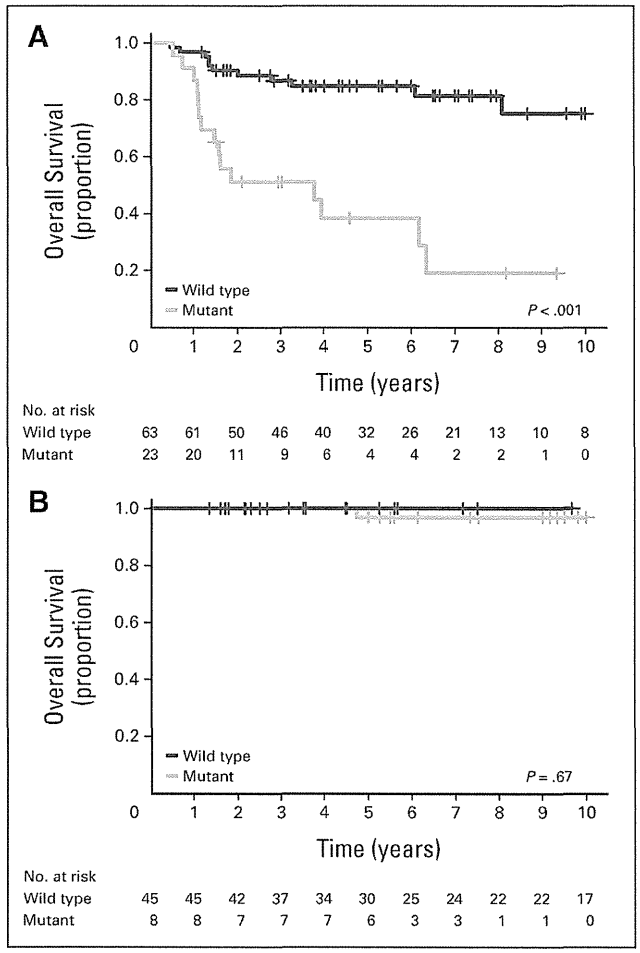

Fig A2. Kaplan-Meier estimates of overall survival for children and infants by group. (A) Sonic hedgehog (SHH) tumors from the discovery cohort. (B) Wingless (WNT) tumors from the discovery cohort.

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CLINICAL STUDY

Role of surgery, radiotherapy and chemotherapy in papillary tumors of the pineal region: a multicenter study

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Abstract Papillary tumor of the pineal region (PTPR), recently described as a distinct clinicopathological entity, can show aggressive biological behavior. The optimal therapeutic approach of PTPR has not been well defined. The role of surgery, radiotherapy, and chemotherapy in the treatment of PTPR was analyzed in a large multicenter series. In order to determine factors that influence prognosis, outcome data of a series of 44 patients with histopathologically proven PTPR were retrospectively analyzed. Of the 44 patients, 32 were still alive after a median follow-up of 63.1 months. Twelve patients experienced progressive disease, with seven undergoing two relapses and five more than two. Median overall survival (OS) was not achieved. Median progression-free survival (PFS) was 58.1 months. Only gross total resection and younger age were associated with a longer OS, radiotherapy and chemotherapy having no significant impact. PFS was not influenced by gross total resection. Radiotherapy and chemotherapy had no significant effect. This retrospective series confirms the high risk of recurrence in PTPR and emphasizes the importance of gross total resection. However, our data provide no evidence for a role of adjuvant radiotherapy or chemotherapy in the treatment of PTPR.

Keywords Papillary tumor of the pineal region · Pediatric · Prognosis · Radiosurgery

Introduction

Tumors located in the pineal gland region are rare neoplasms, constituting 0.5–1 % of all intracranial tumors [1],

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and comprise a variety of entities, including pineal parenchymal tumors, germ cell tumors, glial tumors, and meningiomas. Papillary tumor of the pineal region (PTPR) is a neuroectodermal tumor thought to be derived from cells of the subcommissural organ. This entity was first described in 2003 [2] and subsequently included in the World Health Organization (WHO) classification of central nervous system tumors [3]. Because data on long-term outcome are lacking, definite histopathological grading of PTPR has not yet been established. Furthermore, the role of adjuvant radiotherapy and chemotherapy in treatment of PTPR remains uncertain. We therefore examined outcome and response to therapy in a series of 44 PTPR patients from France, Germany, Great Britain, Switzerland, Japan, and Slovenia; data for 37 have already been published [4–13], but have been updated.

Materials and methods

Patients

The study was based on a series of 44 patients (21 males and 23 females) diagnosed with PTPR and treated between January 1979 and November 2008. These cases were treated at 28 neurosurgical centers in Europe and two in Japan. Some of the cases have been previously reported [4–13]. The histopathology was reviewed according to current WHO criteria [3]. Cases had often been previously misinterpreted as choroid plexus tumors (7 cases), pineal parenchymal tumors (8 cases), ependymomas (13 cases), or miscellaneous other tumor entities (6 cases); only 10 were initially diagnosed as PTPR. Routine immunohistochemical studies included reactivity with antibodies against neurofilament, synaptophysin, chromogranin-A, neuron-specific enolase, epithelial membrane antigen, glial fibrillary acidic protein, S-100 protein, vimentin, and cytokeratins. Additionally, in some cases, studies included staining for microtubule-associated protein 2 as well as choroid plexus tumor markers Kir 7.1 and stanniocalcin-1 [5]. Data on clinical course and treatment were collected by the participating centers and submitted in an anonymous format following good clinical practice and respecting local laws. Data were updated until the last follow-up or death. One patient was lost to follow-up 15 months after diagnosis. Tumor size was assessed using magnetic resonance imaging (MRI) and/or computerized axial tomography (CT) scans in the 32 cases for which imaging data were available for review. The median diameter of tumors prior to treatment was calculated. The presence of hydrocephalus and tumor extension toward the peduncles, third ventricle, thalamus, lateral ventricles, and cerebellum was assessed. In some cases, postoperative imaging was available for assessment of residual disease before further treatment.

Statistics

Quantitative data are presented as the median and range. When no information was available, status was coded as missing data. Censored data were described using Kaplan–Meier estimation and consisted of the number of patients, number of events, percentage survival, and the 95 % confidence interval (CI). Survival intervals were censored at the date of the last known patient contact. Progression-free survival (PFS) and overall survival (OS) were measured from the date of the first treatment for PTPR (surgical resection or radiotherapy or chemotherapy) to, respectively, the date of first recurrence or death (not considered as a relapse). Statistical comparisons of censored data were performed using the Log-Rank test or Cox proportional hazard model. Smoothing splines were used to predict death risk versus age. Statistical analyses were two sided and performed using R-2.5.0 for Windows.

Assessment of tumor response

Tumor response was based on serial measurements of post-contrast enhanced zones on the post operative CT scan or MRI. When possible, chemotherapy was also correlated with response. Criteria for response to radiotherapy or chemotherapy were defined as follows: a complete response (cr) as the disappearance of the tumor, a partial response (pr) as a 50 % or greater decrease in tumor size, progression of disease (pd) as a greater than 25 % increase in tumor size or any appearance of new tumor sites, and stable disease (sd) as all other situations. The reported site of relapse was the first site of disease progression observed during follow-up.

Results

Patient characteristics

The characteristics of individual patients and the group as a whole are summarized in Tables 1 and 2, respectively. The age of the patients at first treatment ranged from 5 to 66 years (median 29 years), the group comprised 36 adults and 8 children (under 18 years). On average, more than 9 months had passed from the appearance of the first symptoms until the establishment of diagnosis. The most common symptom at presentation was an increase in intracranial pressure with hydrocephalus ($n = 31$); other frequent symptoms were Parinaud's syndrome, ataxia, and

Table 1 Characteristics, treatments, and status of the individual patients

Case	Age (years)	Sex	Size (mm)	Follow up (months)	Surgery	Initial treatment	Dose (pineal) (grays)	Recurrences				Status
								1st	2nd	3rd	4th	
1	19	M	27	106	B, GTR			L	L			Alive
2[a]	28	F	23	138	B, PR	RT	54	L	Brain			Alive pr
3	56	F	35	23	GTR	RT	60	L				Dead
4	53	M	32	91	B	SRS	12	L	L			Dead
5	34	F	40	16	PR			Sp				Dead
6	42	F	26	36	B, GTR	RT, CT	54	L	L			Dead
7[a]	43	M	27	18	PR	RT	na	L				Dead (suicide)
8[a]	14	M	na	0	GTR							LTF
9	32	M	30	198	GTR			L				Alive
10[a]	66	M	na	104	GTR	RT	na	L				Dead
11[a]	62	M	25	25	PR	RT	56					Dead
12	38	F	na	15	GTR			L				Alive
13	23	F	40	111	B, PR			L	L			Alive
14	24	F	na	61	B, GTR	CT, RT	na	L				Alive
15	29	F	25	7	B	RT	50					Dead
16	27	M	50	4	B	RT	55					Dead
17	33	F	20	77	B, PR	CT, RT	60	L				Alive
18	22	F	22	58	B, PR			L				Alive
19	46	F	na	99	B, GTR	RT	60					Alive
20	45	F	18	47	2 B, PR			L	Brain			Alive
21	24	M	na	44	GTR							Alive
22	5	F	28	61	GTR	CT		L,	LV + Sp	Sp		Dead
23[b]	13	M	na	33	B, GTR	RT	50					Alive
24[a]	14	M	50	122	GTR	CT		L	L	L	L	Alive
25[a]	35	M	28	58	PR	RT	56					Alive
26[a]	29	M	48	21	B	RT	42	Brain, Sp				Dead
27[a]	11	F	32	115	B, GTR	RT	54	L	L			Alive pd
28[a]	28	F	5	43	B, GTR							Alive cr
29[a]	26	F	30	166	B	Brachy	60	L + 4^{th}V	L	L		Alive
30[a]	29	F	17	82	GTR	RT	57.5					Alive
31[a]	25	M	40	178	GTR			L	L + 4^{th}V,	L + 4^{th}V		Alive
32	7	F	na	95	GTR	RT, CT	54					Alive cr
33[c]	15	M	30	3	B, GTR							Alive cr
34	16	F	na	21	PR	CT, RT	na					Alive sd
35[d]	21	M	25	198	B, GTR	RT	50	Cereb	Cereb	L		Alive pd
36[e]	33	M	45	67	PR	RT	55	L				Alive pr
37[f]	29	F	36	41	B, GTR	Presurg RT	50					Alive cr
38	36	F	na	45	PR, GTR	RT	na	L				Alive pd
39[g]	42	F	na	14	B							Alive
40	44	M	20	17	GTR	RT	54					Alive cr
41	48	M	27	2	B, GTR	RT	50					Alive cr
42	52	F	na	36	B, PR	SRS	na	L				Alive
43	56	M	20	53	GTR							Alive cr

Table 1 continued

Case	Age (years)	Sex	Size (mm)	Follow up (months)	Surgery	Initial treatment	Dose (pineal) (grays)	Recurrences				Status
								1st	2nd	3rd	4th	
44[h]	43	M	25	28	GTR	CT, RT	60					Alive cr

Cases 1–31 have been reported by Fevre Montange et al. [4] using the same case numbers

Cases 1–23, 25, 35, 39, 42 were treated in France; cases 24–32, 34, 38, 43 in Germany; case 33 in Slovenia; cases 36, 41 in Great Britain; cases 37, 44 in Japan; and case 40 in Switzerland

B biopsy, *Brachy* brachytherapy, *Cereb* cerebellar, *cr* complete response, *GTR* gross total resection, *CT* chemotherapy, *L* local recurrence, *LTF* lost to follow up, *LV* lateral ventricle, *na*, not available, *pd* progressive disease, *pr* partial response, *PR* partial resection, *Presurg*, presurgical, *RT* radiotherapy, *sd* stable disease, *Sp* spinal recurrence, *SRS* stereotactic radiosurgery, $4^{th}V$ fourth ventricle

[a] cases also reported by Hasselblatt et al. [5]

[b] case also reported by Buffenoir et al. [6]

[c] case reported by Jeruc and Popovic [7]

[d] case reported by Lechapt-Zalcman et al. [8]

[e] case reported by Santarius et al. [9]

[f] case reported by Amemiya et al. [10] and Shibahara et al. [11]

[g] case reported by Fevre Montange et al. [12]

[h] case reported by Inoue et al. [13]

Table 2 Grouped characteristics of the patients and treatments

	Median (range)	Number of patients	Percent
All patients		44	100
Sex			
Male		21	47.7
Female		23	52.3
Tumor size (mm)	27.5 (5–50)		
Age at first treatment (years)	29 (5–66)		
Type of surgery			
Biopsy		6	13.6
Gross total resection		26	59.1
Partial resection		12	27.3
Chemotherapy			
Yes		8	18.2
No		36	81.8
Radiotherapy			
Yes		28	63.6
No		16	36.4
Radiotherapy dose (Grays)	54 (12–60)		

isolated diplopia. On MRI, most cases showed a lesion of the pineal region (case 22 is illustrated in Fig. 1) with strong contrast enhancement (Fig. 1a). The median tumor diameter was 27.5 mm (5–50 mm, $n = 32$). Extension toward the peduncles, the third ventricle (Fig. 1b, d), thalamus, lateral ventricles, or cerebellum was noted in three cases initially and in four cases at recurrence. Three cases (case 5 initially, cases 22 and 26 at recurrence) showed evidence of spinal cord seeding of tumor cells (case 22 is illustrated in Fig. 1d, e).

Surgery

Whether surgery was performed was determined by the clinical history of the patient and the local preferences and policies of the participating centers. Of the 44 patients, 28

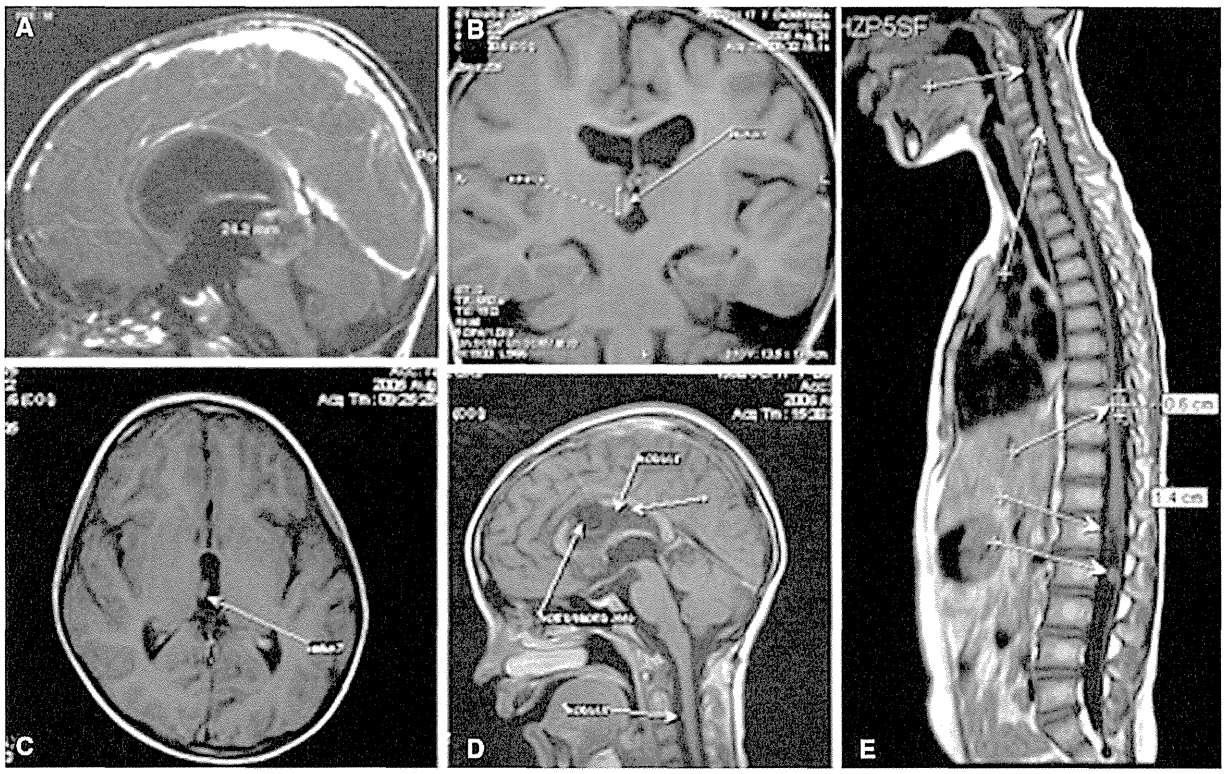

Fig. 1 Magnetic resonance imaging features in case 22. All images are T1-weighted gadolinium-enhanced magnetic resonance imaging scans. **a** Sagittal image of the lesion at presentation, showing a 28 mm (largest diameter) mass in the pineal region prior to first surgery (total tumor removal). Coronal (**b**) and axial (**c**) images showing a first recurrence in the right part of the third ventricle 2 years after the first surgery. Four years after the second surgery, the patient developed multiple nodules in both lateral ventricles (**d**) and in the spine (**e**) visible on sagittal images

underwent either cerebrospinal fluid shunt procedures ($n = 22$) or ventriculocisternostomy ($n = 6$). Tumor resection was more often performed via an infratentorial-supracerebellar approach than via a transcallosal approach. Gross total resection was achieved in 26 patients (11 after biopsy and 1 after partial resection) and partial resection was achieved in 12 (6 after biopsy). In 6 patients, only a biopsy was performed. Because of local recurrence, 14 patients had a second surgical intervention, 10 of which resulted in gross total resection and 4 in partial resection. Eight patients underwent a third operation because of tumor recurrence (gross total resection in 5 and partial resection in 3).

Radiotherapy

The method of radiotherapy delivery was based on the protocol of each center and on the initial diagnosis. Treatments consisted of craniospinal irradiation with a boost to the primary site, whole brain radiotherapy with a boost to the primary site, focal irradiation of the pineal area only, and radiosurgery. Twenty-eight patients received radiotherapy: 3 craniospinal irradiation with a boost applied to the primary site, 1 whole brain radiotherapy with a boost applied to the primary site, 22 focal irradiation of the pineal area (one received brachytherapy with 125-iodine), and 2 radiosurgery. The median pineal dose (which was known in 22 of the 26 irradiated cases) was 54 Gy (CI 95 %: 12.0–60.0 Gy). Despite relatively high cumulative doses (more than 100 Gy in 3 cases), irradiation-related side effects were rare. In one case, thalamic radionecrosis was associated with diplopia and hypersomnia, while, in another, radiosurgery of the recurrence (without prior irradiation) caused thalamo-tectal radionecrosis, leading to motor deficiency and Parinaud's syndrome.

Chemotherapy

Adjuvant chemotherapy (mainly based on cisplatinum-VP16 protocols) was applied in 4 adults and 4 children. Following resection, 4 patients without residual tumor received adjuvant chemotherapy with carboplatin-VP16-vincristine ($n = 3$) or temozolomide ($n = 1$). Two patients (cases 17 and 34) who underwent incomplete tumor

resection were treated with carboplatin plus either etoposide or vincristine. One patient (case 44), who had complete tumor removal, underwent combined radiotherapy and ACNU (3-[(4-amino-2-methyl-5-pyrimidinyl) methyl]-1-(chloroethyl)-1-nitrosourea) chemotherapy. At relapse, chemotherapy was used in a few cases following the same scheme.

Prognosis

Of the 44 patients, 32 were still alive. The median follow-up period for the whole population was 63.1 months. The follow-up period was longer than 4 years for 21 patients (48 %) and longer than 10 years for six (14 %). OS was 84.5 % (CI 95 %: 73.7–96.7) after 24 months and 71.6 % (CI 95 %: 55.7–92.0) after 10 years (Fig. 2a). On univariate analysis, OS was not influenced by sex, tumor size, radiotherapy, or chemotherapy, but was influenced by type of surgery (biopsy vs partial resection vs gross total resection) ($p = 0.04$) (Table 3). Older age was associated with a shorter OS ($p = 0.03$). Median PFS was 38.4 months (CI 95 %: 29.2–72.5). PFS was 68.9 % (CI 95 %: 55.6–85.2) after 24 months, 37.9 % (CI 95 %: 24.0–69.9) after 48 months, and 26.6 % (CI 95 %: 13.4–52.7) after 72 months (Fig. 2b). On univariate analysis, PFS was not influenced by age, sex, chemotherapy, or radiotherapy (Table 3). In total, 25 patients (56.8 %) experienced at least one relapse, the primary site being affected in 22. Of the 22 patients presenting with local recurrences, 13 were irradiated and nine were not. Two patients underwent radiosurgery (cases 4 and 42), 10 localized conformal radiotherapy (cases 2, 3, 7, 10, 14, 17, 27, 29, 36 and 38), and one craniospinal irradiation with a boost to the primary site (case 6). One patient had local recurrence and fourth ventricle spread (case 29). Three other patients had distant relapse, one with spinal spread (case 5), one with simultaneous spinal and meningeal spread (case 26), and one with cerebellar spread (case 35). Twelve patients experienced a second relapse, which was local in 7 cases (cases 1, 4, 6, 13, 24, 27, and 29) or extended in 5 cases: bulbar (case 2), cerebellar and frontal ventricular (case 20), spinal and entire ventricular system (case 22), local with posterior fossa extension (case 31), and cerebellar (case 35). Of the 7 cases with local recurrence, 5 had adjuvant treatments after the first surgery: radiotherapy, chemotherapy alone or with craniospinal irradiation, stereotactic radiosurgery, or brachytherapy. Five patients had a third relapse and one patient a fourth. After the second or subsequent relapses, 2 patients had chemotherapy: one (case 22) received gemcitabine–oxaliplatine (GEMOX) as second line chemotherapy for a spinal and ventricular relapse and showed a partial response after 9 cycles, while the other (case 35) showed a partial response after 9 cycles of carboplatin plus etoposide.

Discussion

The present series of 44 cases represents the largest retrospective series of PTPRs to date, extending previously published data [4] by increasing the number of patients and doubling the median follow-up period to 63.1 months. This tumor occurs across a wide age range (from 5 to 66 years in our series), with the vast majority occurring in young adults, in agreement with other reports [14–16]. Some cases have already been described in children [4, 17–19], one being a 15 month-old boy, the youngest patient to present with PTPR [18].

Radiological descriptions of PTPR are quite rare. In our series, MRI results was not always available, so a detailed review of imaging findings was not performed. Radiologically, PTPRs are mildly lobulated, partially cystic,

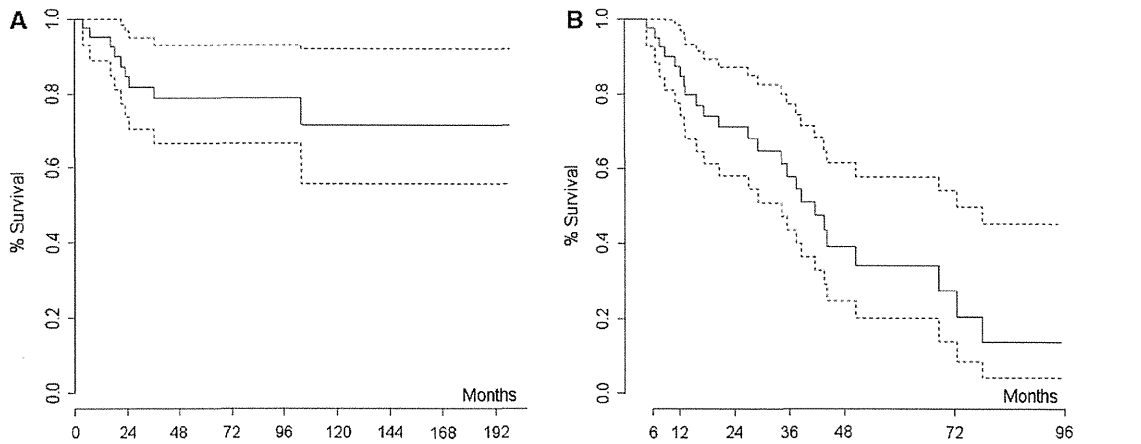

Fig. 2 Kaplan–Meier estimation of overall survival and the 95 % confidence intervals (**a**) and of relapse-free survival and the 95 % confidence intervals (**b**)

Table 3 Univariate analysis of overall and progression-free survival for the qualitative data

	Overall survival		Progression-free survival	
	Events/Patients (%)[a]	*p* value	Events/Patients (%)[a]	*p* value
Sex		0.57		0.89
Male	5/21 (23.8 %)		10/21 (47,6 %)	
Female	4/23 (17,4 %)		14/23 (60.9 %)	
Type of surgery		0.04		0.18
Biopsy	3/6 (50.0 %)		3/6 (50.0 %)	
Gross total resection	3/26 (11.5 %)		13/26 (50.0 %)	
Partial resection	3/12 (25.0 %)		9/12 (75.0 %)	
Chemotherapy		0.49		0.86
Yes	1/8 (12.5 %)		5/8 (62.5 %)	
No	8/36 (22.2 %)		20/36 (55.5 %)	
Radiotherapy		0.12		0.15
Yes	8/28 (28.6 %)		15/28 (53.6 %)	
No	1/16 (6.2 %)		10/16 (62.5 %)	

[a] Percentage of events (death or relapse), *p* values in the Log-Rank test <0.05 were considered significant

heterogeneously enhancing masses, and are usually associated with obstructive hydrocephalus [10, 11, 14, 20, 21]. They are difficult to distinguish from other clinical entities, especially pineocytomas [10, 14]. T2-weighted MRI often reveals heterogeneous, hyperintense regions, whereas T1-weighted imaging often reveals low-to-intermediate intensities [11, 13, 22]. However, hyperintensity on non-contrast T1-weighted sequences has been described in some patients presenting with PTPR, which might be explained by high concentrations of proteins in the small cystic spaces seen in these neoplasms [23–28]. However, this hyperintensity was not found in other cases [11, 25], suggesting a variation in radiographic findings in different lesions. In two reported cases, proton MR spectroscopy revealed increased choline and decreased N-acetyl aspartate peaks and a slightly increased lactate peak [13, 26]. Positron emission tomography has shown increased 18-fluorodeoxyglucose uptake at the site of the lesion, suggesting a malignant tumor with increased glucose metabolism [13]. Further studies are required to validate these results obtained in case reports of PTPR.

Data on treatment options and their outcomes have only been published for small series, and prognostic factors and formal consensus guidelines for optimal management of patients presenting with PTPR are not yet well established. Our study showed that the extent of surgery was identified on univariate analysis as the only clinical factor significantly associated with a better OS, highlighting the importance of surgery as the primary mainstay of therapy for PTPR. This result confirms the tendency for gross total resection to be associated with better OS and PFS previously reported with fewer patients [4]. As PTPRs may be derived from subcommissural modified ependymocytes, the clinical behavior of PTPRs can be compared to that of ependymomas. The effect of the extent of tumor removal has been well documented in intracranial ependymomas in adults by some authors [29–34], more particularly when the extent of resection was adequately evaluated. The initial treatment of choice for PTPRs, as in ependymomas, seems to be complete resection whenever safely feasible.

The role of radiotherapy in the treatment of patients presenting with PTPR is not established. Our results showed that radiotherapy did not influence OS or PFS, but the number of patients treated was quite low. This negative result may be due to the retrospective nature of our study, which involved heterogeneous radiotherapy regimens in the different centers. As the prognosis for PTPRs seems to be similar to that of ependymomas, with a propensity for local recurrence, many institutions treat the patient with post operative radiotherapy. The precise role of radiotherapy in ependymomas, more particularly the optimal dose, timing, and field size, is still debated, but its seems that adjuvant radiotherapy may be beneficial to control disease locally [34, 35]. Adjuvant radiotherapy was frequently used in the PTPR cases reported [8, 9, 13, 14, 20–22, 24, 27, 36–43]. Prospective studies are required to analyze the role of radiotherapy in PTPRs, more particularly after incomplete resection or when surgery cannot be performed.

The role of chemotherapy in the treatment of patients presenting with PTPR is also poorly documented. Only a few cases of PTPRs treated with chemotherapy have been reported previously [4, 11, 16, 44]. Our results showed that chemotherapy did not influence OS or PFS. One patient with a PTPR that recurred after surgery and radiotherapy was treated with temozolomide and is still symptom-free 9 years after the first treatment [44]. This alkylating drug can inhibit all stages of tumor cell growth and may present some advantages in patients with PTPRs. Although hypermethylation of the

promotor of the O6-methylguanine-DNA-methyltransferase gene has not been described in PTPRs, in other brain tumors, such as glioblastoma, it has been shown to be associated with improved outcome and may be a predictive marker of sensitivity to alkylating agents [45].

Our series further confirms the high rate of recurrence in PTPR, which accounted for 58 % at 5 years and greater than 70 % at 6 years. This high rate of recurrence is similar to that reported for intracranial ependymomas [46]. Recurrences were more often local and 13 patients underwent only one relapse (local or not). Spinal dissemination seems to be rare and was only seen in 3 cases. Some cases of PTPR may be more aggressive and have a potential for seeding into the spinal canal, particularly if complete tumor resection cannot be achieved [4, 47].

In conclusion, these data confirm the high risk of recurrence in PTPR and emphasize the importance of gross total resection, while adjuvant radiotherapy or chemotherapy failed to exert significant effects on OS and PFS.

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Ethical standards The present experiments comply with the current laws of the countries in which they were performed.

Conflict of interest The authors declare that they have no conflict of interest.

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ORIGINAL PAPER

TERT promoter mutations are highly recurrent in SHH subgroup medulloblastoma

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Abstract *Telomerase reverse transcriptase* (*TERT*) promoter mutations were recently shown to drive telomerase activity in various cancer types, including medulloblastoma. However, the clinical and biological implications of *TERT* mutations in medulloblastoma have not been described. Hence, we sought to describe these mutations and their impact in a subgroup-specific manner. We analyzed the *TERT* promoter by direct sequencing and genotyping in 466 medulloblastomas. The mutational distributions were determined according to subgroup affiliation, demographics, and clinical, prognostic, and molecular features. Integrated genomics approaches were used to identify specific somatic copy number alterations in *TERT* promoter-mutated and wild-type tumors. Overall, *TERT* promoter mutations were identified in 21 % of medulloblastomas. Strikingly, the highest frequencies of

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TERT mutations were observed in SHH (83 %; 55/66) and WNT (31 %; 4/13) medulloblastomas derived from adult patients. Group 3 and Group 4 harbored this alteration in <5 % of cases and showed no association with increased patient age. The prognostic implications of these mutations were highly subgroup-specific. *TERT* mutations identified a subset with good and poor prognosis in SHH and Group 4 tumors, respectively. Monosomy 6 was mostly restricted to WNT tumors without *TERT* mutations. Hallmark SHH focal copy number aberrations and chromosome 10q deletion were mutually exclusive with *TERT* mutations within SHH tumors. *TERT* promoter mutations are the most common recurrent somatic point mutation in medulloblastoma, and are very highly enriched in adult SHH and WNT tumors. *TERT* mutations define a subset of SHH medulloblastoma with distinct demographics, cytogenetics, and outcomes.

Keywords TERT promoter mutations · SHH pathway · Adult · Medulloblastoma

Introduction

Medulloblastoma is a highly malignant embryonal brain tumor located in the posterior fossa [6, 29, 33, 35]. While this tumor comprises the most common malignant brain tumor in children, it only accounts for approximately 1 % of primary CNS tumors in adults [18, 20]. The current consensus recognizes four core molecular subgroups (WNT, SHH, Group 3, and Group 4) with distinct molecular, demographic, clinicopathological, and prognostic characteristics [5, 15, 16, 26, 27, 37, 38, 41, 42]. The defining features of medulloblastoma subgroups differ dramatically according to age at diagnosis [15, 27, 41]. Specifically, Group 3 tumors are largely confined to non-adults, SHH tumors are most frequent in infants and adults, while WNT and Group 4 medulloblastomas are mostly observed in pediatric cohorts [15, 24, 27, 38, 41]. Particularly within SHH tumors, age-associated heterogeneity was observed regarding the transcriptional characteristics, somatic copy number alterations (SCNA), and the prognostic implications of biomarkers [15, 18, 38, 40]. Delineation of tumorigenic features characteristic for these age-related

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differences, particularly within SHH tumors, are highly desirable to understand these clear biological and prognostic discrepancies.

Telomere maintenance is fundamentally important to normal self-renewing stem cells and cancer cells [3, 7, 9, 14, 22]. It has been suggested that tumors derived from cell populations with low self-renewal capacity generally rely on alterations that restore telomerase activity, while epigenetic mechanisms maintain telomerase activity in tumor types derived from self-renewing stem cells [13]. The identification of recurrent *telomerase reverse transcriptase* (*TERT*) promoter mutations in 21 % of 91 medulloblastomas [13] is intriguing, since other mechanisms converging on increased telomerase activity including alternative lengthening of telomeres (ALT) [8] or mutations affecting the *ATRX/DAXX* complex are excessively uncommon in medulloblastoma [12, 25, 32, 34, 39]. Although *TERT* mutations have been reported in several cancers [2, 10, 11, 13, 19, 43], their putative association with distinct biological behavior and clinical or even prognostic characteristics has not been comprehensively studied. The initial analyses of *TERT* mutations in medulloblastoma [12] mainly catalogued the mutational frequency rather than correlating the molecular and clinical features of these mutations in a subgroup-specific manner.

In this study, we analyzed a representative set of 466 medulloblastomas for *TERT* promoter mutations. Subsequently, we correlated the mutational distribution with clinicopathological features, outcome, and molecular characteristics in a subgroup-specific manner. We demonstrate that *TERT* promoter mutations comprise the most recurrent mutation in adult SHH tumors identified to date and potentially define distinct prognostic subgroups in SHH and Group 4 medulloblastoma patients.

Materials and methods

Tumor material and patient characteristics

All tissues and clinicopathological information were serially collected in accordance with institutional review boards

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from various contributing centers to this study. Nucleic acid extractions were carried out as previously described [28]. The clinicopathological characteristics of the investigated patient cohort are outlined in Table 1. The median follow-up was 44.06 months (range 0.7–301.5 months).

Gene expression and copy number analysis

Subgroup affiliation was determined using nanoString limited gene expression profiling as previously described [31]. Somatic copy number alterations were assessed on the Affymetrix Single Nucleotide Polymorphism (SNP) 6.0 array platform in 418 of 466 cases to identify SCNAs specific for *TERT* mutant and wild-type tumors. Raw copy number estimates were obtained in dChip, followed by CBS segmentation in R as previously described [30]. Somatic copy number alterations were identified using GISTIC2 [21]. *TERT* expression levels were compared using R2 (www.r2.amc.nl). Differences in expression were tested using one-way ANOVA.

Sanger sequencing

Isolated DNA (25 ng) from all 466 tumors and 7 matched germline samples (25 ng) was amplified by PCR. PCRs contained 1 μl DNA template, 10 μM forward (5′-CAG GGC ACG CAC ACC AG-3′) and reverse (5′-GTC CTG CCC CTT CAC CTT C-3′) TERT-specific primers, and 12.5 μl HotStar Taq Plus Master Mix (Qiagen, Gaithersburg, Maryland, USA) in a 25 μl total reaction volume. Cycle parameters comprised 95 °C × 15 min; 28 cycles of 98 °C × 40 s, 65 °C × 30 s, 72 °C × 1 min; 72 °C × 10 min. PCRs were carried out using the C1000 Thermal Cycler (BioRad, Hercules, CA, USA). PCR products were purified with the PureLink PCR Micro kit (Life Technologies, Burlington, ON, Canada). In all experiments, controls were included in the absence of DNA to rule out contamination by PCR products. Templates for Sanger sequencing were analyzed with forward (5′-CAG CGC TGC CTG AAA CTC-3′) and reverse (5′-GTC CTG CCC CTT CAC CTT C-3′) sequencing primers using dGTP BigDye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (Life Technologies), and 5 % DMSO on the ABI3730XL capillary genetic analyzer (Life Technologies).

Genotyping assay

Two primers (forward primer, 5′-CAG CGC TGC CTG AAA CTC-3′; reverse primer, 5′-GTC CTG CCC CTT CAC CTT C-3′) were designed to amplify a 163-bp product encompassing C228T and C250T hotspot mutations in the *TERT* promoter—corresponding to the positions 124 and 146 bp, respectively, upstream of the ATG start site. Two fluorogenic LNA probes were designed with different fluorescent dyes to allow single-tube genotyping. One probe was targeted to the WT sequence (*TERT*

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Table 1 Clinicopathological and molecular characteristics according to *TERT* mutational status

Characteristic	*TERT* MUT	*TERT* WT	*p* value
Age (years)			
Median	22.00	7.08	**<0.0001**[#]
Range	0.66–49.00	0.24–56.32	
NA	1	0	
Gender			
Male	56	236	0.47[Φ]
Female	37	129	
NA	3	5	
Histology			
MBEN	3	8	0.59[χ]
Desmoplastic	10	59	
Classic	46	217	
LC/A	11	38	
NA	26	48	
M-stage			
M0	58	240	**0.03**[Φ]
M1-3	12	103	
NA	26	27	
TP53 status			
MUT	4	12	0.78[Φ]
WT	42	97	
NA	50	261	
Subgroup			
WNT	6	47	**<0.0001**[χ]
SHH	80	133	
Group 3	2	48	
Group 4	8	142	

F female, *LC/A* large cell/anaplastic, *M* male, *MB* medulloblastomal, *MBEN* medulloblastoma with extensive nodularity, *NA* not available (data were excluded from statistical comparison)

Bold values indicate $p < 0.05$

[#] Mann–Whitney *U* test

[Φ] Fisher's exact test

[χ] Chi-square test

WT, 5′-5HEX-CCC CTC CCG G-3IABkFQ-3′), and one was targeted to either of the two mutations (*TERT* mut, 5′-56FAM-CCC CTT CCG G-3IABkFQ). Primer and probes were custom designed by Integrated DNA Technologies (Coralville, Iowa, USA) using internal SNP design software, and sequence homogeneity was confirmed by comparison to all available sequences on the GenBank database using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Primers were optimized to avoid for hairpins and homo- and heterodimers. Primers and probes were obtained from Integrated DNA Technologies.

Real-time PCR was performed in 25 μl reaction mixtures containing 12.5 μl of TaqMan Universal Master Mix II with UNG (Applied Biosystems), 900 nM concentrations of each primer, 250 nM *TERT* WT probe, 250 nM *TERT* MUT probe, and 1 μl (25 ng) of sample DNA. Thermocycling was performed on the StepOnePlus (Applied Biosystems) and consisted of 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 95 °C for 15 s and 60 °C for 1 min.

Analysis was performed using StepOne Software, version 2.1. Samples were considered mutant if they had CT values of ≤39 cycles. Each sample was verified visually by examining the PCR curves generated to eliminate false positives due to aberrant light emission. End-point allelic discrimination genotyping was performed by visually inspecting a plot of the fluorescence from the WT probe versus the MUT probe generated from the post-PCR fluorescence read.

Statistical analysis

Survival time according to *TERT* mutational status was assessed using the Kaplan–Meier estimate and a log-rank test. Comparisons of binary and categorical patient characteristics between subgroups and cohorts were performed using the two-sided Fisher's exact test or Chi-squared test. Continuous variables were analyzed using the Mann–Whitney *U* test. *p* values <0.05 were considered statistically significant. Multivariate Cox proportional hazards regression was used to adjust for additional covariates using the survival R package (v.2.36). All other statistical analyses were performed using StataSE 12 (Stata Corp. College Station, TX, USA) and Graphpad Prism 5 (La Jolla, CA, USA).

Results

Characteristics of *TERT*-mutated medulloblastomas

We performed Sanger sequencing on a clinically well-annotated medulloblastoma cohort ($n = 466$), reflecting the spectrum of demographics and histological subtypes of the disease (Table 1; Supplementary Figure 1A). Our results were verified using a Taqman-based genotyping assay that detects both of the most highly recurrent *TERT* promoter mutations (C228T and C250T). Since both mutational hotspots are located in highly homologous sequences, C228T and C250T mutations result in an identical binding sequence for the mutation-specific probe (CCCGGAAGGGG; Supplementary Figure 1B). A total of 21 % of medulloblastomas harbored *TERT* mutations (Fig. 1a). In line with a previous report, these mutations were enriched in older patients (Table 1; $p < 0.0001$), all mutations were heterozygous, and none of the available matched germline controls displayed this mutation [13]. Interestingly, we found that *TERT*-mutated medulloblastomas present less

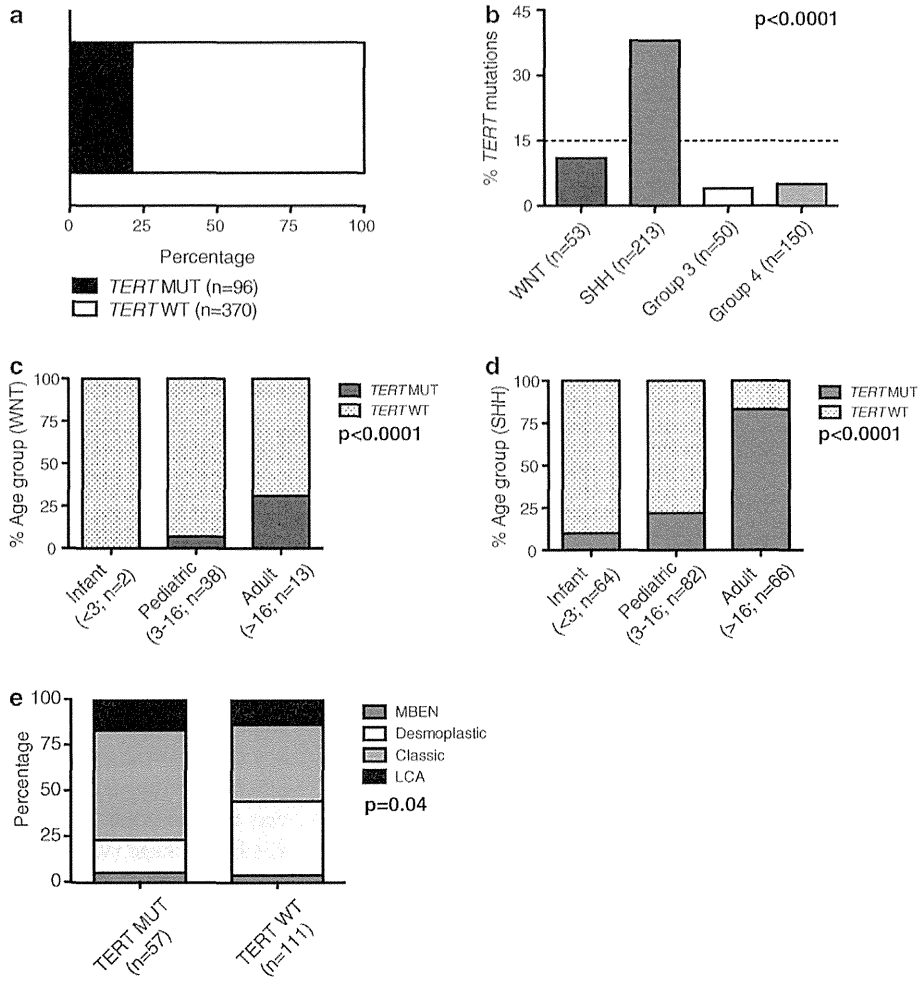


Fig. 1 *TERT* promoter-mutated medulloblastomas display distinct demographics, histology, and subgroup affiliation. **a** *Bar graph* indicating the frequency of *TERT* mutations in 466 primary medulloblastomas. **b** Prevalence of *TERT* mutations according to medulloblastoma subgroups, and within, **c** WNT and **d** SHH subgroups according to age groups. Distribution of histological variants within SHH tumors according to *TERT* mutational status (**e**). *MUT* mutation, *OS* overall survival, *WT* wild-type

frequently with metastatic dissemination at diagnosis compared to *TERT* wild-type tumors ($p = 0.03$).

TERT mutations are specifically enriched in SHH medulloblastomas

In a subgroup-specific analysis, we revealed that *TERT* mutations were significantly enriched in SHH tumors (80/213; 38 %; $p < 0.0001$) compared to WNT (6/53; 11 %) and Group 3 (2/50; 4 %) or Group 4 tumors (8/150; 5 %). *TERT* mutations in both WNT and SHH medulloblastomas were positively correlated with age. *TERT* mutations were significantly enriched in adult patients (Fig. 1c, d, both $p < 0.0001$). Increasing age was not associated with increased mutational frequency in either Group 3 or Group 4 tumors (n.s.). While histopathological features were similar between *TERT*-mutated and wild-type tumors across subgroups, we observed that classic histology was more commonly observed in *TERT* mutant SHH tumors, and desmoplastic histology in wild-type SHH tumors (Fig. 1e; Table 2; $p = 0.04$), respectively.

Prognostic implications of *TERT* mutations

When medulloblastoma patients across all subgroups were stratified by *TERT* mutational status, we observed no significant differences in survival (Fig. 2a; $p = 0.45$). Further after normalizing the subgroup composition to reported subgroup ratios, a statistical difference was still not revealed (data not shown; $p = 0.36$) [1, 15, 26, 41]. However, when *TERT* mutational status is re-analyzed in a subgroup-specific manner, several important survival associations are observed. *TERT* mutations had no prognostic impact within WNT tumors (Fig. 2b; $p = 0.17$). However, a significant association between *TERT* promoter mutations and outcomes was noted in SHH and Group 4 medulloblastomas. Specifically, the 5-year overall survival of SHH tumors with and without *TERT* mutations was 77.6 ± 7 %

Table 2 Clinicopathological and molecular characteristics of SHH medulloblastoma according to *TERT* mutational status

Characteristic	*TERT* MUT	*TERT* WT	*p* value
Age (years)			
Median	25.00	3.00	**<0.0001**[#]
Range	0.66–49.00	0.24–52.00	
NA	1	0	
Gender			
Male	46	80	0.77[Φ]
Female	31	48	
NA	3	5	
Histology			
MBEN	3	4	**0.04**[χ]
Desmoplastic	10	44	
Classic	34	47	
LC/A	10	16	
NA	23	22	
M-stage			
M0	46	87	0.84[Φ]
M1-3	10	22	
NA	24	24	
TP53 status			
MUT	4	8	1[Φ]
WT	38	71	
NA	38	54	

F female, *LC/A* large cell/anaplastic, *M* male, *MB* medulloblastoma, *MBEN* medulloblastoma with extensive nodularity, *NA* not available (data were excluded from statistical comparison)

Bold values indicate $p < 0.05$

[#] Mann–Whitney *U* test

[Φ] Fisher's exact test

[χ] Chi-square test

and 64.1 ± 5.1 %, respectively (Fig. 2c; $p = 0.04$). In contrast to the improved prognosis of *TERT* mutant SHH tumors, we observed the inverse pattern in Group 4 tumors where the 5-year overall survival for patients without and with *TERT* mutations was 73.3 % ± 4.3 % and 62.5 % ± 17.1 % (Fig. 2d; $p = 0.04$). Similar to the unfavorable prognosis of *TERT* mutations in Group 4 tumors, both of the patients with *TERT*-mutated Group 3 tumors died after 7 and 45 months of follow-up, respectively (Supplementary Table 1). Thus, we conclude that *TERT* mutations define distinct prognostic patient cohorts in a subgroup-specific fashion with good prognosis in SHH and poor prognosis in Group 4 medulloblastomas.

Survival analysis restricted to specific age groups

As *TERT* mutations are predominantly observed in non-infant medulloblastomas, we evaluated the prognostic implications of these promoter mutations across all four medulloblastoma subgroups in an age-dependent manner. *TERT* mutational status across subgroups had no prognostic impact among patients older than 3 years of age at diagnosis (Fig. 3a; $p = 0.59$). Interestingly, the prognostic impact of *TERT* mutation was more pronounced in the non-infant SHH population with a 5-year overall survival of 76.9 % ± 7.6 % and 59.3 % ± 6.9 % of non-infants with and without *TERT* promoter mutations, respectively (Fig. 3b; $p = 0.019$). These prognostic implications were similar in adult medulloblastoma patients and in the adult SHH subgroup (Supplementary Figure 2). In a subset of 76 SHH cases with known *TP53* mutational status [44], we revealed that *TP53* mutations identify non-infant SHH tumors with a particularly poor prognosis, while in contrast *TERT* mutations identify a subsets with good prognosis (Fig. 3c; $p = 0.047$). Mutations of both *TERT* and *TP53* were observed in 4/12 SHH tumors (Supplementary Table 2). Non-infant Group 4 showed an inverse prognostic association with poor outcome of *TERT*-mutated cases (Fig. 3d; $p = 0.024$). Lastly, we analyzed the overall survival of SHH patients under a multivariate Cox proportional hazards model comprising age at diagnosis, *TERT* mutational status, M-stage, and histology. In addition to the known prognostic significance of M-stage ($p < 0.001$) and histology ($p = 0.02$), we revealed that *TERT* status continued to be associated with good prognosis (HR 0.17, CI 0.04–0.69, $p = 0.01$), independent of other prognostic factors including age at diagnosis ($p = 0.35$).

Distinct somatic copy number alterations of *TERT*-mutated medulloblastomas

To identify additional genetic features associated with these distinct demographic and clinical differences, we evaluated broad and focal copy number alterations according to subgroup affiliation and *TERT* promoter mutations. Notably, only 1/6 (17 %) of *TERT*-mutated WNT tumors harbored monosomy 6, while this alteration is observed in approximately 80 % of *TERT* wild-type medulloblastomas of the WNT subgroup (Fig. 4a; $p = 0.005$). Loss of chromosome 2 and 10q loss were significantly enriched in *TERT* wild-type SHH tumors, while 3q loss was more frequently observed in their *TERT* mutant counterparts (Fig. 4b). Previously described focal alterations characteristic for SHH tumors including amplification of *MYCN/GLI2/CDK6/YAP1/PPM1D*, and deletions targeting *PTCH1/CDKN2A/CDKN2B/PTEN* were largely confined to *TERT* wild-type SHH medulloblastomas, while *TERT* mutant SHH (Fig. 5) and Group 4 (Supplementary Figure 3) showed very few focal SCNAs. Consistent with the higher frequency of *TERT* mutations in SHH tumors, we observed increased *TERT* expression in the SHH subgroup compared to Group

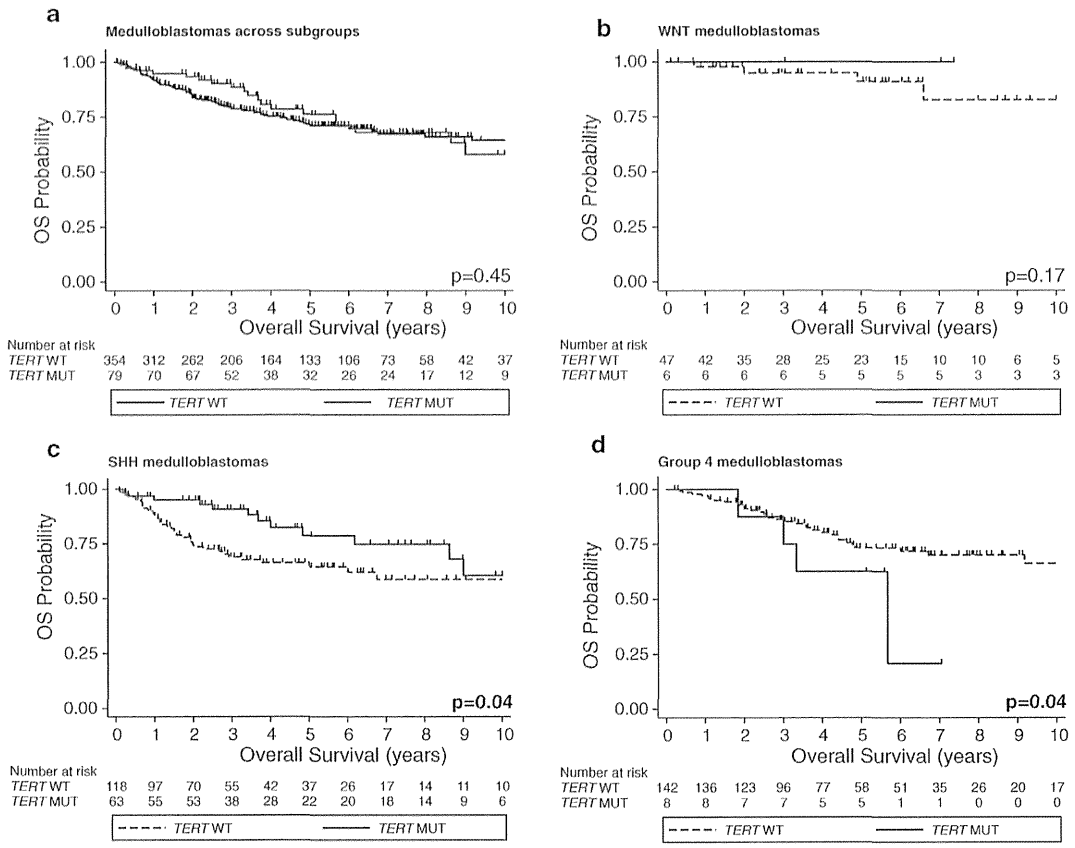


Fig. 2 Prognostic impact of *TERT* promoter mutations varies according to medulloblastoma subgroups. Kaplan–Meier estimate displaying overall survival (OS) according to *TERT* mutational status in primary medulloblastomas (**a**), within WNT (**b**), SHH (**c**), and Group 4 (**d**) subgroups. Survival differences were calculated using continuous log-rank tests. *MUT* mutation, *OS* overall survival, *WT* wild-type

4 tumors in two independent gene expression profiling studies ($p < 0.001$; Supplementary Figure 4). Furthermore, we observed *TERT* amplification in two tumors included in the entire cohort of 1,088 previously studied tumors [30]. Both of these cases with *TERT* amplification were SHH-driven medulloblastomas with wild-type *TERT* status, which were derived from pediatric patients who were both alive after 15 and 83 months of follow-up (Supplementary Figure 5). Thus, broad and focal SCNAs underline that *TERT* mutations define a genetically distinct subset within SHH tumors and possibly within the WNT and Group 4 tumors.

Discussion

The underlying biology of adult medulloblastomas remains poorly understood. Next-generation sequencing studies have revealed a broad spectrum of novel, potentially tumorigenic mutations in the recent past, but none of these studies focused on adult medulloblastomas [12, 25, 32, 34, 39]. In addition, the vast majority of these mutations are not recurrent enough to stratify patients into distinct clinical and prognostic subgroups.

In this study, we demonstrate that *TERT* promoter mutations, initially described in melanoma [10, 11], comprise the most recurrent mutation described so far across medulloblastoma subgroups, with a particular enrichment in older patient cohorts. These somatic mutations are especially common in older patients with SHH tumors (83 %) and to a lesser extent in adults with WNT medulloblastomas (11 %). Based on the transcriptional heterogeneity of SHH tumors in infant and adult patients, we suspect that the adult cluster mainly comprised *TERT*-mutated medulloblastomas [24]. According to the initial classification of tumor types with *TERT* mutations at frequencies over 15 % (*TERT*-high) vs. below this threshold (*TERT*-low) [13], our report suggests distinct baseline telomerase activity of the cell of origin in each of the subgroups (Group 3 $\geq$ Group 4 > WNT >> SHH). Furthermore, the identification of

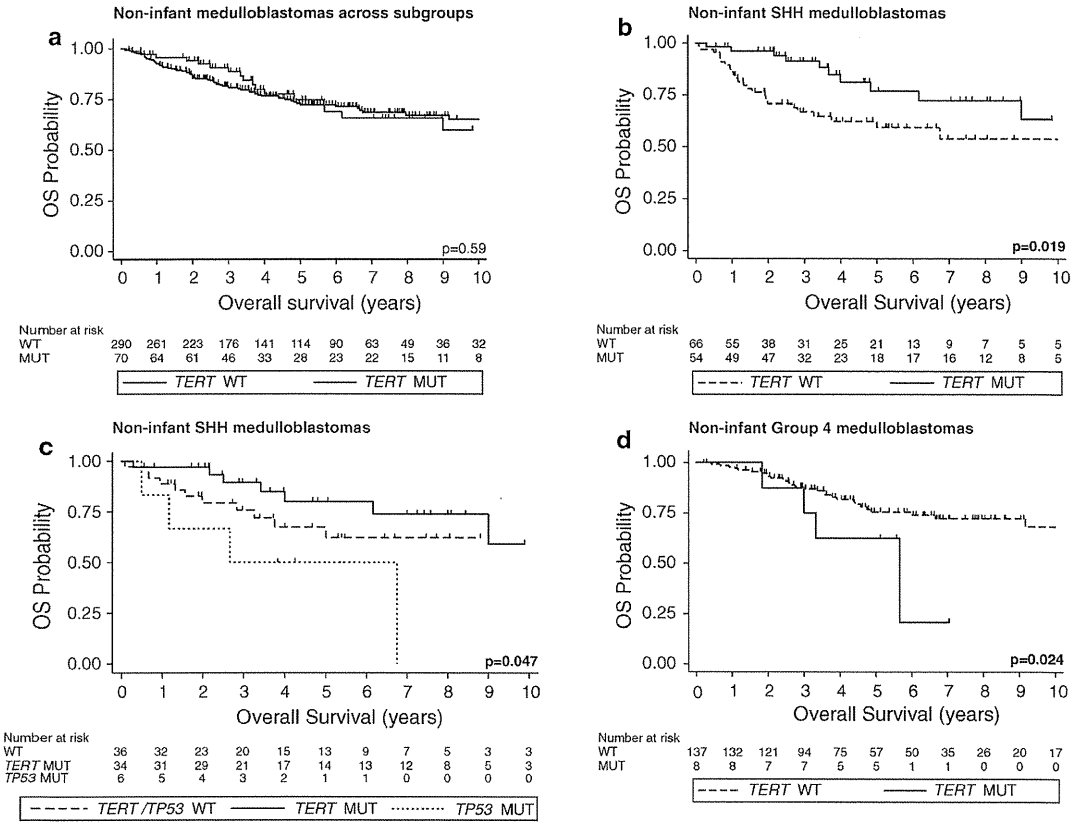


Fig. 3 *TERT* promoter mutations delineate prognostic subsets within non-infant SHH and Group 4 medulloblastomas. Kaplan–Meier estimate displaying overall survival (OS) in non-infant medulloblastomas (>3 years of age at diagnosis) according to *TERT* mutational status across subgroups (**a**), in SHH tumors (**b**), in SHH tumors (*TP53* mutated/wild-type) (**c**), and Group 4 (**d**). Survival differences were calculated using continuous log-rank tests

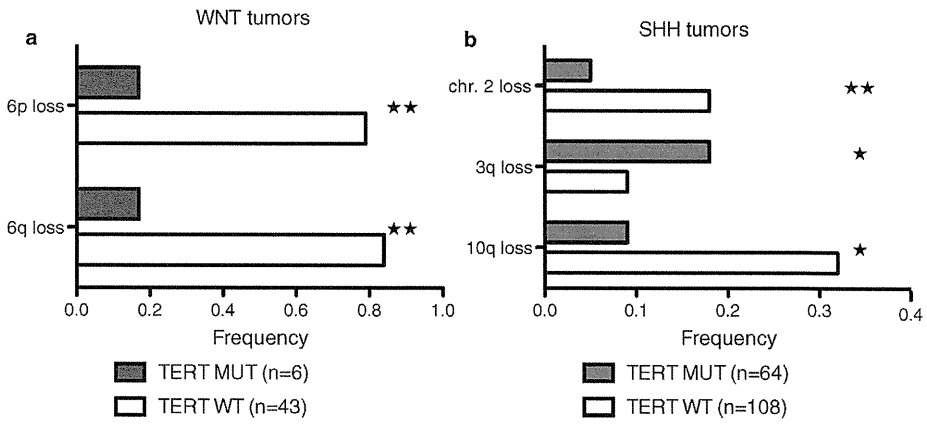
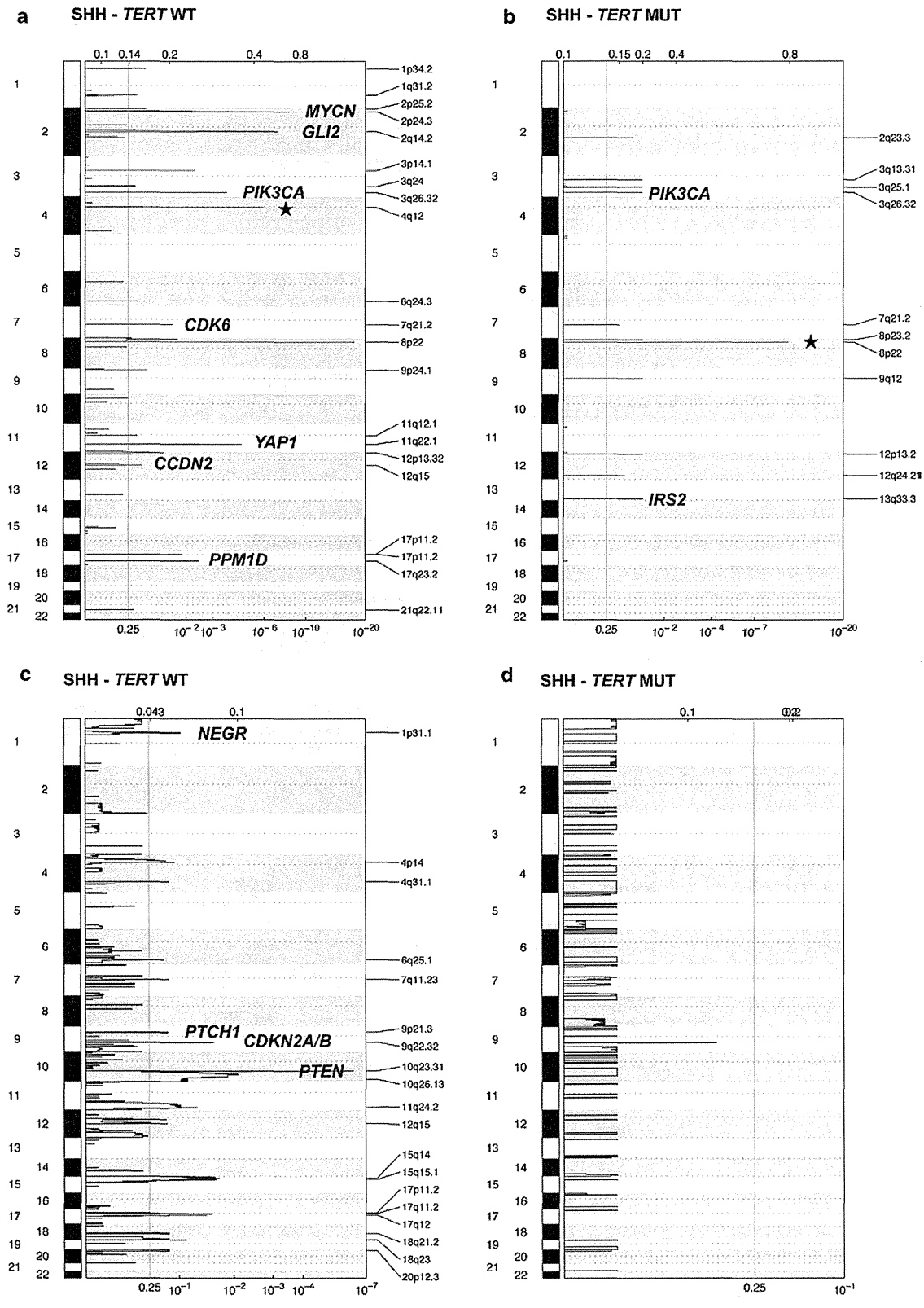


Fig. 4 WNT and SHH medulloblastoma harbor distinct broad genomic imbalances depending on the mutational status of *TERT*. *Bar graphs* indicating the frequency of broad cytogenetic alterations in WNT (**a**), and SHH (**b**) tumors. ★★ $p < 0.01$; ★ $p < 0.05$; *MUT* mutation, *WT* wild-type

recurrent *TERT* promoter mutations makes a compelling argument that the increasing availability of whole-genome sequencing results may substantially add to a refined understanding of the mutational landscape of different biological and age-driven medulloblastoma subgroups, since earlier next-generation sequencing studies focusing on the protein-coding regions had not encompassed gene-regulatory regions including promoter mutations.

In this study, we demonstrate that the mutational status of the *TERT* promoter can segregate individuals with SHH


a
SHH - TERT WT
MYCN
GLI2
PIK3CA
CDK6
YAP1
CCDN2
PPM1D
b
SHH - TERT MUT
PIK3CA
IRS2
c
SHH - TERT WT
NEGR
PTCH1
CDKN2A/B
PTEN
d
SHH - TERT MUT