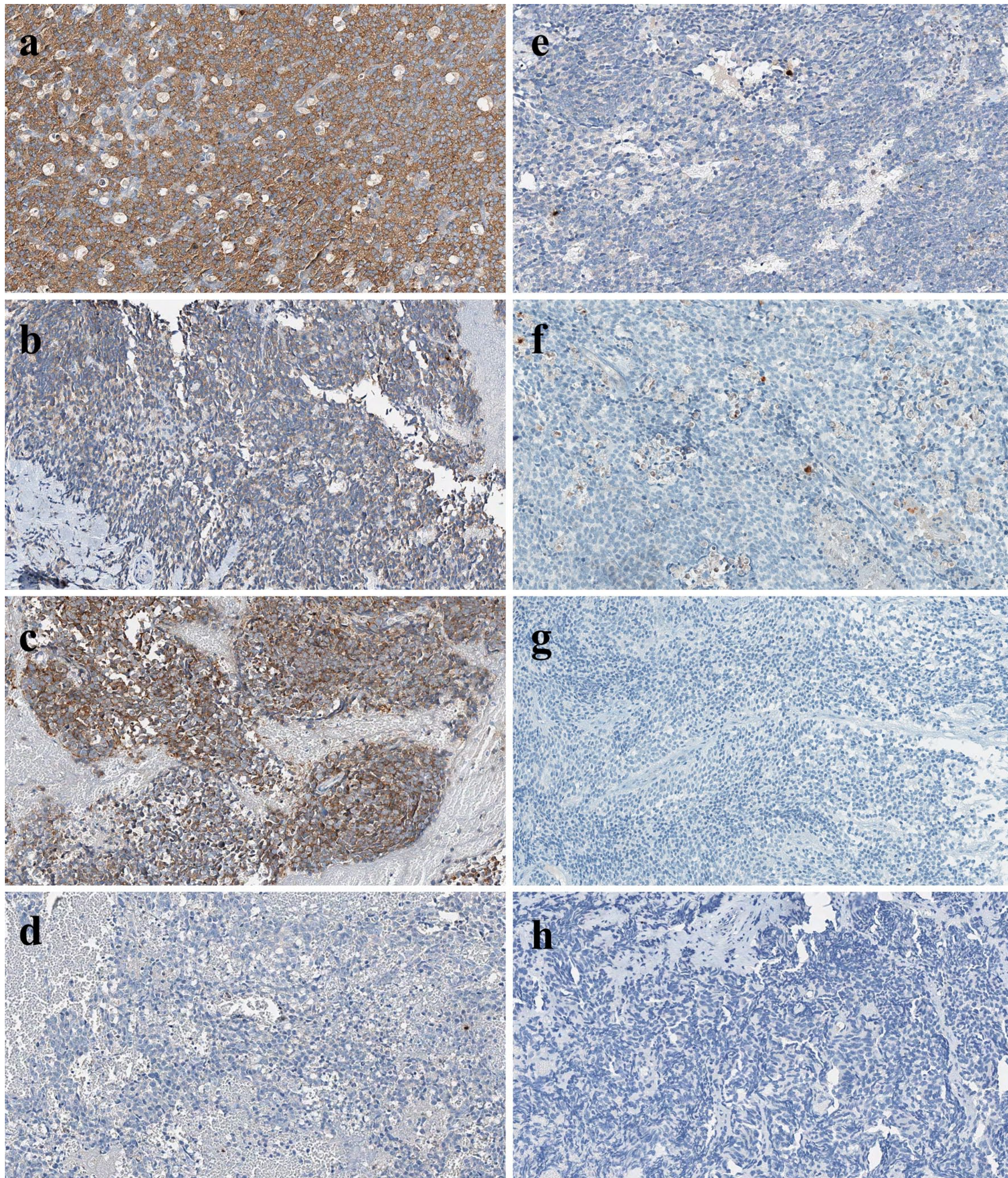

Fig. 2 Anti-DICER1 immunostaining (magnification = 20×). Immuno-reactivity for DICER1 retained: *panels* **a**, **b** and **c** (cases 9, 14, 13, respectively); and DICER1 immuno-reactivity lost: *panels* **d**, **e**, **f, g** and **h** (cases 12, 8, 10, 11, 19, respectively)

tumour. In a fourth case (case 12), two nonsense mutations, c.3280_3281delTT (p.(Leu1094Argfs*9)) and c.3675C>G (p.(Tyr1225*)) were identified within FFPE tumour gDNA, both of which are predicted to prematurely truncate the DICER1 protein (Fig. 3). Without a germ-line gDNA sample available from this case, we were unable to determine whether either of these mutations was in the germ line. Nevertheless, the loss of protein expression in this case suggests bi-allelic inactivation (Table 1). Thus, germ-line mutations were present in three out of eighteen previously untested PinBs. Unexpectedly, there were no somatic missense mutations identified that affected the *DICER1* RNase IIIb domain in any of the 19 tumour samples evaluated.

We also studied three PinBs from children with previously identified, but unpublished germ-line *DICER1* mutations (cases 19–21). These cases were included as they

Table 2 Summary of mutation data

PinB case	Germ-line *DICER1* mutation	LOH demonstrated in tumour?	DICER1 IHC analysis
8	c.4754C>G, p.(Ser1585*)	No	Loss of staining
10	c.5103C>A, p.(Tyr1701*)	Yes	Loss of staining
11	c.4633dupT, p.(Ser1545Phefs*7)	Yes	Loss of staining
12	c.3280_3281delTT, p.(Leu1094Argfs*9)[a]; c.3675C>G, p.(Tyr1225*)[a]	No	Loss of staining
19	c.1498A>T, p.(Lys500*)	Yes	Loss of staining
20	c.4050+1G>A	ND	ND
21	c.4407_4410delTTCT, p.(Ser1470Leufs*19)	ND	ND

IHC immunohistochemistry, *ND* not done, *LOH* loss of heterozygosity

[a] Mutation identified in tumour DNA, but germ-line vs somatic origin of mutation not determined

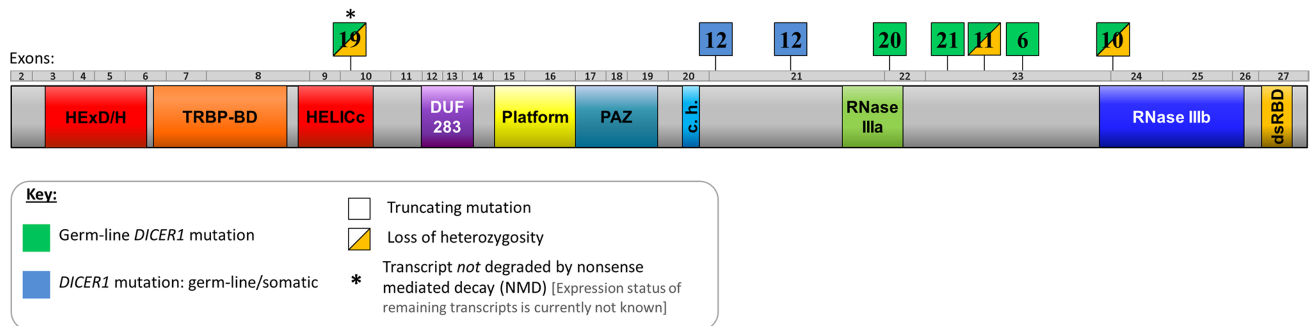

Fig. 3 Graphic representation of the unfolded DICER1 protein structure (NP_001258211.1) indicating the approximate positions of the germ-line *DICER1* mutations observed in the 21 PinB cases being reported. Mutations shaded in blue represent mutations that were identified within tumour gDNA, but are not confirmed to be somatic in origin. Case number indicated at the position of each mutation. DICER1 domains, defined as follows: *DExD/H* DExD/H box helicase domain, *TRBP-BD* trans-activating response RNA-binding protein binding domain, *HELICc* helicase conserved C-terminal domain, *DUF283* domain of unknown function, *Platform* platform domain, *PAZ* polyubiquitin-associated zinc-finger domain, *c.h.* connector helix, *RNase IIIa* Ribonuclease IIIa domain, *RNase IIIb* Ribonuclease IIIb domain, *dsRBD* double-stranded RNA-binding domain. Mutations: Case 8: germ-line DICER1 amino acid change, p.(Ser1585*); Case 10: germ-line DICER1 amino acid change, p.(Tyr1701*); somatic DICER1 change, loss of heterozygosity (LOH); Case 11: germ-line DICER1 amino acid change, p.(Ser1545Phefs*7); somatic DICER1 change, LOH; Case 12: DICER1 amino acid changes, p.(L1094Rfs*9) and p.(Y1225X)—not confirmed to be germ-line or somatic in origin; Case 19: germ-line DICER1 amino acid change, p.(Lys500*); somatic DICER1 change, LOH; Case 20: germ-line DICER1 amino acid change, c.4050+1G>A; Case 21: germ-line DICER1 amino acid change, p.(Ser1470Leufs*19)

afforded us the opportunity to study PinB in the context of personal medical history and/or family history. These cases are described in detail in Table 1, the pedigrees are shown in Fig. 4 and the mutation data are summarized in Table 2. Notably, case 19 carries a c.1498A>T (p.(Lys500*)) germ-line *DICER1* mutation which induces a premature stop codon in the sequence encoding the Helicase domain of the protein (Fig. 3). This mutated transcript was found to be present on analysis of cDNA synthesized from tumour RNA (Fig. 5a, panel II). This suggests that the transcript is not degraded by nonsense-mediated decay (NMD) and subsequent translation thereof would result in the expression of a severely truncated protein. This germ-line *DICER1* mutation was accompanied by LOH of the wild-type allele within the tumour (Fig. 5a). Three of five tested family members have been found to carry the c.1498A>T mutation and a family history of hyperthyroidism exists (Fig. 4a).

Case 20 carries the c.4050+1G>A germ-line *DICER1* variant which is suspected to deleteriously affect transcriptional expression due to the abolition of a donor splice site as predicted by Human Splicing Finder (http://www.umd.be/HSF/4DACTION/input_SSF#). Following the diagnosis of PinB at 10 years of age, this girl was diagnosed with multiple other lesions between the ages of 15 and 21 years. These included vaginal and cervical fibroepithelial polyps diagnosed at 15 years of age, a Sertoli–Leydig cell tumour (SLCT) of the left ovary diagnosed at 16 years of age, a cervical embryonal rhabdomyosarcoma (cERMS) diagnosed at 17 years of age, and a brainstem ERMS diagnosed at 21 years of age. SLCT and cERMS are characteristic manifestations of a germ-line *DICER1* mutation. The family history includes reports of pulmonary and thyroid abnormalities (Fig. 4b). The PinB tumour tissue was not available from case 20 to allow for somatic analysis of the second allele.

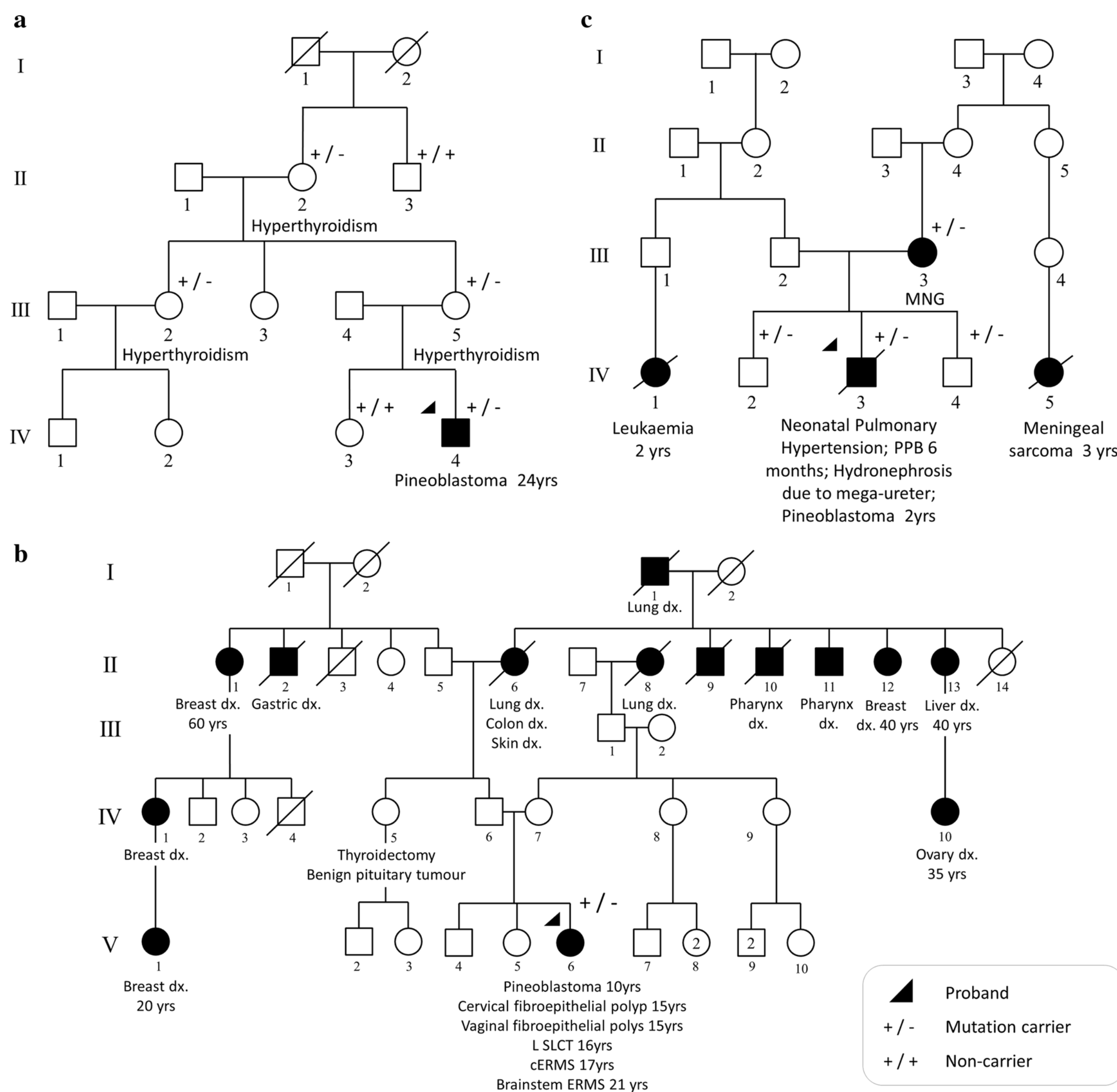

Fig. 4 **a** Case 19: the proband, individual IV-4, was diagnosed with a PinB at the age of 24 years and was found to carry the germ-line mutation, c.1498A>T, in *DICER1*. Of the five family members tested, three were found to carry the same germ-line *DICER1* mutation and all three individuals had hyperthyroidism (individuals II-2, III-2 and III-5). **b** Case 20: the proband, individual V-6, was diagnosed at 10 years of age with a pineoblastoma, at 15 years of age with cervical and vaginal fibroepithelial polyps, at 16 years of age with a Sertoli–Leydig cell tumour (SLCT) of the left ovary, at 17 years of age with a cervical embryonal rhabdomyosarcoma (cERMS) and a brainstem ERMS at 21 years of age. She was found to carry the germ-line *DICER1* mutation, c.4050+1G>A. Several family members had pulmonary and thyroid abnormalities. **c** Case 21: the proband (individual IV-3, deceased) was diagnosed at the age of 2 years with a pineoblastoma. At 6 months of age, multiple pulmonary bullae were detected and congenital bullous emphysema was diagnosed. The lung pathology was later reviewed in the light of the whole exome sequencing results and a revision of the diagnosis to pleuropulmonary blastoma (PPB) was made. Both the proband, his mother (individual III-3) and his two brothers (individuals IV-2 and IV-4) were found to carry the c.4407_4410delTTCT germ-line *DICER1* mutation. The mother is affected by a multinodular goitre (MNG). Individual IV-5 was diagnosed with a meningeal sarcoma at 3 years of age. Meningeal sarcoma is not definitively associated with the DICER1 syndrome and the patient is untested

Case 21 carried an inherited germ-line *DICER1* mutation (c.4407_4410delTTCT, p.(Ser1470Leufs*19)), which is predicted to truncate the protein subsequent to the RNase IIIa domain if the mutant transcript were to forego NMD (Fig. 3). The proband's mother, who is affected by multinodular goitre, and two brothers were found to carry the c.4407_4410delTTCT mutation (Fig. 4c). Tumour tissue was not available from this case to allow for somatic analysis.

For eight of the 21 cases (cases 8–14 and case 19), we had sufficient material to carry out IHC studies of DICER1 [36]. The results were consistent with the molecular findings, in that for cases 9, 13 and 14, we did not identify any deleterious *DICER1* mutations and all cases showed

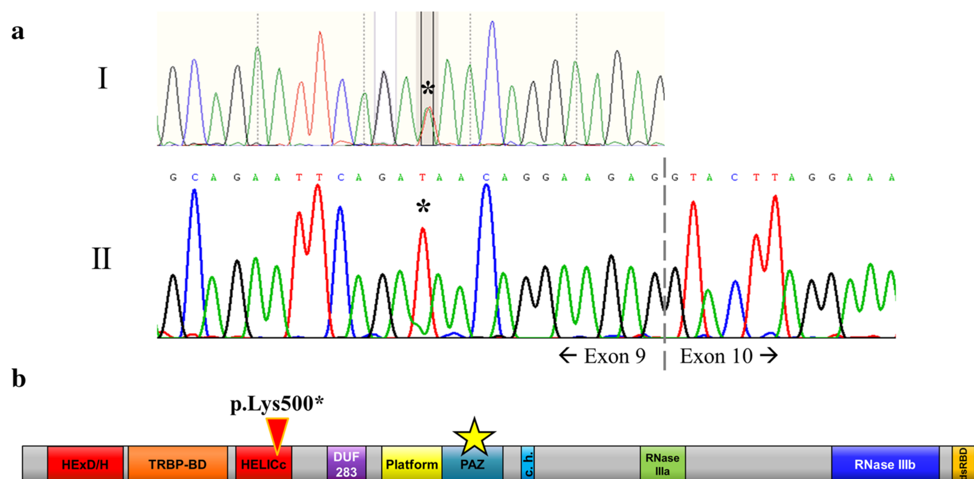

Fig. 5 Case 19 somatic analysis: **a** *Panel I* the germ-line *DICER1* mutation, **c**.1498A>T, indicated by an *asterisk*. *Panel II* Loss of heterozygosity (LOH) of the wild-type allele evident at the position of the germ-line mutation (*asterisk*) in cDNA synthesized from tumour RNA. **b** The position of the germ-line *DICER1* mutation predicted to truncate the protein (indicated by *red arrow*), relative to the anti-DICER1 antibody binding site (indicated by a *yellow star*)

retained staining of DICER1 (Fig. 2a, b, c). In contrast, in cases 8, 10, 11, 12 and 19, there was no DICER1 expression detected by IHC (Fig. 2d–h). Tumours from case 10 and case 11 carried two inactivating mutations (one germ-line truncating mutation and LOH within the tumour) and in case 12, the absence of staining for DICER1 strongly suggests the two predicted truncating mutations are present *in trans* (Fig. 2d). In case 8, we only found one likely deleterious mutation and no second somatic hit, but notably, DICER1 staining was absent (Fig. 2e). This suggests that the wild-type allele has been inactivated by some other mechanism resulting in the absence of full-length, functional DICER1 protein in this tumour. In case 19, as described above, we demonstrated that the mutated transcript was not subjected to NMD and expression of a severely truncated protein is predicted. The binding site of the anti-DICER1 antibody is downstream of the predicted truncation site (Fig. 5b) and therefore the expression of the mutant protein was not detected on IHC analysis (Fig. 2h).

Discussion

The results from this study establish *DICER1* as an important susceptibility gene for PinB, a tumour which we have now shown to be a manifestation of the DICER1 syndrome

The pineocyte and retinal receptor cells share a common embryonic origin in humans [29], explaining the rare syndrome of "trilateral Rb" in *RB-1* mutation carriers. Interestingly, DICER1 has not been demonstrated to have any tumourigenic role in Rb, although, for a small percentage of Rb's, the genetic underpinnings remain unexplained [37].

In addition to the molecular results reported here, PinB has been clinically associated with a particular neuraxis manifestation of *DICER1* mutations: ciliary body medulloepithelioma (CBME) [31, 44]. Two children with both PinB and CBME have been reported [24, 34]. Intracranial medulloepithelioma has also been reported in a kindred that likely harbours a *DICER1* mutation [5]. These observations suggest a possible cell-of-origin relationship between anterior elements in the globe and the pineal gland.

All six germ-line *DICER1* mutations identified (three identified in this study and three previously identified) (Table 2) are loss-of-function mutations that inactivate one allele of *DICER1*. The mutations identified in cases 11, 19 and 21 are confirmed to be inherited. Notably, the father of patient 11 carries the c.4633dupT mutation and was affected by a Wilms tumour in childhood (Table 1). Three cases (case 10, 11 and 19) were found to exhibit loss of the wild-type allele in addition to the deleterious germ-line *DICER1* mutation (Table 2 and Supplementary Figure S2) resulting in the complete loss of DICER1 expression within the tumours (Fig. 2). These preliminary findings indicate that the mechanism by which the second allele is inactivated in PinB may differ from that in other DICER1 syndrome diseases. Almost all reported somatic mutations observed in DICER1-related tumour types affect the metal ion-binding residues of the RNase IIIa or IIIb domains (e.g. Glu1705, Asp1709, Asp1810 and Glu1813) [1, 15]. These so-called "hotspot" missense mutations have been shown to shift the expression of mature miRNAs within the tumours towards 3p-derived miRNAs as a consequence of reduced 5p miRNA-strand processing [1, 14]. We observed no such missense mutations in six PinBs with available data. This was compared with 59 hotspot mutations in 60 DICER1-related tumours occurring at other sites ($P = 7.7 \times 10^{-8}$, Fisher's exact test) (Supplementary Table S1). These data suggest that the absence of missense RNase IIIb mutations in PinB is unlikely to be a chance finding. In contrast to what is seen in other DICER1-related tumours, LOH of the *DICER1* locus is the most frequent "second hit" in PinBs.

This phenomenon of *DICER1* LOH shown here in three PinBs and reported only once previously [38], contests the

hypothesis based on a murine model that complete loss of DICER1 is disadvantageous to tumour development in humans. Results from in vivo analysis conducted by two independent research groups demonstrate that Dicer1 may function as a haplo-insufficient tumour suppressor: the loss of one *Dicer1* allele in both a retinoblastoma mouse model [22] and a Kras-driven lung cancer mouse model [21] enhanced tumourigenesis; however, deletion of the second *Dicer1* allele did not further promote tumour proliferation or initiation, but instead impeded it [21, 22]. LOH of the wild-type allele, as now seen in a total of four PinBs, suggests DICER1 functions as a conventional tumour suppressor in the pineal gland, whereby both alleles are inactivated, initiating tumour development. LOH has been identified in two cases of pituitary blastoma [8], but has not been seen in any other DICER1-related tumours [3, 13, 16, 44]. Furthermore, conditional inactivation of Dicer1 in murine retinal cells results in progressive and extensive retinal degeneration [6]. However, inactivation of DICER1 in pineocytes as a result of a truncating mutation in *DICER1* coupled with LOH of the wild-type allele (as seen in this study) does not seem to have the same degenerative effect. Interestingly, inactivation of Dicer1 in mouse radial glial cells results in the over-production of cortical neurons [28]. This enhanced proliferation may be more in keeping with the tumourigenic events that take place within the pineal gland subsequent to DICER1 inactivation. Overall, the complete loss of DICER1 is seemingly selected against in most cell lineages, but is tolerated in the pineal gland, permitting the progression to PinB. We suspect that other gain- or loss-of-function mutations of other cancer genes may also be required to permit or facilitate the complete loss of DICER1 in PinB. The mechanism of tumourigenesis as a consequence of total loss of DICER1 expression within these tumours remains to be explored. Further studies on this rare tumour will focus on mRNA, miRNA and gDNA profiling.

Also of note is the significantly lower median age of onset of PinB in our cohort (2 years), relative to the previously reported mean age of onset of 12.6 years [12, 26]. This disparity is likely due to the ascertainment of 13 cases from children's hospitals (Cases 1–11, 17–18 and 20). The age of onset of PinBs found to harbour *DICER1* mutations is far less defined than other tumour types occurring within the DICER1 syndrome.

Numerous diseases occur in the DICER1 predisposition syndrome and most *DICER1* mutations are inherited. Thus, finding a germ-line mutation in a PinB patient may have implications for the patient and family. Our recommendations include genetic counselling, family education and sequencing of the parents and, if indicated, other family members. Careful re-examination of the extended family medical history and of pathology specimens can reveal previously unrecognized associated conditions. The advisability of prospective screening for various phenotypes is uncertain, given that syndrome diseases are rare, generally not life threatening and may present over the first three to four decades of life. Screening particularly for pleuropulmonary blastoma, which is highly curable in an early form in infancy but may progress to an aggressive, much-less-curable sarcoma after age 2 years, may be advisable [41].

To our knowledge, this is the first detailed study to interrogate the possible involvement of *DICER1* in PinB pathogenesis. Limitations of the study include the small number of cases recruited and possible bias in the selection of cases: although we did not include patients known to carry germ-line *DICER1* mutations in calculating the prevalence of *DICER1* mutations, we are aware that clinic-based ascertainment schemas have their own biases. For this reason, larger studies with more complete ascertainment will be needed to confirm and extend our findings.

Conclusion

This study suggests that germ-line *DICER1* mutations make a clinically significant contribution to PinB, establishing *DICER1* as an important susceptibility gene for PinB. The means by which the second allele is inactivated seems to differ from other DICER1-related tumours. The total loss of DICER1 protein in the cells challenges the haplo-insufficiency model of DICER1 action. These data, combined with the other reported instance of PinB occurring in a germ-line *DICER1* mutation carrier, indicate that PinB is a recognized manifestation of a germ-line *DICER1* mutation. To determine the true prevalence of *DICER1* mutations in PinB, analysis of a larger unselected series of PinBs is required. From a clinical perspective, the importance of these findings is that *DICER1* genetic testing should be considered for all patients diagnosed with a PinB. Furthermore, these children and their immediate family members (in a setting of an inherited *DICER1* mutation) may be susceptible to other DICER1-associated conditions, and as such, referral to genetic counsellors and surveillance for early detection may be considered. DICER1 IHC may also serve as an easily applicable screening tool for the presence of *DICER1* mutations in PinB.

Acknowledgments We thank the Children's Cancer and Leukaemia Group Tumour Bank for samples, Lisa Storer, Laura Zahavich, Dr Ute Bartels, Dr John-Paul Kilday, Dr Paul Nathan, Dr Armando Lorenzo, Dr Nalin Gupta and Dr Sharon Plon for their assistance with ascertainment and analysis of their respective cases and members of the Baylor-Hopkins Center for Mendelian Genomics their help with the exome sequencing of case 21. We also thank Pierre Lepage and the MUGQIC staff for designing primers for, optimizing and performing the Fluidigm Access Array capture and sequencing, and N. Benlimame (George and Olga Minarik Research Pathology Facility, Jewish General Hospital) for help with immunohistochemical analysis. This research was made

possible thanks to the support of Alex's Lemonade Stand Foundation and The Brain Tumour Charity who fund the CNS PNET research at CBTRC. The identification of a *DICER1* mutation in one patient was made by whole exome sequencing conducted at the Center for Mendelian Genomics, funded by The National Institutes of Health (NIH) and supported by the National Human Genome Research Institute grant U54HG006542 to the Baylor-Hopkins Center for Mendelian Genomics.

Conflict of interest The authors have no conflicts of interest to disclose.

References

1. Anglesio MS, Wang Y, Yang W, Senz J, Wan A, Heravi-Moussavi A, Salamanca C, Maines-Bandiera S, Huntsman DG, Morin GB (2013) Cancer-associated somatic DICER1 hotspot mutations cause defective miRNA processing and reverse-strand expression bias to predominantly mature 3p strands through loss of 5p strand cleavage. J Pathol 229(3):400–409. doi:10.1002/path.4135
2. Bahubeshi A, Bal N, Rio Frio T, Hamel N, Pouchet C, Yilmaz A, Bouron-Dal Soglio D, Williams GM, Tischkowitz M, Priest JR, Foulkes WD (2010) Germline DICER1 mutations and familial cystic nephroma. J Med Genet 47(12):863–866. doi:10.1136/jmg.2010.081216
3. Bahubeshi A, Tischkowitz M, Foulkes WD (2011) miRNA processing and human cancer: DICER1 cuts the mustard. Sci Transl Med 3(111):111ps146. doi:10.1126/scitranslmed.3002493
4. Blach LE, McCormick B, Abramson DH, Ellsworth RM (1994) Trilateral retinoblastoma–incidence and outcome: a decade of experience. Int J Radiat Oncol Biol Phys 29(4):729–733
5. Cross SF, Arbuckle S, Priest JR, Marshall G, Charles A, Dalla Pozza L (2010) Familial pleuropulmonary blastoma in Australia. Pediatr Blood Cancer 55(7):1417–1419. doi:10.1002/pbc.22592
6. Damiani D, Alexander JJ, O'Rourke JR, McManus M, Jadhav AP, Cepko CL, Hauswirth WW, Harfe BD, Strettoi E (2008) Dicer inactivation leads to progressive functional and structural degeneration of the mouse retina. J Neurosci 28(19):4878–4887. doi:10.1523/JNEUROSCI.0828-08.2008
7. de Kock L, Plourde F, Carter MT, Hamel N, Srivastava A, Meyn MS, Arseneau J, Soglio DB, Foulkes WD (2013) Germ-line and somatic DICER1 mutations in a pleuropulmonary blastoma. Pediatr Blood Cancer 60(12):2091–2092. doi:10.1002/pbc.24692
8. de Kock L, Sabbaghian N, Plourde F, Srivastava A, Weber E, Bouron-Dal Soglio D, Hamel N, Choi JH, Park SH, Deal CL, Kelsey MM, Dishop MK, Esbenshade A, Kuttesch JF, Jacques TS, Perry A, Leichter H, Maeder P, Brundler MA, Warner J, Neal J, Zacharin M, Korbonits M, Cole T, Traunecker H, McLean TW, Rotondo F, Lepage P, Albrecht S, Horvath E, Kovacs K, Priest JR, Foulkes WD (2014) Pituitary blastoma: a pathognomonic feature of germ-line DICER1 mutations. Acta Neuropathol. doi:10.1007/s00401-014-1285-z
9. de Kock L, Sabbaghian N, Soglio DB, Guillerman RP, Park BK, Chami R, Deal CL, Priest JR, Foulkes WD (2014) Exploring the association between DICER1 mutations and differentiated thyroid carcinoma. J Clin Endocrinol Metab. doi:10.1210/jc.2013-4206
10. Doros L, Yang J, Dehner L, Rossi CT, Skiver K, Jarzembowski JA, Messinger Y, Schultz KA, Williams G, Andre N, Hill DA (2012) DICER1 mutations in embryonal rhabdomyosarcomas from children with and without familial PPB-tumor predisposition syndrome. Pediatr Blood Cancer 59(3):558–560. doi:10.1002/pbc.24020
11. Doros LA, Rossi CT, Yang J, Field A, Williams GM, Messinger Y, Cajaiba MM, Perlman EJ, A Schultz K, Cathro HP, Legallo RD, Lafortune KA, Chikwava KR, Faria P, Geller JI, Dome JS, Mullen EA, Gratias EJ, Dehner LP, Hill DA (2014) DICER1 mutations in childhood cystic nephroma and its relationship to DICER1-renal sarcoma. Mod Pathol. doi:10.1038/modpathol.2013.242
12. Fauchon F, Jouvet A, Paquis P, Saint-Pierre G, Mottolese C, Ben Hassel M, Chauveinc L, Sichez JP, Philippon J, Schlienger M, Bouffet E (2000) Parenchymal pineal tumors: a clinicopathological study of 76 cases. Int J Radiat Oncol Biol Phys 46(4):959–968
13. Foulkes WD, Bahubeshi A, Hamel N, Pasini B, Asioli S, Baynam G, Choong CS, Charles A, Frieder RP, Dishop MK, Graf N, Ekim M, Bouron-Dal Soglio D, Arseneau J, Young RH, Sabbaghian N, Srivastava A, Tischkowitz MD, Priest JR (2011) Extending the phenotypes associated with DICER1 mutations. Hum Mutat 32(12):1381–1384. doi:10.1002/humu.21600
14. Gurtan AM, Lu V, Bhutkar A, Sharp PA (2012) In vivo structure-function analysis of human Dicer reveals directional processing of precursor miRNAs. RNA 18(6):1116–1122. doi:10.1261/rna.032680.112
15. Heravi-Moussavi A, Anglesio MS, Cheng SW, Senz J, Yang W, Prentice L, Fejes AP, Chow C, Tone A, Kalloger SE, Hamel N, Roth A, Ha G, Wan AN, Maines-Bandiera S, Salamanca C, Pasini B, Clarke BA, Lee AF, Lee CH, Zhao C, Young RH, Aparicio SA, Sorensen PH, Woo MM, Boyd N, Jones SJ, Hirst M, Marra MA, Gilks B, Shah SP, Foulkes WD, Morin GB, Huntsman DG (2012) Recurrent somatic DICER1 mutations in nonepithelial ovarian cancers. N Engl J Med 366(3):234–242. doi:10.1056/NEJMoa1102903
16. Hill DA, Ivanovich J, Priest JR, Gurnett CA, Dehner LP, Desruisseau D, Jarzembowski JA, Wikenheiser-Brokamp KA, Suarez BK, Whelan AJ, Williams G, Bracamontes D, Messinger Y, Goodfellow PJ (2009) DICER1 mutations in familial pleuropulmonary blastoma. Science 325(5943):965. doi:10.1126/science.1174334
17. Karaca E, Weitzer S, Pehlivan D, Shiraishi H, Gogakos T, Hanada T, Jhangiani SN, Wiszniewski W, Withers M, Campbell IM, Erdin S, Isikay S, Franco LM, Gonzaga-Jauregui C, Gambin T, Gelowani V, Hunter JV, Yesil G, Koparir E, Yilmaz S, Brown M, Briskin D, Hafner M, Morozov P, Farazi TA, Bernreuther C, Glatzel M, Trattnig S, Friske J, Kronnerwetter C, Bainbridge MN, Gezdirici A, Seven M, Muzny DM, Boerwinkle E, Ozen M, Clausen T, Tuschl T, Yuksel A, Hess A, Gibbs RA, Martinez J, Penninger JM, Lupski JR (2014) Human CLP1 mutations alter tRNA biogenesis, affecting both peripheral and central nervous system function. Cell 157(3):636–650. doi:10.1016/j.cell.2014.02.058
18. Kees UR, Biegel JA, Ford J, Ranford PR, Peroni SE, Hallam LA, Parmiter AH, Willoughby ML, Spagnolo D (1994) Enhanced MYCN expression and isochromosome 17q in pineoblastoma cell lines. Genes Chromosomes Cancer 9(2):129–135
19. Kees UR, Spagnolo D, Hallam LA, Ford J, Ranford PR, Baker DL, Callen DF, Biegel JA (1998) A new pineoblastoma cell line, PER-480, with der(10)t(10;17), der(16)t(1;16), and enhanced MYC expression in the absence of gene amplification. Cancer Genet Cytogenet 100(2):159–164
20. Kivela T (1999) Trilateral retinoblastoma: a meta-analysis of hereditary retinoblastoma associated with primary ectopic intracranial retinoblastoma. J Clin Oncol 17(6):1829–1837
21. Kumar MS, Pester RE, Chen CY, Lane K, Chin C, Lu J, Kirsch DG, Golub TR, Jacks T (2009) Dicer1 functions as a haploinsufficient tumor suppressor. Genes Dev 23(23):2700–2704. doi:10.1101/gad.1848209
22. Lambertz I, Nittner D, Mestdagh P, Denecker G, Vandesompele J, Dyer MA, Marine JC (2010) Monoallelic but not biallelic loss of Dicer1 promotes tumorigenesis in vivo. Cell Death Differ 17(4):633–641. doi:10.1038/cdd.2009.202
23. Lesnick JE, Chayt KJ, Bruce DA, Rorke LB, Trojanowski J, Savino PJ, Schatz NJ (1985) Familial pineoblastoma.

Report of two cases. J Neurosurg 62(6):930–932. doi:10.3171/jns.1985.62.6.0930
24. Mamalis N, Font RL, Anderson CW, Monson MC, Williams AT (1992) Concurrent benign teratoid medulloepithelioma and pineoblastoma. Ophthalmic Surg 23(6):403–408
25. Marees T, Moll AC, Imhof SM, de Boer MR, Ringens PJ, van Leeuwen FE (2010) Re: more about second cancers after retinoblastoma. J Natl Cancer Inst 102(11):831–832. doi:10.1093/jnci/djp207
26. Mena H, Rushing EJ, Ribas JL, Delahunt B, McCarthy WF (1995) Tumors of pineal parenchymal cells: a correlation of histological features, including nucleolar organizer regions, with survival in 35 cases. Hum Pathol 26(1):20–30
27. Moll AC, Imhof SM, Bouter LM, Kuik DJ, Den Otter W, Bezemer PD, Koten JW, Tan KE (1996) Second primary tumors in patients with hereditary retinoblastoma: a register-based follow-up study, 1945–1994. Int J Cancer 67(4):515–519. doi:10.1002/(SICI)1097-0215(19960807)67:4<515:AID-IJC9>3.0.CO;2-V
28. Nowakowski TJ, Mysiak KS, O'Leary T, Fotaki V, Pratt T, Price DJ (2013) Loss of functional Dicer in mouse radial glia cell-autonomously prolongs cortical neurogenesis. Dev Biol 382(2):530–537. doi:10.1016/j.ydbio.2013.08.023
29. Olsson R (1961) Subcommissural ependyma and pineal organ development in human fetuses. Gen Comp Endocrinol 1:117–123
30. Peyster RG, Ginsberg F, Hoover ED (1986) Computed tomography of familial pineoblastoma. J Comput Assist Tomogr 10(1):32–33
31. Priest JR, Williams GM, Manera R, Jenkinson H, Brundler MA, Davis S, Murray TG, Galliani CA, Dehner LP (2011) Ciliary body medulloepithelioma: four cases associated with pleuropulmonary blastoma–a report from the International Pleuropulmonary Blastoma Registry. Br J Ophthalmol 95(7):1001–1005. doi:10.1136/bjo.2010.189779
32. Priest JR, Williams GM, Mize WA, Dehner LP, McDermott MB (2010) Nasal chondromesenchymal hamartoma in children with pleuropulmonary blastoma–a report from the International Pleuropulmonary Blastoma Registry registry. Int J Pediatr Otorhinolaryngol 74(11):1240–1244. doi:10.1016/j.ijporl.2010.07.022
33. Pugh TJ, Yu W, Yang J, Field AL, Ambrogio L, Carter SL, Cibulskis K, Giannikopoulos P, Kiezun A, Kim J, McKenna A, Nickerson E, Getz G, Hoffher S, Messinger YH, Dehner LP, Roberts CW, Rodriguez-Galindo C, Williams GM, Rossi CT, Meyerson M, Hill DA (2014) Exome sequencing of pleuropulmonary blastoma reveals frequent biallelic loss of TP53 and two hits in DICER1 resulting in retention of 5p-derived miRNA hairpin loop sequences. Oncogene. doi:10.1038/onc.2014.150
34. Ramasubramanian A, Correa ZM, Augsburger JJ, Sisk RA, Plager DA (2013) Medulloepithelioma in DICER1 syndrome treated with resection. Eye (Lond) 27(7):896–897. doi:10.1038/eye.2013.87
35. Ramasubramanian A, Kytasty C, Meadows AT, Shields JA, Leahey A, Shields CL (2013) Incidence of pineal gland cyst and pineoblastoma in children with retinoblastoma during the chemoreduction era. Am J Ophthalmol 156(4):825–829. doi:10.1016/j.ajo.2013.05.023
36. Rio Frio T, Bahubeshi A, Kanellopoulou C, Hamel N, Niedziela M, Sabbaghian N, Pouchet C, Gilbert L, O'Brien PK, Serfas K, Broderick P, Houlston RS, Lesueur F, Bonora E, Muljo S, Schimke RN, Bouron-Dal Soglio D, Arseneau J, Schultz KA, Priest JR, Nguyen VH, Harach HR, Livingston DM, Foulkes WD, Tischkowitz M (2011) DICER1 mutations in familial multinodular goiter with and without ovarian Sertoli-Leydig cell tumors. JAMA 305(1):68–77. doi:10.1001/jama.2010.1910
37. Rushlow DE, Mol BM, Kennett JY, Yee S, Pajovic S, Thériault BL, Prigoda-Lee NL, Spencer C, Dimaras H, Corson TW, Pang R, Massey C, Godbout R, Jiang Z, Zacksenhaus E, Paton K, Moll AC, Houdayer C, Raizis A, Halliday W, Lam WL, Boutros PC, Lohmann D, Dorsman JC, Gallie BL (2013) Characterisation of retinoblastomas without RB1 mutations: genomic, gene expression, and clinical studies. Lancet Oncol 14(4):327–334. doi:10.1016/S1470-2045(13)70045-7
38. Sabbaghian N, Hamel N, Srivastava A, Albrecht S, Priest JR, Foulkes WD (2012) Germline DICER1 mutation and associated loss of heterozygosity in a pineoblastoma. J Med Genet 49(7):417–419. doi:10.1136/jmedgenet-2012-100898
39. Sabbaghian N, Srivastava A, Hamel N, Plourde F, Gajtko-Metera M, Niedziela M, Foulkes WD (2013) Germ-line deletion in DICER1 revealed by a novel MLPA assay using synthetic oligonucleotides. Eur J Hum Genet. doi:10.1038/ejhg.2013.215
40. Schild SE, Scheithauer BW, Schomberg PJ, Hook CC, Kelly PJ, Frick L, Robinow JS, Buskirk SJ (1993) Pineal parenchymal tumors. Clinical, pathologic, and therapeutic aspects. Cancer 72(3):870–880
41. Schultz KA, Harris A, Williams GM, Baldinger S, Doros L, Valusek P, Frazier AL, Dehner LP, Messinger Y, Hill DA (2014) Judicious DICER1 testing and surveillance imaging facilitates early diagnosis and cure of pleuropulmonary blastoma. Pediatr Blood Cancer. doi:10.1002/pbc.25092
42. Seki M, Yoshida K, Shiraishi Y, Shimamura T, Sato Y, Nishimura R, Okuno Y, Chiba K, Tanaka H, Kato K, Kato M, Hanada R, Nomura Y, Park MJ, Ishida T, Oka A, Igarashi T, Miyano S, Hayashi Y, Ogawa S, Takita J (2014) Biallelic DICER1 mutations in sporadic pleuropulmonary blastoma. Cancer Res. doi:10.1158/0008-5472.CAN-13-2470
43. Shields CL, Meadows AT, Shields JA, Carvalho C, Smith AF (2001) Chemoreduction for retinoblastoma may prevent intracranial neuroblastic malignancy (trilateral retinoblastoma). Arch Ophthalmol 119(9):1269–1272
44. Slade I, Bacchelli C, Davies H, Murray A, Abbaszadeh F, Hanks S, Barfoot R, Burke A, Chisholm J, Hewitt M, Jenkinson H, King D, Morland B, Pizer B, Prescott K, Saggar A, Side L, Traunecker H, Vaidya S, Ward P, Futreal PA, Vujanic G, Nicholson AG, Sebire N, Turnbull C, Priest JR, Pritchard-Jones K, Houlston R, Stiller C, Stratton MR, Douglas J, Rahman N (2011) DICER1 syndrome: clarifying the diagnosis, clinical features and management implications of a pleiotropic tumour predisposition syndrome. J Med Genet 48(4):273–278. doi:10.1136/jmg.2010.083790
45. Tischkowitz M, Xia B, Sabbaghian N, Reis-Filho JS, Hamel N, Li G, van Beers EH, Li L, Khalil T, Quenneville LA, Omeroglu A, Poll A, Lepage P, Wong N, Nederlof PM, Ashworth A, Tonin PN, Narod SA, Livingston DM, Foulkes WD (2007) Analysis of PALB2/FANCN-associated breast cancer families. Proc Natl Acad Sci 104(16):6788–6793. doi:10.1073/pnas.0701724104
46. Tomiak E, de Kock L, Grynspan D, Ramphal R, Foulkes WD (2014) DICER1 mutations in an adolescent with cervical embryonal rhabdomyosarcoma (cERMS). Pediatr Blood Cancer 61(3):568–569. doi:10.1002/pbc.24826
47. Turaka K, Shields CL, Meadows AT, Leahey A (2012) Second malignant neoplasms following chemoreduction with carboplatin, etoposide, and vincristine in 245 patients with intraocular retinoblastoma. Pediatr Blood Cancer 59(1):121–125. doi:10.1002/pbc.23278
48. Witkowski L, Mattina J, Schonberger S, Murray MJ, Choong CS, Huntsman DG, Reis-Filho JS, McCluggage WG, Nicholson JC, Coleman N, Calaminus G, Schneider DT, Arseneau J, Stewart CJ, Foulkes WD (2013) DICER1 hotspot mutations in non-epithelial gonadal tumours. Br J Cancer 109(10):2744–2750. doi:10.1038/bjc.2013.637
49. Wu MK, Sabbaghian N, Xu B, Addidou-Kalucki S, Bernard C, Zou D, Reeve AE, Eccles MR, Cole C, Choong CS, Charles A, Tan TY, Iglesias DM, Goodyer PR, Foulkes WD (2013) Biallelic DICER1 mutations occur in Wilms tumours. J Pathol 230(2):154–164. doi:10.1002/path.4196