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TERT promoter mutations in gliomas, genetic associations and clinico-pathological correlations

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Background: The role of telomerase reverse transcriptase (*TERT*) in gliomagenesis has been recently further strengthened by the frequent occurrence of *TERT* promoter mutations (*TERT*p-mut) in gliomas and evidence that the *TERT* SNP genetic rs2736100 influences glioma risk. *TERTp*-mut creates a binding site for Ets/TCF transcription factors, whereas the common rs2853669 polymorphism disrupts another Ets/TCF site on *TERT* promoter.

Methods: We sequenced for *TERT*p-mut in 807 glioma DNAs and in 235 blood DNAs and analysed *TERT* expression by RT-PCR in 151 samples. *TERT*p-mut status and *TERTp* polymorphism rs2853669 were correlated with histology, genomic profile, *TERT* mRNA expression, clinical outcome and rs2736100 genotype.

Results: *TERT*p-mut identified in 60.8% of gliomas (491 out of 807) was globally associated with poorer outcome (Hazard ratio (HR) = 1.50). We defined, based on *TERT*p-mut and *IDH* mutation status, four prognostic groups: (1) *TERT*p-mut and *IDH*-mut associated with 1p19q codeletion, overall survival (OS) > 17 years; (2) *TERT*p-wt and *IDH*-mut, associated with *TP53* mutation, OS = 97.5 months; (3) *TERT*p-wt and *IDH*-wt, with no specific association, OS = 31.6 months; (4) *TERT*p-mut and *IDH*-wt, associated with *EGFR* amplification, OS = 15.4 months. *TERT*p-mut was associated with higher *TERT* mRNA expression, whereas the rs2853669 variant was associated with lower *TERT* mRNA expression. The mutation of *CIC* (a repressor of ETV1-5 belonging to the Ets/TCF family) was also associated with *TERT* mRNA upregulation.

Conclusions: In addition to *IDH* mutation status, defining the *TERT*p-mut status of glial tumours should afford enhanced prognostic stratification of patients with glioma. We also show that *TERT*p-mut, rs2853669 variant and *CIC* mutation influence Tert expression. This effect could be mediated by Ets/TCF transcription factors.

The telomerase reverse transcriptase (*TERT*) gene encodes a highly specialised reverse transcriptase, which adds hexamer repeats to the 3' end of chromosomes (Aubert and Lansdorp, 2008; Cesare and Reddel, 2010). The increased telomerase activity seen in cancer leads to preservation of telomeres, allowing tumours to avoid induction of

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senescence (Smogorzewska and de Lange, 2004; Shay and Wright, 2011).

Somatic mutations of the *TERT* promoter (*TERT*p-mut) have recently been documented in various cancers (Griewank *et al*, 2013; Horn *et al*, 2013; Huang *et al*, 2013; Liu *et al*, 2013), but particularly in glioma (Aapola *et al*, 2000; Arita *et al*, 2013; Killela *et al*, 2013; Liu *et al*, 2013). The two most common mutations in *TERT*, C228T and C250T, map −124 and −146 bp, respectively, upstream of the *TERT* ATG site (chr5, 1,295,228 C>T and 1,295,250 C>T, respectively), creating binding sites for Ets/TCF transcription factors that are associated with a two- to four-fold increased transcriptional activity (Brennan *et al*, 2013; Huang *et al*, 2013).

There is increasing evidence that *TERT* variation also influences cancer susceptibility. Notably, the SNP rs2736100 is associated with glioblastoma (GBM) risk, especially for *IDH1* wild-type GBM (Shete *et al*, 2009; Simon *et al*, 2010; Di Stefano *et al*, 2013). Recently, germline mutation of the TERT promoter at position −57 has been shown to cause familial melanoma (Horn *et al*, 2013).

Here, we have (1) determined the prevalence and prognostic impact of *TERT* promoter mutations, in 807 patients with glioma (WHO grades II, III and IV). (2) examined the relationship between *TERT* promoter mutation and tumour subtype and (3) assessed the contribution of germline mutations in these patients and in familial glioma and patients with glioma and melanoma.

PATIENTS AND METHODS

Patients and tissue samples. Collection of patient samples and clinico-pathological information was undertaken with informed consent and ethical board approval in accordance with the tenets of the Declaration of Helsinki. Patients studied fulfilled the following criteria: histologic diagnosis of primary glial tumour according to the WHO classification; complete clinical data and follow-up information available within in the neuro-oncology database (Onconeurothèque Paris). Blood DNAs from 80 patients with familial glioma requited through the Onconeurothèque service were also studied. For controls we made use of data previously generated on 1090 French individuals, which have been described previously (Shete *et al*, 2009).

Molecular analysis. DNA was extracted from fresh-frozen tumours or formalin-fixed paraffin-embedded (FFPE) tumours using the QIAmp DNA minikit (Qiagen, Courtaboeuf, France) and the iPrep ChargeSwitch Forensic kit (Life Technologies, Saint Aubin, France), respectively, DNA was extracted from EDTA-venous blood samples using a standard saline method. DNAs were quantified using Nanodrop (Thermo Fisher Scientific, Villebon sur Yvette, France).

Genomic profiling was performed by CGH-array analysis or SNP array, as previously described (Idbaih *et al*, 2008; Gonzalez-Aguilar *et al*, 2012). Mutational status of *IDH1*, *IDH2* and *TP53* was determined by Sanger sequencing, as described (Sanson *et al*, 2009). *MGMT* promoter methylation status was determined by bisulphite modification and subsequent two-stage nested methylation-specific PCR (Everhard *et al*, 2006).

Mutation analysis of exons 1–20 of *CIC* was undertaken using 454 Sequencing Technology (Roche Applied Science, Meylan, France). Details of PCR primers are shown in Supplementary Table 1. All variations were then validated by Sanger sequencing using the same primers.

Genotyping of rs2736100 has been previously described (Shete *et al*, 2009).

The *TERT* promoter was amplified using GGCCGATTC GACCTCTCT (GTCCTGCCCCTTCACCTT for FFPE samples) and AGCACCTCGCGGTAGTGG primers and Sanger sequencing performed using an ABI Prism 3730 DNA Analyzer (Applied Biosystems, Villebon sur Yvette, France).

To determine *TERT* mRNA expression, tumours were lysed using Lysing Matrix D tube and FastPrep instrument (MP Biomedicals, Illkirch, France) and RNA extracted using the iPrep Trizol Plus RNA Kit (Life Technologies). In all, 300 ng of RNA was retrotranscribed with the Maxima First-Strand cDNA Synthesis Kit (Thermo Scientific, Villebon sur Yvette, France). The cDNA obtained was used as a template for the determination of *TERT* mRNA expression by qPCR using a QuantiFast assay (Qiagen). The ΔΔCp method was applied to normalise to the expression of *TERT* mRNA, using both the expression of β actin and a non-tumour brain tissue sample.

Statistical analysis. The χ^2 test was used to compare the distribution of categorical variables and unpaired *t*-test or Mann–Whitney test associations with continuous variables.

Overall survival (OS) was defined as the time between the diagnosis and death or last follow-up. Patients who were still alive at last follow-up were considered as a censored event in the analysis. Progression-free survival (PFS) was defined as the time between the diagnosis and recurrence or last follow-up. Patients who were recurrence free at last follow-up were considered as a censored event in analysis. To identify clinical and/or genomic factors associated with OS or PFS, survival curves were calculated by the Kaplan–Meier method and differences between curves assessed using the log-rank test. Variables with a significant *P*-value were then used to develop a multivariate Cox model. In all analyses a *P*-value of $\leqslant 0.05$ (two-sided) was considered to be statistically significant.

RESULTS

Somatic and constitutional *TERT*p-mut status. Tumours from 807 patients (451 male; median age at diagnosis 51.0 years, range, 17.3–89.1; 206 grade II, 206 grade III and 395 grade IV) were screened for *TERT*p-mut. Complete patient characteristics are shown in Supplementary Table 2.

Tumours from 491 of the 807 patients (60.8%) were *TERT*p-mut–355 C228T (72.3%) and 136 C250T (27.7%). One GBM and two grade II oligodendrogliomas carried both C250T and C228T. These three cases were considered as *TERT* C228T mutant in all subsequent analyses. To confirm the mutations were somatic, we screened germline DNA of 91 of the cases. No mutation was detectable in germline DNA. We also investigated for the presence of *TERT*p-mut, in 80 familial glioma patients and 64 glioma patients with a second cancer −14 with melanoma (Supplementary Table 2). In none of the cases was a −149, −124 or −57 mutation identified.

rs2853669 genotypes were available for 385 of the tumours. The distribution of genotypes showed no significant departure from HWE (39 CC, 161 CT, 185 TT $P=0.650$). There was no difference in the distribution of genotypes between the *TERTp* mut and the *TERTp* wt cases (TT 45.8 *vs* 53.5%, CT 44.3 *vs* 36.0% and CC: 10.0 *vs* 10.5%, respectively).

We then investigated a purported association between somatic *TERT*p-mut and rs2736100 genotype in 518 glioma patients, finding no association in the whole group (allele A frequency 371 out of 616 = 60% *vs* 249 out of 420 = 59%, $P=0.9$) or when stratifying by *IDH* status and tumour class (Supplementary Table 3).

Case–control comparison of showed a stronger association with rs2736100 with *IDH*-wt gliomas but not with *TERT*p-mut gliomas (Supplementary Table 4). Collectively, these data imply there is no association between *TERT*p-mut and rs2736100 genotype. In

addition, we did not find any significant association between *TERT* promoter mutation and the other gliomas susceptibility SNPs rs11979158, rs2252586, rs4295627, rs4977756, rs498872 and rs6010620 (data not shown).

***TERT*p-mut is associated with GBM and *EGFR* amplification, and with oligodendroglioma, *1p19q* codeletion and *CIC* mutation.** *TERT*p-mut was associated with an older age at diagnosis in all gliomas (median age 56.1 years for *TERT* mutated patients *vs* 40.0 years; *t*-test $P<0.0001$) and when stratified by grade (median age at diagnosis 40.4 years *vs* 36.1 for grade II, $P=0.008$; 53.3 *vs* 37.8 for grade III, $P<0.0001$ and 59.6 *vs* 53.6 years for grade IV, $P<0.0001$).

*TERT*p-mut was more frequent in GBM than in grade II or III tumours (299 out of 395 = 75.8% *vs* 189 out of 412 = 45.9%; χ^2 test $P<10^{-17}$), more frequent in oligodendrogliomas than in astrocytomas/oligoastrocytomas for grade III (52 out of 81 = 64.2% *vs* 46 out of 125 = 30.7%: χ^2 test $P=0.0001$) and for grade II (70 out of 119 = 58.8% *vs* 21 out of 87 = 24.1%; χ^2 test $P<10^{-6}$). Additionally, there was no difference in the ratio of C228T/C250T mutations among the different grades (Table 1).

Table 1. Distribution of *TERT* promoter mutations in gliomas according to WHO histological classification

		C250T mutations (%)	C228T mutations (%)	All TERT mutations (%)
Grade II		**26/206 (12.5)**	**65/206 (31.4)**	**91/206 (44.0)**
	AII	0/13 (0.0)	1/13 (7.7)	1/13 (7.7)
	OAII	7/74 (9.5)	13/74 (17.6)	20/74 (27.0)
	OII	19/119 (16.0)	51/119 (42.9)	70/119 (58.8)
Grade III		**30/206 (14.6)**	**68/206 (33.1)**	**98/206 (47.8)**
	AII	0/30 (0.0)	12/30 (40.0)	12/30 (40.0)
	OAIII	15/95 (15.8)	19/95 (20.0)	34/95 (35.8)
	OIII	15/81 (18.5)	37/81 (45.7)	52/81 (64.2)
Grade IV	GBM	**77/395 (19.5)**	**222/395 (56.2)**	**299/395 (75.7)**

Abbreviations: AII = diffuse astrocytoma; AIII = anaplastic astrocytoma; GBM = glioblastoma; OII = oligodendroglioma; OIII = anaplastic oligodendroglioma; OAII = oligoastrocytoma; OAIII = anaplastic oligoastrocytoma; TERT = telomerase reverse transcriptase; WHO = world health organisation.

*TERT*p-mut was identifiable in 87.9% (94 out of 107 of gliomas with 1p19q codeletion (90 oligodendrogliomas, 17 oligoastrocytomas; 26 (24.3%) on C250T and 68 (63.6%) on C228T) as compared with 58.8% of non-codeleted gliomas (341 out of 580, χ^2 test $P<0.0001$). *EGFR* amplification was present in 183 tumours (142 GBM) and was mutually exclusive with 1p19q codeletion: 163 (89.1%) having *TERT*p-mut (121, C228T) as compared with 51.8% (323 out of 624) of *EGFR* non-amplified tumours (χ^2 test $P<0.0001$). The association of *TERT* promoter mutations with other molecular alterations commonly seen in glioma is detailed in Supplementary Table 5 and Supplementary Figure 1. We investigated whether there was a relationship between *CIC* inactivating mutations and *TERT*p-mut in grades II and III. *CIC* mutation was associated with *TERT*p-mut in 85% of the cases (28 out of 33), compared with 61% (25 out of 41) in *CIC*-wt tumours (χ^2 test $P<0.04$).

***TERT*p-mut is associated with increased *TERT* mRNA expression.** We investigated the transcriptional consequences of *TERT*p-mut in 153 tumours for which mRNA was available. We found a three-fold increase in mRNA expression between *TERT*p-mut and non-mutated groups (mean ± s.e.m. 1.03 ± 0.37 *vs* 3.44 ± 0.88 AU; Mann–Whitney test $P<0.0001$. Figure 1A).

Since the presence of the rs2853669 -C allele disrupts an Ets2 binding site (Rachakonda *et al*, 2013), we investigated the effect of rs2853669 genotype on *TERT* mRNA expression. Tumours harbouring the variant allele (CC + CT) showed a two-fold reduction in *TERT* expression, as compared with TT homozygotes (respective means ± s.e.m. 2.97 ± 1.01 and 6.57 ± 2.04 AU; Mann–Whitney test $P=0.005$). This relationship was also seen in the *TERTp* mutant cohort, however we did not evidence any significant association in *TERTp* wt tumours (Figure 1B and C).

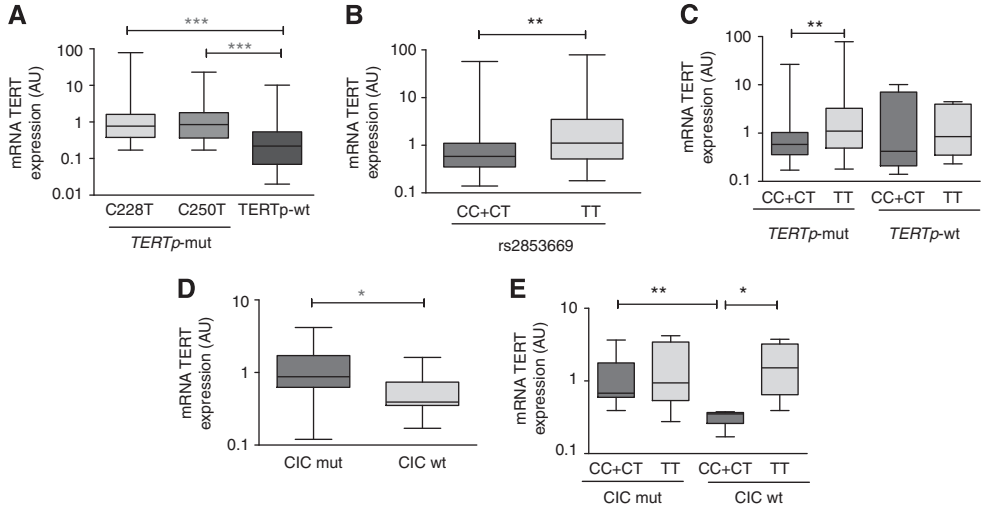


Figure 1. Expression of *TERT* mRNA in gliomas. The Mann–Whitney test was used to compare the expression of the different groups. (**A**) Expression of *TERT* mRNA according to *TERT* promoter mutation status. *TERTp* mutation (C228T $n=88$ or C250T $n=30$) is associated with higher *TERT* mRNA expression compared with *TERTp*-wt group ($n=35$) ($P\leqslant 0.0001$ in both cases). (**B**) Expression of *TERT* mRNA according to rs2853669 status. Variant allele carriers ($n=70$) present a lower *TERT* expression than TT homozygotes ($n=66$) ($P=0.0053$). (**C**) Expression of *TERT* mRNA according to *TERTp* and rs2853669 status. *TERT* mRNA expression is lower for the variant allele carriers ($n=62$) compared with TT ($n=56$) in *TERTp*-mut subgroup ($P=0.0079$). For *TERTp*-wt group, only seven CC + CT samples and eight TT samples were available. (**D**) Expression of *TERT* mRNA according to *CIC* mutation status. *TERT* mRNA expression is increased in *CIC* mutant tumours ($n=18$) compared with *CIC* wild type ($n=11$; $P=0.043$). (**E**) Impact of rs2853669 and *CIC* mutational status *TERT* expression. In the *CIC*-wt cohort, *TERT* expression was lower in CC + CT subgroup, as compared with TT subgroup ($P=0.0159$). For the variant allele carriers (CC + CT), expression of TERT was increased in the *CIC* mutant group ($n=8$), as compared with *CIC* wt ($n=5$) ($P=0.0016$). *$P<0.05$; **$P<0.01$; ***$P<0.0001$.

Since Ets/TCF transcription factors, including ETV1-4 transcription factors are controlled by *CIC* (Dissanayake *et al*, 2011), we also investigated a specific relationship with *CIC* mutation. We found *TERT* mRNA expression was two-fold higher in *CIC* mutant tumours, compared with *CIC* wild-type gliomas (Mann–Whitney test $P=0.043$) for the whole group (Figure 1D), and for the carriers of the variant allele (Figure 1E). The variant allele C was also associated with a decrease in *TERT* mRNA expression in the *CIC* wt group.

Prognostic impact of *TERT*p-mut is dependent on tumour grade. For patients with grade III and IV gliomas *TERT*p-mut was associated with a significantly shorter PFS and OS (Figure 2; Supplementary Table 6). For example in grade III gliomas, median OS of *TERT* promoter normal patients was twice longer (62.6 *vs* 29.4 months) than OS of *TERT* promoter mutated (log-rank test $P=0.013$). This was in sharp contrast with low-grade gliomas, where OS was better for patients with *TERT*p-mut (>16 years *vs* 97.5 months, $P=0.013$). There was no difference in outcome between C228T and C250T *TERT*p-mut in any of the analyses.

In a multivariate Cox model analysis incorporating *IDH* mutation, age at diagnosis, 1p19q codeletion, *MGMT* promoter methylation, Karnofsky performance status, WHO grade and extension of surgery (Table 2) *TERT*p-mut was seen to be an independent negative prognostic factor for OS (Hazard ratio (HR) = 1.50; 95% CI: 1.07–2.09, $P=0.018$).

***TERT*p-mut is associated with specific prognostic and molecular subgroups.** Given *TERT*p-mut is associated with both 1p19q codeletion and *EGFR* amplification, which are mutually exclusive alterations with opposite prognostic effects and *TERT*p-mut had a different effect in low- and high-grade gliomas, prompted us to refine our survival analysis (Figure 3). Gliomas can be stratified into four distinct prognostic groups according to *IDH* and *TERT*p-mut status: (1) *TERT*p-mut and *IDH*-mut, highly associated with 1p19q codeletion (83.9%, 94 out of 11), OS> 17 years; (2) *TERT*p-wt and *IDH*-mut, associated with *TP53* mutation (67.7%, 67 out of 99, OS = 97.5 months); (3) *TERT*p-wt and *IDH*-wt, with no specific association (all negative), OS = 31.6 months; (4) *TERT*p-mut and *IDH*-wt, highly associated with *EGFR* amplification (44.1%, 161 out of 365, OS = 15.0 months) (Figure 4).

***TERT*p-mut confers a poor prognosis except if associated with 1p19q codeletion.** We considered the prognostic impact of the above classification in grades II, III and IV (Figure 5; Supplementary Table 7). In grades II and III, *TERT*p-mut was predictive of a longer survival in the *IDH* mutated group, but shorter survival in the *IDH* wt group. This finding can be explained by the fact that 94 out of 114 of *TERT*p-mut-*IDH*-mut are 1p19q codeleted. Indeed in the GBM group that do not include any 1p19q codeletion, *TERT*p-mut is associated with a particularly poor prognosis in *IDH*-wt tumours (OS = 13.8 *vs*16.5 months, $P=0.006$) but surprisingly also in *IDH*-mut (OS = 13.8 *vs* 29.1

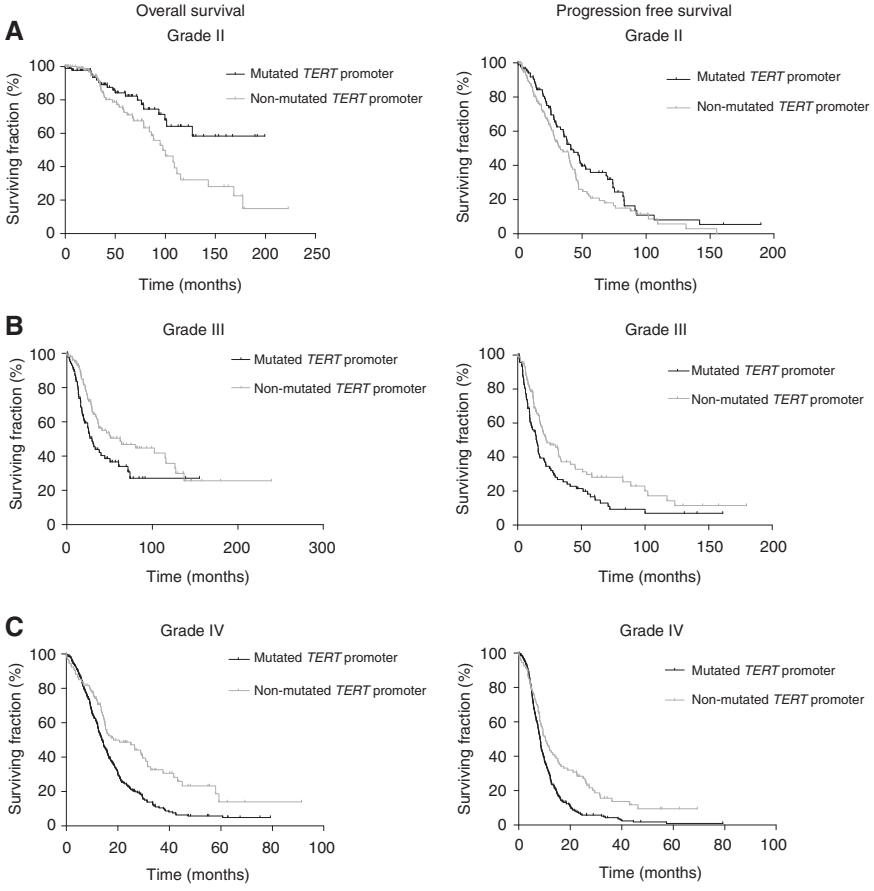


Figure 2. Prognostic impact of *TERT* promoter mutation status on overall survival and PFS, according to grade. Survivals were compared using the log-rank test (Mantel Cox). In grade II gliomas ($n=206$), *TERT*p mutation is associated with better survival (median >16 years *vs* 97.5 months; $P=0.013$). There is also a trend for better PFS (median 41.3 *vs* 33.3 months; $P=0.068$) (**A**) whereas in grade III (**B**; $n=206$) and grade IV gliomas (**C**; $n=395$), *TERT*p mutation is associated with poorer survival (median 29.4 *vs* 62.6 months $P=0.013$ and 13.8 *vs* 18.4 months $P<0.0001$) and PFS (median 15.1 *vs* 22.4 months $P=0.006$ and 8.3 *vs* 10.4 months $P<0.0001$).

Table 2. Cox model for overall survival and progression-free survival

	Overall survival			Progression-free survival		
Parameters	**HR**	**95% CI for HR**	***P***	**HR**	**95% CI for HR**	***P***
Age at diagnosis < 60 years	0.631	0.470–0.848	*0.002*	0.798	0.610–1.044	*0.0991*
IDH mutation	0.586	0.369–0.930	*0.023*	0.707	0.473–1.056	*0.090*
1p19q codeletion	0.182	0.076–0.436	*< 0.0001*	0.433	0.257–0.730	*0.002*
Surgery *vs* biopsy	0.586	0.435–0.791	*< 0.0001*	0.896	0.682–1.178	*0.432*
MGMT promoter methylation	0.652	0.497–0.855	*0.002*	0.691	0.539–0.887	*0.004*
KPS > 70	0.553	0.404–0.757	*< 0.0001*	0.567	0.421–0.763	*< 0.0001*
Grade	1.850	1.400–2.444	*< 0.0001*	1.215	0.989–1.494	*0.064*
TERT promoter mutation	1.497	1.071–2.092	*0.018*	1.766	1.299–2.401	*< 0.0001*

Abbreviations: CI = confidence interval; HR = hazard ratio: IDH = isocitrate dehydrogenase; KPS = Karnofsky Performance Status; TERT = telomerase reverse transcriptase. The analysis was conducted on 362 tumours with all parameters available.

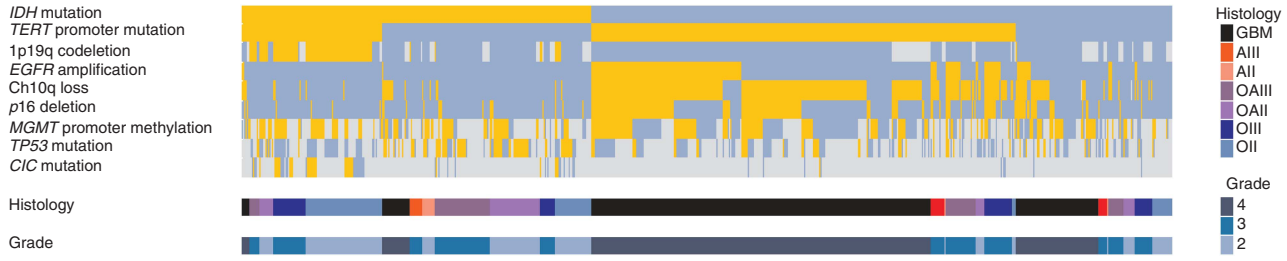


Figure 3. Association of *TERT* promoter mutations with the major genetic alterations in gliomas ($n = 806$). Each tumour is represented by a column. A yellow box indicates the presence of the genetic alteration, the absence in blue, and the cases not assessed are indicated in grey. The stratification has been done using four groups: *IDH* mut-*TERTp* mut, *IDH* mut-*TERTp* wt, *IDH* wt-*TERTp* wt and *IDH* wt *TERTp* mut. *TERT*p mutation is associated with two mutually exclusive alterations: 1p19q codeletion and *EGFR* amplification.

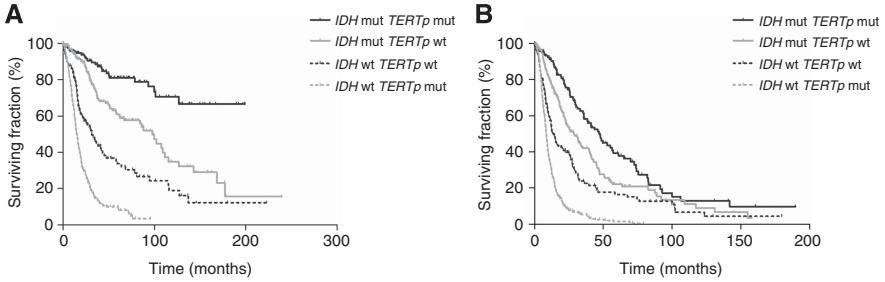


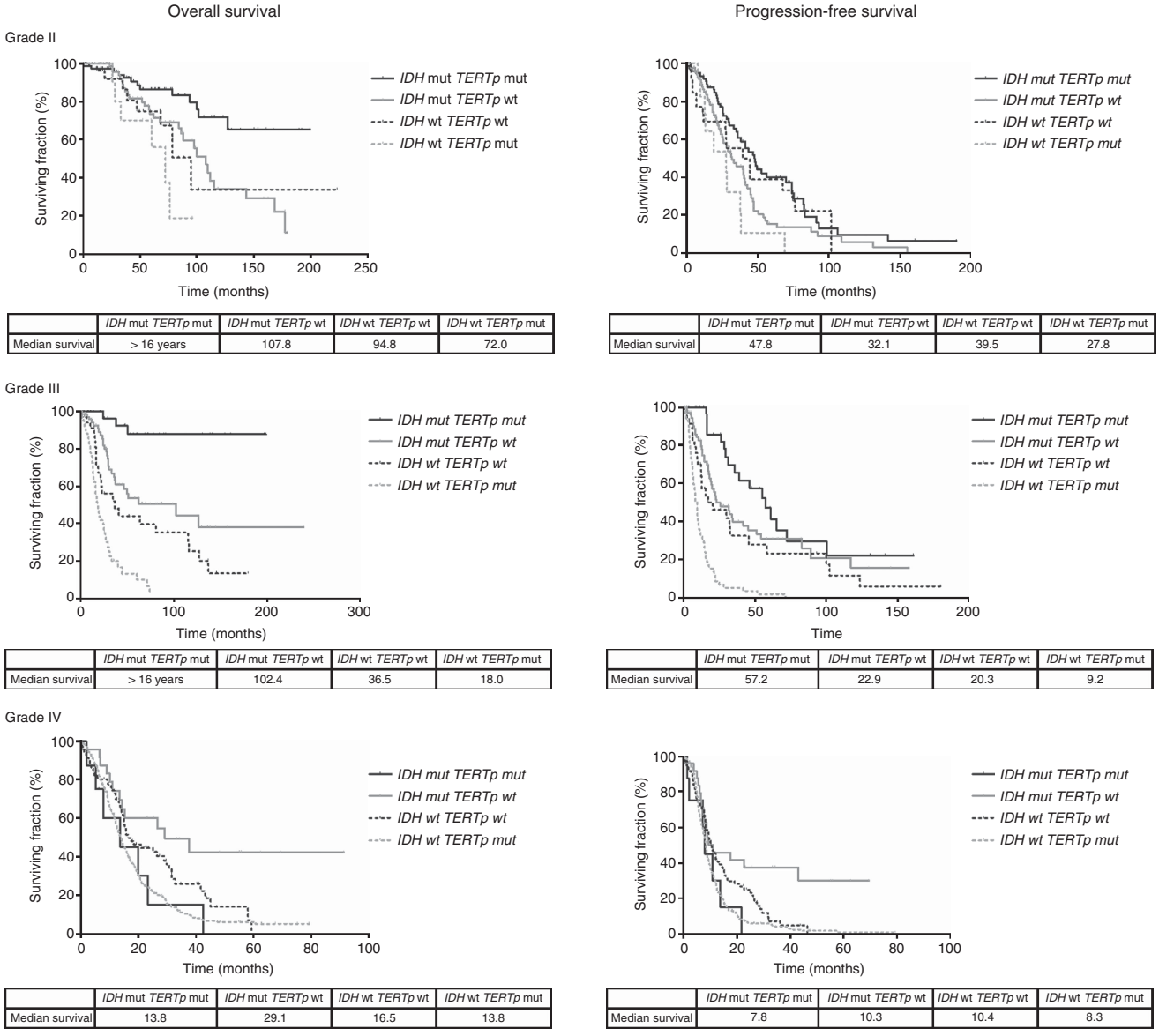
Figure 4. Prognostic stratification of gliomas according to *IDH* and *TERT* promoter mutation status ($n = 804$). (**A**) Overall survival. (**B**) Progression-free survival. We identified four prognostic subgroups, (1) *TERT*p-mut and *IDH*-mut (OS > 17 years, PFS 46.9 months), (2) *TERT*p-wt and *IDH*-mut (OS = 97.5 months, PFS 28.6 months), (3) *TERT*p-wt and *IDH*-wt, (OS = 31.6 months, PFS14.1 months) and (4) *TERT*p-mut and *IDH*-wt (OS = 15.0 months, PFS 8.5 months).

months, $P = 0.022$) (Figure 6). In contrast *TERT*p-mut was associated with a poorer outcome in *IDH*-wt gliomas irrespective of grade (OS: 76.2 *vs* 94.8 months in grade II $P =$ ns; 18.0 *vs* 36.5 months in grade III $P = 0.007$; 13.7 *vs* 17.5 months in grade IV $P = 0.006$).

DISCUSSION

Given that 60% of tumours being *TERT*p-mut, *TERT* is the most frequently mutated gene in gliomas thus far identified (Arita *et al*, 2013; Killela *et al*, 2013; Liu *et al*, 2013). We found *TERT*p-mut glioma patients were older, consistent with previous reports of other malignancies (Griewank *et al*, 2013; Killela *et al*, 2013). Unlike melanomas, in which germline *TERTp* mutations have been reported to cause familial melanoma (Horn *et al*, 2013), we found no evidence that *TERT*p-mut contributes substantially to predisposition to gliomas or the glioma/melanoma syndrome.

Our data showed that *TERT*p-mut is generally associated with poorer outcome in high-grade gliomas, consistently with previous data, on glioma (Killela *et al*, 2013, 2014), and other tumours (Rachakonda *et al*, 2013). In contrast, however we observed a trend for better outcome in low-grade gliomas. Stratifying tumours by both *IDH1/2* and *TERT*p-mut status provides insight into this apparent paradox, identified four molecular subtypes of gliomas with distinct prognosis, In *IDH* mutated tumours, *TERT*p-mut is largely confined to 1p19q codeleted oligodendroglial tumours that have the best outcome (Kaloshi *et al*, 2007; van den Bent *et al*, 2013). Mutation of *CIC*, recently identified (Bettegowda *et al*, 2011;



Grade II

	IDH mut *TERTp* mut	*IDH* mut *TERTp* wt	*IDH* wt *TERTp* wt	*IDH* wt *TERTp* mut
Median survival	> 16 years	107.8	94.8	72.0

	IDH mut *TERTp* mut	*IDH* mut *TERTp* wt	*IDH* wt *TERTp* wt	*IDH* wt *TERTp* mut
Median survival	47.8	32.1	39.5	27.8

Grade III

	IDH mut *TERTp* mut	*IDH* mut *TERTp* wt	*IDH* wt *TERTp* wt	*IDH* wt *TERTp* mut
Median survival	> 16 years	102.4	36.5	18.0

	IDH mut *TERTp* mut	*IDH* mut *TERTp* wt	*IDH* wt *TERTp* wt	*IDH* wt *TERTp* mut
Median survival	57.2	22.9	20.3	9.2

Grade IV

	IDH mut *TERTp* mut	*IDH* mut *TERTp* wt	*IDH* wt *TERTp* wt	*IDH* wt *TERTp* mut
Median survival	13.8	29.1	16.5	13.8

	IDH mut *TERTp* mut	*IDH* mut *TERTp* wt	*IDH* wt *TERTp* wt	*IDH* wt *TERTp* mut
Median survival	7.8	10.3	10.4	8.3

Figure 5. Prognostic stratification of gliomas according to *IDH* and *TERT* promoter mutation status (four subgroups) in grade II ($n=205$), grade III ($n=206$) and grade IV ($n=394$). Median survivals are indicated in months if not otherwise stated. *TERT*p mutation is associated with a poorer outcome in *IDH*-wt gliomas whatever the grade. *TERT*p mutation is associated with better outcome in *IDH* mut grades II and III (OS: >16 years *vs* 107.8 months in grade II $P=0.004$; >16 years *vs* 102.4 in grade III $P=0.0005$). In contrast, the survival of grade IV with *TERT*p mutation and *IDH*-mut is extremely poor compared with *TERT*p-wt (13.8 *vs* 29.1 months; $P=0.022$).

Yip *et al*, 2012) is also primarily a feature of this group (Figure 3). In contrast, if *TERT*p-mut is associated with *IDH1/2* wild-type tumours, then it is mainly seen in the context of GBM (276 out of 340) with almost half of them (124 out of 276) having an *EGFR* amplification which is associated with poor outcome. In our study, this subgroup also included 54 grade III gliomas that had a particularly poor OS of 20.1 months. Taken together, our data show that the prognostic impact of *TERT*p-mut is highly contextual and depends on the histologic and genomic background of the tumour.

From a mechanistic point of view, *TERTp* mutation leads to the creation of a putative binding site for Ets/TCF transcription factors (Huang *et al*, 2013), leading to a two- to four-fold higher expression of telomerase (Arita *et al*, 2013; Huang *et al*, 2013; Nault *et al*, 2013; Rachakonda *et al*, 2013). The activity of telomerase reverse transcriptase is closely correlated with *TERT* mRNA level. The expression of *TERT* is regulated by many transcription factors binding motives located in its promoter and by epigenetic and chromatin remodelling mechanisms (Kyo *et al*, 2008; Zhu *et al*, 2010). Among the complex regulation of telomerase expression, rs2853669 has been shown to modulate both *TERT* expression and impact on prognosis in bladder cancer (Rachakonda *et al*, 2013). Indeed, the presence of the variant allele disrupts a pre-existing Ets2 binding site and results in the decrease of *TERT* expression in our series. However, unlike bladder cancer, rs2853669 variant does not modify the prognostic impact of *TERTp* mutation in our glioma series (data not shown). Our data also suggest a link between *CIC* mutation and *TERT* expression in the context of glial tumours. Indeed, the presence of the variant allele of rs2853669 did not result in a reduction of *TERT* expression in the *CIC* mutant subgroup.

Among the 40% gliomas lacking *TERTp* mutation, ~50% harbour an *IDH* mutation (mostly astrocytomas (43 out of 180) and oligoastrocytomas (91 out of 180), which are frequently *TP53* mutated. In this group, mutations in the *ATRX* gene (alpha thalassaemia/mental retardation syndrome X-linked), or in its

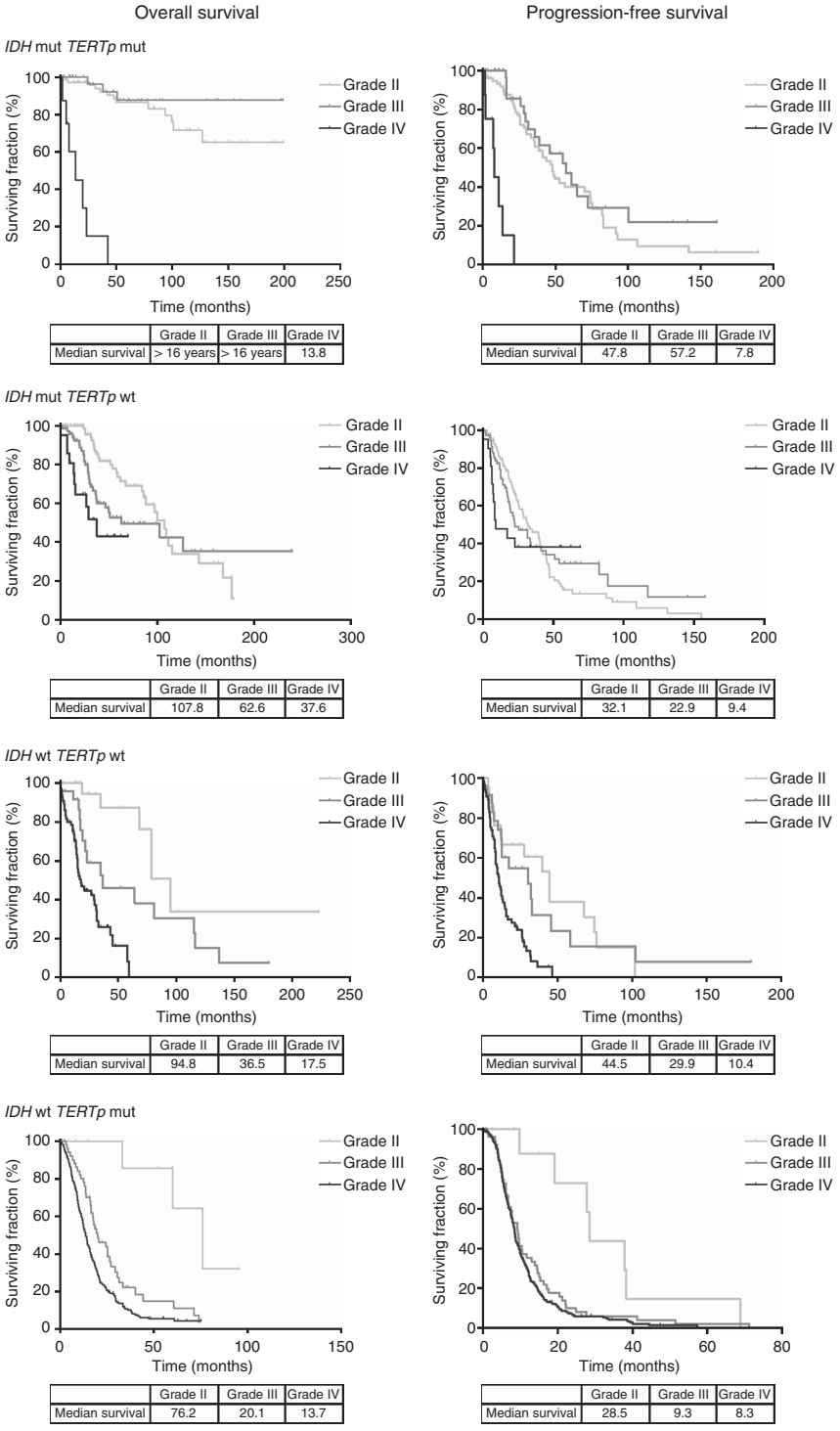


Figure 6. Prognostic stratification of gliomas according to grade in the four molecular subgroups (*IDH* mut *TERTp* mut $n=122$; *IDH* mut *TERTp* wt $n=180$; *IDH* wt *TERTp* wt $n=114$, *IDH* wt *TERTp* mut $n=340$). There is a dramatic difference of survival between grade IV (OS = 13.8 months) and grades II + III (OS > 16 years) in the *IDH*-mut-*TERT*-mut group ($P<0.0001$). In contrast, the group 2 (*IDH*-mut-*TERT*-wt) is much more homogeneous through grades II–IV.

partner death domain-associated protein (*DAXX*), which are involved in alternative lengthening telomere (ALT) phenotype, have been frequently documented (Jiao *et al*, 2012; Kannan *et al*, 2012; Liu *et al*, 2012; Killela *et al*, 2013) and are mutually exclusive with telomerase reactivation. The *IDH*-wt and *TERT*p-wt group includes mostly GBM tumours (58%, 66 out of 114). The 'triple negative' low-grade gliomas, characterised by a poorer outcome also conform to these categories (Metellus *et al*, 2010). Telomere maintenance mechanism has not been investigated yet in this subgroup.

A more detailed analysis shows the four group classification, recently reported (Killela *et al*, 2014) is an oversimplification (see Supplementary Figure 2 and Figure 5); for example, the *TERT*p-mut-*IDH*-mut is indicative of better outcome for grades II and III with an OS > 17 years, but is associated with a poorer outcome in GBM (OS = 13.8 months), whereas in GBM the best group

prognostic is those patients with *TERT*p-wt-*IDH*-mut group (OS = 29.1 *vs* 13.8 months for the *TERTp*-mut-*IDH* mut group). This discrepancy is unlikely to be solely explained by the relationship between *TERT*p mutation and 1p19q codeletion, present in 89% (94 out of 106) of our *TERT*p-mut-*IDH*-mut grades II and III, but none of our grade IV tumours. In fact, the survival of patients with *TERT*p-mut-*IDH*-mut grades II and III, without 1p19q codeletion was not significantly different from those with 1p19q codeleted tumours (median OS = 10 years) (Supplementary Figure 3).

In conclusion, our data confirm the high frequency of *TERT*p-mut in glioma and show that these mutations clusterise into specific entities, with distinct clinical significances. *TERT*p mutations are mostly associated with poor outcome, except for 1p19q codeleted grade II and grade III, and for *EGFR* amplified grade III and grade IV (Supplementary Figure 2A and B, respectively). A telomere maintenance mechanism (either *TERTp* mutation or *ATRX/DAXX* mutations) is involved in >80% of gliomas and appears therefore as a unique feature in these tumors, offering the prospect of new therapeutic approaches.

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