RESEARCH ARTICLE

TERT promoter mutations in primary and secondary WHO grade III meningioma

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Abstract

Purpose: *TERT* promoter mutation (*TERT*p^{Mut}) has a strong association to recurrence and has been suggested to act as a driver mutation for malignant transformation of WHO grade I and II meningiomas. *TERT*p^{Mut} has been investigated in selected high-grade meningioma samples. The existence of *TERT*p^{Mut} across recurrent tumors in a population-based cohort needs to be investigated in order to identify when *TERT*p^{Mut} emerges across recurrent samples and to validate prognostic impact among WHO grade III tumors. **Methods:** We gathered material from a consecutive single-center cohort of 40 patients with malignant meningioma (WHO grade III) treated between 2000 and 2018, including specimens from primary and secondary malignant meningiomas with the corresponding earlier benign specimens and later malignant recurrences. In total 107 tumor samples were studied by Sanger sequencing for *TERT* promoter mutational status. **Results:** Seven of 40 patients (17.5%) harbored *TERT*p^{Mut} thus validating the incidence of *TERT*p^{Mut} in previous non-population-based cohorts. In 6/7 patients, the *TERT*p^{Mut} was present at initial surgery (WHO grade I–III) while in one patient the *TERT*p^{Mut} was found *de novo* when the meningioma became malignant. The incidences were 2/1.000.000/year for *TERT*-p^{Mut} WHO grade III meningioma and 8/1.000.000/year for *TERT*p^{wt} WHO grade III meningioma in our catchment area. We found a 1.7 times higher recurrence rate (CI 95% 0.65–4.44) and a 2.5 higher mortality rate per 10 person-years (CI 95% 1.01–6.19) for *TERT*p^{Mut} compared to *TERT*p^{wt}. **Conclusion:** *TERT*p^{Mut} can occur independently of malignant progression in meningioma and was most often present from the first tumor sample across recurring tumors. *TERT*p^{Mut} in WHO grade III may represent a marker of an aggressive subset of tumors.

INTRODUCTION

Meningiomas account approximately for 37% of all intracranial tumors (17). A subset of meningiomas, WHO grade III, are malignant. These tumors comprise approximately 1%–3% of all primary meningiomas, with a dismal prognosis with significant morbidity and mortality (9, 17). Better knowledge of pathophysiology is desirable to improve management of these patients, as hitherto published retrospective data show limited efficacy of aggressive surgery and adjuvant therapy once malignant criteria are met (18).

WHO grade III meningiomas comprise of primary malignant meningiomas and secondary that develop through malignant transformation of grade I or II meningiomas. Previous reports have described *CDKN2A/B* loss (4), accumulation of chromosome gains and losses (6), and telomerase reverse transcriptase (*TERT*) gene alterations (5). *TERT* gene alterations (*TERT*-alt) may enforce cell immortalization by counteracting telomere shortening, thus promoting growth (1). *TERT*-alt comprise, but are not limited to, promoter mutations, gene translocations and DNA amplifications (1). The most common alterations are specific point mutations (C250T and C228T) of the *TERT* promoter region (*TERT*p^{Mut}) (24). *TERT* promoter mutations either occur already in initial low-grade meningiomas or emerge during malignant transformation of meningiomas (5). A recent meta-analysis of

individual patient data including 677 patients with grade I–III meningiomas provided evidence that TERT-alt is a negative prognostic biomarker independent of the tumors' WHO-grade (11). The previous studies (8, 18, 20, 21) included 21–58 patients possessing WHO grade III meningiomas with 14%–23% expressing *TERT*p^{Mut}. *TERT*p^{Mut} was reported to accumulate in grade III tumors and thought to contribute to malignant progression. *TERT*p^{Mut} was a negative prognostic factor among grade III tumors in one study (20). The previous studies were neither population based nor included consecutive patients with clinical follow-up. Only one study (18) compared expression of *TERT*p^{Mut} in primary (5/28) and secondary malignant meningiomas (3/29), but did not include preceding grade I–II samples from the secondary grade III meningiomas. Observational data do not indicate *TERT*p^{Mut} as a common cause for WHO grade shifts although accumulation in higher grades has been observed and considered a possible driver mutation (20). Serial samples that include pre-malignant meningiomas of patients with secondary grade III tumors allow the analysis of how *TERT*p^{Mut} is associated with the shift to a grade III tumor.

We have investigated the frequency of the two point mutations C228T and C250T (*TERT*p^{Mut}) in a consecutive cohort of 40 WHO grade III meningiomas and occurrence of *TERT*p^{Mut} in a series of tumor samples obtained during malignant transformation of secondary WHO grade III meningiomas.

The aims were to validate the previously detected 14%–23% expression of *TERT*p^{Mut} in WHO grade-III meningiomas in a consecutive, population-based clinical material and to analyze detection of *TERT*p^{Mut} in longitudinal series of tumor samples from patients with secondary and primary WHO grade III meningiomas.

MATERIALS AND METHODS

Patient cohort

We recruited patients diagnosed and treated for WHO grade III meningioma between October 2000 and September 2018 at the Departments of Neurosurgery and Pathology at the Copenhagen University Hospital Rigshospitalet in Denmark (catchment area 2.2 million). We identified 40 consecutive patients, a subset of whom have been clinically described in a previous report (12). The linkage between Danish social security system (CPR number) and WHO grade III specimen numbers in the Danish Pathology Biobank enabled us to localize serial tumor tissue from initial as well as all subsequent surgeries for all 40 patients, in total 119 potential paraffin-embedded formalin-fixed tumor specimens. Tissue was successfully retrieved from 108/119 (91%) specimens in the pathology archive. The remaining specimens had either been destroyed or lost. A senior consultant in neuropathology (HB) classified each specimen according to the WHO 2016 classification (11) before moving further with the *TERT* analysis. After TERT analysis, the slides were reviewed again in order to identify histopathological distinctions in *TERT*p^{Mut} tumors (histological subtypes, necrosis, brain invasion, mitoses).

Ethical approval for the current study was obtained by the Danish Ethics Committee (approval number H-6-2014-010).

Statistics

We applied time since first WHO grade III meningioma to radiologically verified recurrence according to the RANO criteria (23) or death as underlying time scale, whichever appeared first. The term "malignant progression" is used for WHO grade I/II tumors switching to grade III. Tumor "recurrence" was used to denote radiological progression or reappearance of a previously operated tumor. For investigation of differences in age and sex in *TERT*p^{Mut} and *TERT*p^{wt} groups, we applied a t-test and Fisher's test, respectively.

For *TERT*p^{Mut} versus *TERT*p^{wt}, we (i) applied the Aalen–Johansen estimator and Gray's test to investigate the cumulative incidence of recurrence when considering death without recurrence as a competing risk; and (ii) estimated the recurrence and mortality rate per 10 person-years.

We applied Cox proportional hazards regression analysis to investigate the effect of *TERT*p^{Mut} on death. The Cox regression was adjusted to age at diagnosis. We tested assumption of proportionality by investigating Schoenfeld residuals and found that the assumption was valid. Age at diagnosis was included as a continuous covariate, in which we found a linear association to be adequate after applying a restricted cubic spline regression (χ^2, $P > 0.05$). The probability of overall survival was estimated with Kaplan-Meier curves, and we tested whether the curves were significantly different with a log-rank test. We considered $P < 0.05$ significant. For computing, we used the open-source software R version 3.7.1 to all analyses.

Mutational analysis

DNA extraction

DNA was extracted from 4 × 10 micrometer paraffin-embedded formalin-fixed tissue following the standard protocol. In short, TRIS buffer (TRIS 10 mM + 0.1 mM EDTA, pH 7.4) was added to the tissue and incubated at 95°C for 10 min. The samples were subsequently cooled down to 56°C and 20 µL of Proteinase K (Qiagen, Denmark) was added. The samples were then incubated overnight at 56°C. The following day, Proteinase K was inactivated by incubation for 10 min at 95°C. We measured DNA concentration with NanoDrop (ThermoFisher Scientific, Denmark). One of the 108 samples was excluded because of inadequate DNA quality and the amount leaving the total amount of samples available for Sanger sequencing was found to be 107.

Sanger sequencing

Bidirectional Sanger sequencing of the *TERT* promoter region including the two mutations, C228T and C250T, and was performed using previously applied methodology (10). In short, the target region was amplified by conventional PCR using the previously reported primers

5′-CACCCGTCCTGCCCCTTCACCTT-3′ (sense) and 5′-GGCTTCCCACGTGCGCAGCAGGA-3′ (antisense) in 20 μL reactions using the Platinum™ II Hot-Start PCR Master Mix (Thermo Fisher Catalog #:14000013) with GC-enhancer. The amount of DNA was between 10 ng and 100 ng depending on the quality. PCR conditions consisted of a Touch-Down protocol with annealing temperatures ranging from 60 to 50°C. ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fisher) was used for PCR purification. The sequences were generated by Sanger sequencing at the KiGene core facility, Karolinska Institutet. Samples were sequenced with sense primer initially and subsequently verified with antisense primer in selected cases. All mutations and aberrations at the hotspots were confirmed and re-analyzed with both sense and antisense primers.

RESULTS

The diagnoses of 40 consecutive grade III meningiomas during 18 years in a catchment area of 2.2 million inhabitants correspond to an incidence of 10.1/100.000/year. Among our 40 patients with WHO grade III meningiomas, we found *TERT*p^{Mut} in seven patients (17.5%): 2/20 (10%) primary grade III meningiomas and 5/20 (25%) secondary grade III meningiomas. The patient and treatment characteristics of the *TERTp*^{Mut} and the *TERTp*^{wt} groups are shown in Table 1. There was no significant difference in age (*TERT*p^{Mut}: mean 62 years, *TERT*p^{wt}: mean 59, *t*-test $P = 0.68$). Only one female belonged to the *TERT*p^{Mut} group (1/7 14.3%) compared to 19 in the *TERT*p^{wt} group (19/33, 57.6%). The difference was not statistically significant (Fisher test; $P = 0.09$). Twenty patients (50%) had a primary malignant meningioma and 20 a secondary. Of the 40 WHO grade III meningiomas, four tumors were papillary (two primary and two secondary), four were rhabdoid (three primary and one secondary) and 32 were anaplastic (15 primary and 17 secondary). *TERT*p^{Mut} tumors of all grades did not show any distinctive histopathological features compared to the *TERTp*^{wt} group, apart from *TERT*p^{Mut} being found solely in patients whose tumors were classified as anaplastic meningiomas.

Primary malignant meningiomas (n = 20)

The two patients with papillary and three patients with rhabdoid histology all had meningiomas containing *TERTp*^{wt}. Two patients with primary anaplastic meningiomas (patient #1 and #7) had *TERT*p^{Mut}. Both had a C228T *TERT*p^{Mut} and none had the C250T mutation (Figure 1). Patient #1 was only operated once for the WHO grade III meningioma while patient #7 was operated for a grade III recurrence that retained the C228T *TERT*p^{Mut} (Figure 1).

Secondary malignant meningiomas (n = 20)

The two patients with papillary and one patient with rhabdoid histology had meningiomas containing *TERTp*^{wt}. Five patients with anaplastic meningioma had *TERT*p^{Mut}. The first tumor sample from one of these patients (patient #7) with a C228T *TERT*p^{Mut} contained, however, papillary elements while subsequent tumor samples were purely anaplastic.

Four patients had a C228T (patient #2, 3, 5 and 6) and one had a C250T (patient #4) mutation (Figure 1). The *TERT*p^{Mut} was present already in the first available meningioma sample (WHO grade I meningioma for patient #3 and grade II meningiomas for patients #2, 4 and 5) in four patients. The same mutation was detectable in samples from 2, 4 and 1 subsequent surgeries in patients #2, 4 and 5, respectively. One patient (patient #6) underwent five surgeries for a WHO

Table 1. Patient and treatment characteristics of the *TERT*p^{Mut} and the *TERT*p^{Wt} group.

	*TERT*p^{Mut} (n = 7)	*TERT*p^{Wt} (n = 33)
Age at first WHO grade III diagnosis (mean in years ± SD (range))	62 ± 15 (32–81)	59 ± 16 (10–87)
Female	1 (14%)	19 (58%)
Number of surgeries, all grades (mean, range)	4 (1–11)	3 (1–8)
Tumor size of the first WHO gr. III (widest diameter in mm ± SD (range))*	56 ± 20 (16–88)	49 ± 16 (40–96)
Time from first grade III to death or end of follow-up July 2019 in months (median, (range))	20 (0,3–80)	38 (0,5–201)
Location (first WHO grade III tumor)		
Convexity	4 (57%)	17 (52%)
Falcine/parasagittal (non-convexity)	1 (14%)	9 (27%)
Skull base	2 (29%)	4 (12%)
Intraventricular	0 (0%)	3 (9%)
Simpsongrade (first WHO grade III)		
Grade I	1 (14%)	8 (24%)
Grade II	1 (14%)	13 (39%)
Grade III	2 (29%)	6 (18%)
Grade IV	2 (29%)	4 (12%)
Grade V	0 (0%)	0 (0%)
Missing data (Simpson grade I–III)	1 (14%)	2 (6%)
Received adjuvant radiotherapy (any tumor)	86%	67%

*Not all preoperative MR scans were available for the first WHO grade III tumor. In the *TERT*p^{Mut} group, the tumor size was derived from the first meningioma (WHO grade II) in one patient and from the seventh meningioma, WHO grade III, in another patient. In *TERT*p^{Wt} the first meningioma (WHO grade II) was used in three patients, and in four patients the last WHO grade III preoperative scan was used.

Pt	OP #1		OP #2		OP #3		OP #4		OP #5		OP #6-#10		OP #11	
1	III	C228T												
2	II	C228T	III	C228T	III	C228T								
3	I	C228T	III	wt	III	C228T	III	C228T						
4	II	C250T	II	C250T	III	C250T	II	C250T	III	C250T				
5	II	C228T(10%)	III	C228T										
6	I	NA	I	wt	I	wt	I	wt	I	wt	II	NA	III	C228T
7	III	C228T	III	C228T										

Figure 1. *TERT* promoter mutations (*TERT*p^{Mut}), C228T and C250T, during malignant degeneration in the seven patients with *TERT*p^{Mut} from our cohort of 40 WHO grade III meningioma. [Colour figure can be viewed at wileyonlinelibrary.com]

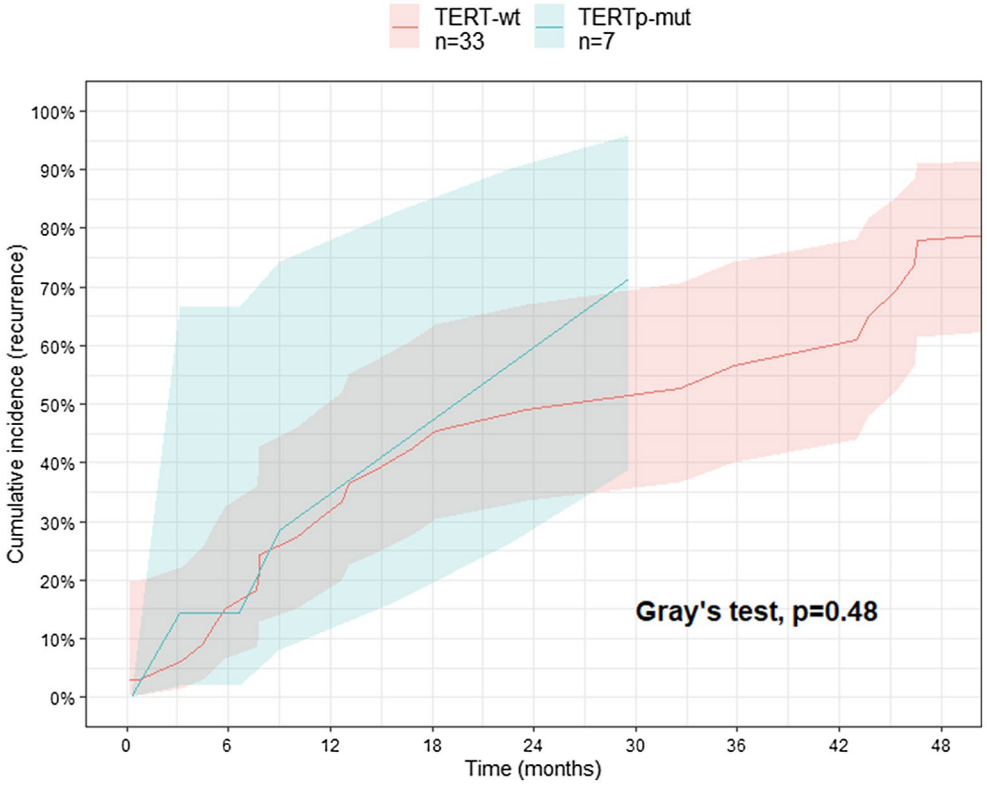

Figure 2. Cumulative incidence of recurrence in *TERT*p^{Mut} and *TERT*p^{wt} in our cohort of 40 WHO grade III meningioma (with death without recurrence as a competing risk). [Colour figure can be viewed at wileyonlinelibrary.com]

grade I tumor where the first tumor sample was unavailable and four subsequent tumor samples showed *TERT*p^{wt}. Material was again unavailable for five subsequent operations for WHO grade II tumors, while the final operation, which revealed a grade III meningiomas, showed *TERT*p^{Mut}.

One patient changed from *TERT*p^{Mut} to *TERT*p^{wt} after the first surgery: Patient #3 had a C228T mutation in the tumor sample from the first surgery of a WHO grade I meningioma, while the first subsequent recurrence, which had progressed to WHO grade III, showed *TERT*p^{wt}. Later recurrences of the grade III tumor were again *TERT*p^{Mut} (Figure 1). We reviewed the tumor blocks again from the *TERT*p^{wt} tumor from patient #3 (two large pieces) and concluded that the sequenced material was representable for the tumor sample.

Recurrence and mortality in *TERT*p^{Mut} and *TERT*p^{wt} patients

The cumulative incidence of recurrence after first WHO grade III diagnosis was 33% (CI 95%: 20–52) for the *TERT*p^{wt} group and 43% (CI 95%: 16–82) for *TERT*p^{Mut} group after 1 year. Gray's test showed no significant difference between cumulative incidence of recurrence in the *TERT*p^{Mut} and *TERT*p^{wt} group (P = 0.48) (Figure 2). In contrast, incidence of recurrence was 6.8 (CI 95% 5.0–8.7)

per 10 person-years for *TERT*p^{Mut} patients compared with 4.0 for *TERT*p^{wt} patients (CI 95% 3.5–4.5), translating into a 1.7 higher recurrence rate in the *TERT*p^{Mut} group (Figure 3).

Cox proportional hazard regression analyses did not show a statistically significant effect on overall survival by *TERT* promoter status. Adjusted to age at diagnosis, the hazard ratio for *TERT*p^{Mut} patients (n = 7) was 1.9 (CI 95% 0.7–5.4) in reference to their wild-type counterparts (n = 33). *TERT*p^{wt} patients had a median survival of 52.0 months (CI 95% 19.9—not reached) compared with *TERT*^{Mut} patients with a median survival of 20.4 (CI 95% 6.67—not reached) (Figure 4). A log-rank test indicated no significant difference (P = 0.2). In contrast, the mortality incidence rate was 3.7 for *TERT*p^{Mut} patients (CI 95% 2.8–4.7) per 10 person-years, and 1.5 in *TERT*p^{wt} patients (CI 95% 1.3–1.7), yielding a 2.5 higher mortality rate in the *TERT*p^{Mut} group (Figure 3).

DISCUSSION

Main findings

We found *TERT*p^{Mut} in seven patients, 2/20 with primary and 5/20 with secondary, WHO grade III meningiomas. Already the first, premalignant, sample from 4/5 patients with secondary malignant meningiomas harbored *TERT*p^{Mut}. Our findings corroborated previous reports (5, 21) of C228T being the most common *TERT*p^{Mut} in meningioma, as we found C228T in six of the seven patients.

The proportion of *TERT*p^{Mut} in this population-based consecutive cohort was 17.5%, which is similar to the 14%–23% in previous series with malignant meningioma, and two- to threefold higher than in series with benign meningioma (14). The incidences were 2/1.000.000/year for *TERT*p^{Mut} WHO grade III meningioma and 8/1.000.000/year for *TERT*p^{wt} WHO grade III meningioma in our catchment area.

We found a higher incidence rate of recurrence and death when comparing incidence rates per 10 person-years for *TERT*p^{Mut} to *TERT*p^{wt} WHO grade III meningioma. *TERT*p^{Mut} were confined to WHO grade III tumors of the anaplastic subtype with one small exception; an anaplastic WHO grade III meningioma that showed some level of papillary morphology in the first tumor and in later recurrence the papillary morphology was absent.

Prognostic impact of *TERT*p^{Mut} in WHO grade III meningioma

We found a 1.7 times higher recurrence rate for *TERT*p^{Mut} compared to *TERT*p^{wt} and a 2.5 higher mortality rate for *TERT*p^{Mut} compared to *TERT*p^{wt}, which is well compatible with an independent prognostic impact by *TERT*p^{Mut}. Surprisingly, however, Cox regression analysis showed a difference that was not statistically significant between overall survival in the *TERT*p^{Mut} and *TERT*p^{wt} groups. Similarly, the cumulative incidences of recurrence from the first WHO grade III tumor failed to show a statistically significant difference between the groups. In contrast, larger cohorts and meta-analyses of WHO grade III meningioma have demonstrated associations also between *TERT*p^{Mut} and survival. Sahm *et al* reported that *TERT*p^{Mut} WHO grade III meningioma patients recurred statistically significantly earlier than *TERTp^{wt}* meningioma patients (20). Our cohort, although large for primary and secondary grade III meningiomas, included only 40 patients. The difference between published literature and our cohort may reflect lower statistical power of the latter. WHO grade III meningiomas comprise a population where all patients experience recurrence and death within a relatively short time span and larger cohorts may be needed to detect statistically significant differences between subgroups. Importantly, however, cumulative incidence of recurrences and incidence rates reflect different qualities. Malignant tumors such as WHO grade III meningiomas recur if follow-up is long enough, while time

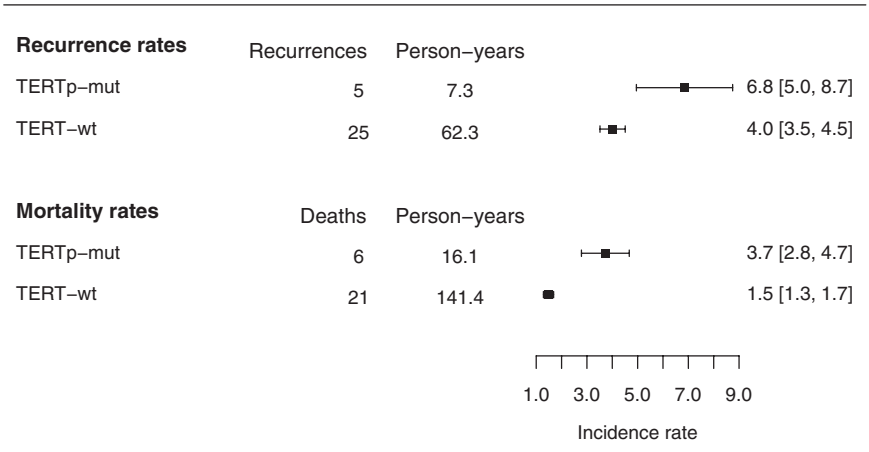

Figure 3. Incidence rates (events of recurrence and mortality) per 10 person-years for *TERT*p^{Mut} and *TERT*p^{wt}. Incidence rate ratio for recurrence 1.7 (6.8/4) and for mortality 2.5 (3.7/1.5). [Colour figure can be viewed at wileyonlinelibrary.com]

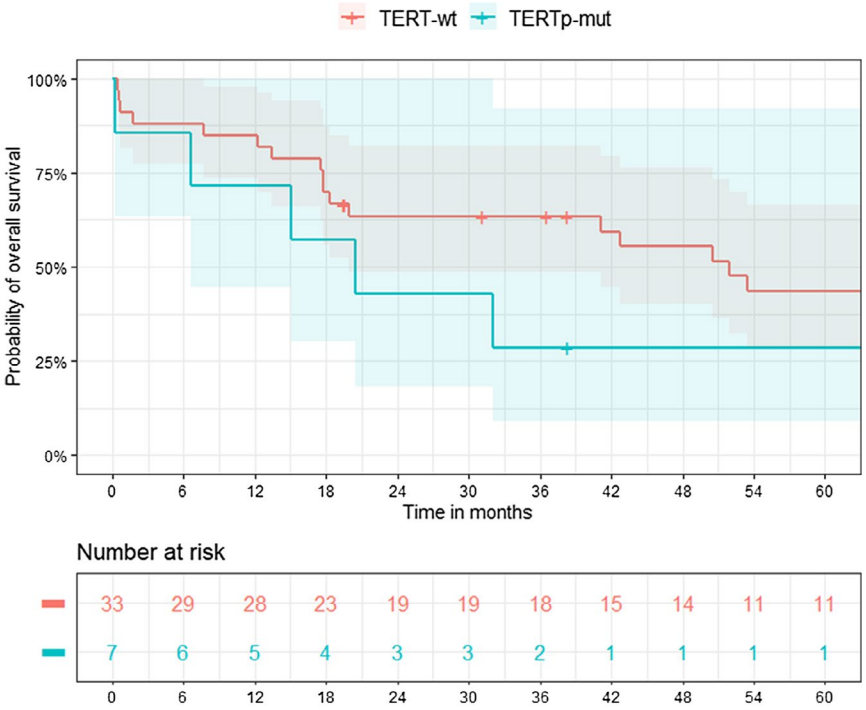

Figure 4. Kaplan–Meier Survival curve. Overall survival for *TERT*p^{Mut} and *TERT*p^{wt} WHO grade III meningioma. *P* value of log-rank test = 0.2. [Colour figure can be viewed at wileyonlinelibrary.com]

to recurrence may differ with the growth rates. In analogy, the Ki-67 proliferation index reflects time to recurrence rather than cumulative incidence of recurrence during long-term follow-up of meningiomas (15). In this context, rates of recurrence and mortality may be preferrable prognostic markers. These rates were higher for *TERT*p^{Mut} meningiomas in our cohort; findings that agree with Mirian *et al* (14) who reported that *TERT* gene alterations (including promoter mutations, gene translocations and DNA amplifications: *TERT*-alt), were independent prognostic markers that defined a subset of aggressive malignant meningiomas.

TERT gene alterations in the natural history of malignant meningioma

*TERT*p^{Mut} are typically somatic mutations (8) which, in our cohort, were already present in the first meningiomas operated not only in the two patients with primary anaplastic meningiomas, but also in the first available non-malignant samples in 4/5 secondary anaplastic meningiomas with *TERT*p^{Mut}. The findings together with prognostic observations agreed that *TERT*p^{Mut} may be a marker of aggressive disease (5, 8, 14). Such a linkage between *TERT*-mutation and aggressive disease could occur in two different ways.

One possibility is that *TERT* would function as a driver mutation in which case the *TERT* mutation would occur simultaneously with change from a benign grade I or II meningioma to grade III. Such mechanisms have been described for hepatocellular cancer (16). Observations in a small number of meningioma patients were compatible with this mechanism in a study by Juratli *et al* (8). They observed switches from *TERT*p^{wt} to *TERT*p^{Mut} that were simultaneous with switches from benign to anaplastic meningiomas while pairs of WHO grade I, II or III tumors that retained their original grades also retained wild-type TERT (8); in this study *TERT*p^{Mut} appeared to be a feature of progression and anaplastic histology.

The other possibility is represented by findings of Goutigny *et al* who described that *TERT*p^{Mut} could be present already in grade I–II samples (5). In this study, *TERT*p^{Mut} was interpreted to represent a meningioma subgroup that was particularly prone to malignant progression and the authors suggested that *TERT*p^{Mut} could serve to drive phenotypes from lower to higher grade meningiomas. Our population-based findings from a similarly sized cohort as those above (5, 8), support the latter possibility; T*ERT*p^{Mut} can be present early in a subgroup of meningiomas with a particularly bad prognosis possibly because of some genetic instability which also conferred an increased risk of malignant progression.

The incidence of T*ERT*p^{Mut} grade III meningiomas is low and research will depend on availability of mutational and clinical data from different sources. Population-based data are particularly useful and publication of our findings will not only support the latter of two above hypotheses but also provide material for meta-analyses of *TERT*p^{Mut} in high-grade meningioma.

Temporal and spatial heterogeneity

Investigation of *TERT*p^{Mut} in serial samples obtained during malignant progression in meningioma is complicated by

spatial heterogeneity of grade II–III meningiomas (2, 13). Previous studies showed that WHO grade II–III meningioma maintained very few mutations across serial recurrences (2, 3). Spatial heterogeneity regarding *TERT*p^{Mut} is evident in other cancers (22). For meningiomas, Juratli *et al* (8) analyzed spatially different tumor samples obtained during one operation from three different patients. The tumors displayed simultaneous presence of both *TERT*p^{Mut} and *TERT*p^{wt} subclones. Findings of heterogeneity suggest that *TERT*p^{Mut} may be an epiphenomenon and a passenger mutation. The association between worsening WHO-grade and negative prognostic impact of *TERT*p^{Mut} (5, 8, 14, 20) could reflect that higher grade tumors simultaneously have worse prognoses and genetic instability with faulty telomere maintenance mechanisms reflected as *TERT*p^{Mut} which are inherent to many types of cancer (1).

Patient #3 (Figure 5) in our cohort clearly showed that biopsies from serial recurrences could show different TERT-mutational status. The temporarily "disappearing" *TERT*p^{Mut} could suggest undersampling or differential dominance of subclones with different *TERT*-mutational status during the course of disease; a mechanism well described by Juratli *et al* (8). The *TERT*p^{Mut} WHO grade I meningioma might have had enough genomic disruption to progress to a WHO grade III, where the dominant clonal component happened to be *TERT*p^{wt}, while the last recurrence was again dominated by tumors with clonal components containing *TERT*p^{Mut}. Small subclones with *TERT*p^{Mut} cells might not have been reproducibly discovered at the first operation of the grade III tumor as a result of the sensitivity of Sanger sequencing (7, 19). Similarly, patient #6 with secondary malignant meningioma and first detection of *TERT*p^{Mut} when the meningioma became malignant, might have harbored a smaller *TERT*p^{Mut} subclone already in the initial tumors. The apparent temporal heterogeneity probably reflected spatial heterogeneity with co-existence, but differential dominance of *TERT*p^{wt} and *TERT*p^{Mut} subclones. Given temporal and spatial heterogeneity, *TERT*p^{Mut} could either be independent of a basic malignant geno-and phenotype or represent a subclone with qualities to maintain malignant behavior and produce recurrences. Our findings and previous publications are compatible with both possibilities: *TERT*p^{Mut} might be either a driver mutation or a passenger mutation—it could also have different roles in different tumors.

Strengths and weaknesses

Malignant meningiomas are rare. Our cohort is large and comprehensive for the pathology but is still too small for high statistical power. A unique strength of our study is the availability of surgical tissues from a consecutive, population-based cohort of primary and secondary WHO grade III meningiomas, including serial samples from pre-malignant precursor meningiomas and recurrent grade III meningiomas. The weakness is the retrospective design which precluded systematic collection of tumor samples to account for spatial heterogeneity. Subsequently, analyses only reflect the tumor subclones that were available for analyses and interpretation may have been obfuscated by

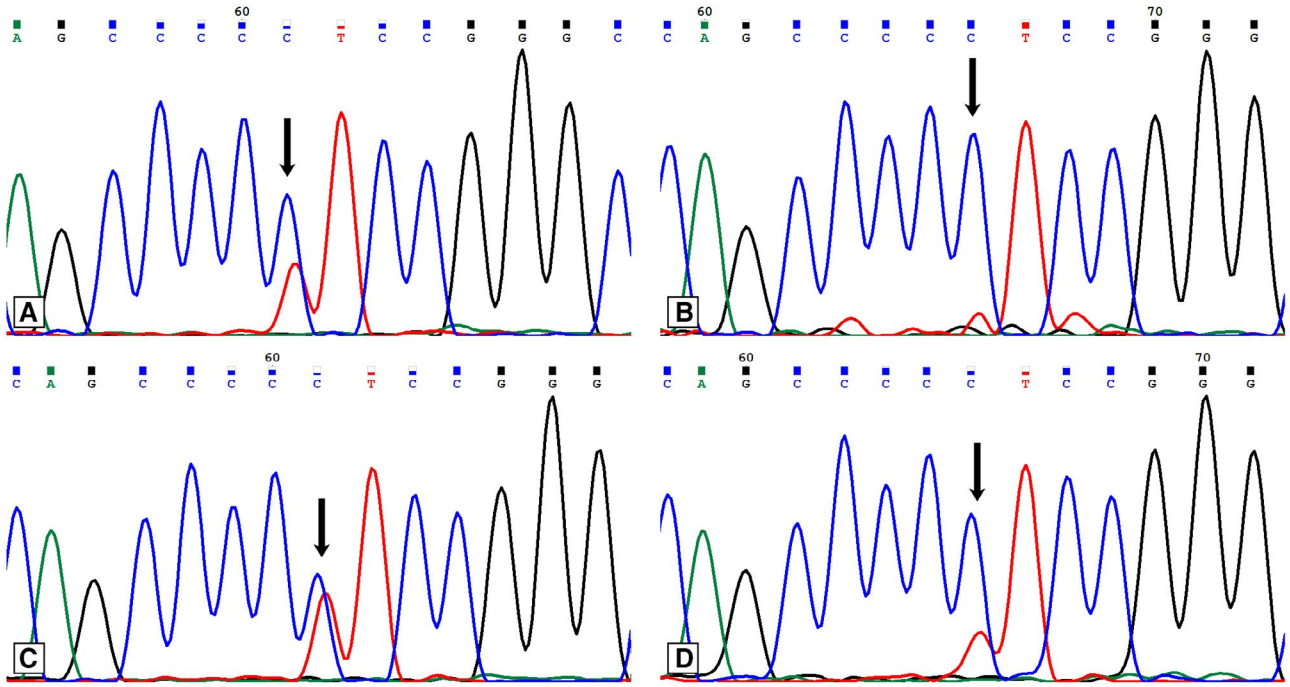

Figure 5. *Chromatograms in patient #3.* **A.** C228T mutation in the patient's first WHO grade I meningioma. **B.** The mutation was not evident in following WHO grade III recurrence. **C,D.** C228T mutation in the later WHO grade III recurrences. [Colour figure can be viewed at wileyonlinelibrary.com]

intratumoral heterogeneity and Sanger sequencing sensitivity.

Finally, we studied only two point mutations by Sanger sequencing. *TERT* promoter mutations comprise the majority of TERT-gene alterations in cancers (1) and a recent systematic review showed that most TERT-alterations in meningiomas were point mutations at C228T and C250T, but 5% still comprised RETREG1-TERT and LPCAT1-TERT fusions (14). Unbiased gene sequencing could thus have revealed other phenotypical relevant *TERT* gene alterations in our cohort of malignant meningioma.

CONCLUSION

We investigated the *TERT* promoter mutation (*TERT*p^{Mut}) status in a population-based consecutive cohort of 40 WHO grade III meningioma patients (20 primary and 20 secondary). Seven of the 40 patients (17.5%) harbored *TERT*p^{Mut}, thus validating earlier reported incidence of *TERT*p^{Mut} in WHO grade III meningioma. The incidences were 2/1.000.000/year for *TERT*p^{Mut} WHO grade III meningioma and 8/1.000.000/year for *TERT*p^{wt} WHO grade III meningioma in our catchment area.

We found a higher recurrence rate and mortality per 10 person-years in the *TERT*p^{Mut} group. TERT-mutations were not typically acquired during malignant progression. *TERT*p^{Mut} could occur independent of malignant progression in meningioma and *TERT*p^{Mut} could represent either driver- or passenger mutations.

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CONFLICT OF INTEREST

The authors declare no conflict of interests.

ETHICS APPROVAL

The study was approved by the Danish Ethics Committee. Approval number H-6-2014-010.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon reasonable request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

REFERENCES

1. Barthel FP, Wei W, Tang M, Martinez-Ledesma E, Hu X, Amin SB *et al* (2017) Systematic analysis of telomere length and somatic alterations in 31 cancer types. *Nat Genet* **49**:349–357. https://doi.org/10.1038/ng.3781.
2. Bi WL, Greenwald NF, Abedalthagafi M, Wala J, Gibson WJ, Agarwalla PK *et al* (2017) Genomic landscape of high-grade meningiomas. *NPJ Genom Med* **2**:15. https://doi.org/10.1038/s41525-017-0014-7.
3. Bi WL, Prabhu VC, Dunn IF (2018) High-grade meningiomas: biology and implications. *Neurosurg Focus* **44**:E2. https://doi.org/10.3171/2017.12.FOCUS17756.
4. Boström J, Meyer-Puttlitz B, Wolter M, Blaschke B, Weber RG, Lichter P *et al* (2001) Alterations of the tumor suppressor genes CDKN2A (p16INK4a), p14ARF, CDKN2B (p15INK4b), and CDKN2C (p18INK4c) in atypical and anaplastic meningiomas. *Am J Pathol* **159**:661–669. https://doi.org/10.1016/S0002-9440(10)61737-3.
5. Goutagny S, Nault JC, Mallet M, Henin D, Rossi JZ, Kalamarides M (2014) High incidence of activating TERT promoter mutations in meningiomas undergoing malignant progression. *Brain Pathol* **24**:184–189. https://doi.org/10.1111/bpa.12110.
6. Goutagny S, Yang HW, Zucman-Rossi J, Chan J, Dreyfuss JM, Park PJ *et al* (2010) Genomic profiling reveals alternative genetic pathways of meningioma malignant progression dependent on the underlying NF2 status. *Clin Cancer Res* **16**:4155–4164. https://doi.org/10.1158/1078-0432.CCR-10-0891.
7. Hagemann IS (2015) Overview of Technical Aspects and Chemistries of Next-Generation Sequencing. Elsevier Inc. https://www.scopus.com/record/display.uri?eid=2-s2.0-84941927536&origin=inward&txGid=5baf662c6d64e11ecc9b2f16899b4ede.
8. Juratli TA, Thiede C, Koerner MVA, Tummala SS, Daubner D, Shankar GM *et al* (2017) Intratumoral heterogeneity and TERT promoter mutations in progressive/higher-grade meningiomas. *Oncotarget* **8**:109228–109237. https://doi.org/10.18632/oncotarget.22650.
9. Kshettry VR, Ostrom QT, Kruchko C, Al-Mefty O, Barnett GH, Barnholtz-Sloan JS (2015) Descriptive epidemiology of World Health Organization grades II and III intracranial meningiomas in the United States. *Neuro Oncol* **17**:1166–1173. https://doi.org/10.1093/neuonc/nov069.
10. Liu T, Brown TC, Juhlin CC, Andreasson A, Wang N, Bäckdahl M *et al* (2014) The activating TERT promoter mutation C228T is recurrent in subsets of adrenal tumors. *Endocr Relat Cancer* **21**:427–434. https://doi.org/10.1530/ERC-14-0016.
11. Louis D, Ohgaki H, Wiestler O, Cavenee W (2016) WHO Classification of Tumours of the Central Nervous System, 4th edn. International Agency for Research on Cancer, World Health Organization, Lyon, France.
12. Maier AD, Bartek J, Eriksson F, Ugleholdt H, Juhler M, Broholm H, Mathiesen TI (2019) Clinical and histopathological predictors of outcome in malignant meningioma. *Neurosurg Rev* **43**:643–653. https://doi.org/10.1007/s10143-019-01093-5.
13. Mei Y, Bi WL, Greenwald NF, Agar NY, Beroukhim R, Dunn GP, Dunn IF (2017) Genomic profile of human meningioma cell lines. *PLoS One* **12**:1–12. https://doi.org/10.1371/journal.pone.0178322.

14. Mirian C, Duun-Henriksen AK, Juratli T, Sahm F, Spiegl-Kreinecker S, Peyre M *et al* (2020) Poor prognosis associated with TERT gene alterations in meningioma is independent of the WHO classification: an individual patient data meta-analysis. *J Neurol Neurosurg Psychiatry* **91**:378–387. https://doi.org/10.1136/jnnp-2019-322257.
15. Mirian C, Skyrman S, Bartek J, Jensen LR, Kihlström L, Förander P *et al* (2020) The Ki-67 proliferation index as a marker of time to recurrence in intracranial meningioma. *Neurosurgery*. :1–10. https://doi.org/10.1093/neuros/nyaa226.
16. Nault JC, Mallet M, Pilati C, Calderaro J, Bioulac-sage P, Laurent C *et al* (2013) High frequency of telomerase reverse-transcriptase promoter somatic mutations in hepatocellular carcinoma and preneoplastic lesions. *Nat Commun* **4**:1–6. https://doi.org/10.1038/ncomms3218.
17. Ostrom QT, Gittleman H, Truitt G, Boscia A, Kruchko C, Barnholtz-Sloan JS (2018) CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2011–2015. *Neuro Oncol* **20**:iv1–iv86. https://doi.org/10.1093/neuonc/noy131.
18. Peyre M, Gauchotte G, Giry M, Froehlich S, Pallud J, Graillon T *et al* (2017) De novo and secondary anaplastic meningiomas: a study of clinical and histomolecular prognostic factors. *Neuro Oncol* **20**:1113–1121. https://doi.org/10.1093/neuonc/nox231.
19. Rohlin A, Wernersson J, Engwall Y, Wiklund L, Björk J, Nordling M (2009) Parallel sequencing used in detection of mosaic mutations: comparison with four diagnostic DNA screening techniques. *Hum Mutat* **30**:1012–1020. https://doi.org/10.1002/humu.20980.
20. Sahm F, Schrimpf D, Olar A, Koelsche C, Reuss D, Bissel J *et al* (2016) TERT promoter mutations and risk of recurrence in meningioma. *J Natl Cancer Inst* **108**:1–4. https://doi.org/10.1093/jnci/djv377.
21. Spiegl-Kreinecker S, Lötsch D, Neumayer K, Kastler L, Gojo J, Pirker C *et al* (2018) TERT promoter mutations are associated with poor prognosis and cell immortalization in meningioma. *Neuro Oncol* **20**:1584–1593. https://doi.org/10.1093/neuonc/noy104.
22. Stenman A, Hysek M, Jatta K, Bränström R, Darai-Ramqvist E, Paulsson JO *et al* (2019) TERT promoter mutation spatial heterogeneity in a metastatic follicular thyroid carcinoma: implications for clinical work-up. *Endocr Pathol* **30**:246–248. https://doi.org/10.1007/s12022-019-09580-7.
23. Wen PY, Macdonald DR, Reardon DA, Cloughesy TF, Sorensen AG, Galanis E *et al* (2010) Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol* **28**:1963–1972. https://doi.org/10.1200/JCO.2009.26.3541.
24. Yuan X, Larsson C, Xu D (2019) Mechanisms underlying the activation of TERT transcription and telomerase activity in human cancer: old actors and new players. *Oncogene* **38**:6172–6183. https://doi.org/10.1038/s41388-019-0872-9.