Hum Genet (2000) 107 : 350–356
DOI 10.1007/s004390000390

ORIGINAL INVESTIGATION

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Molecular analysis of the *TSC1* and *TSC2* tumour suppressor genes in sporadic glial and glioneuronal tumours

Received: 12 June 2000 / Accepted: 21 August 2000 / Published online: 11 October 2000

Abstract Reduced expression of the *TSC2* tumour suppressor gene product, tuberin, has been reported in sporadic astrocytomas, suggesting that the *TSC* genes may play a role in formation of sporadic glial or glioneuronal tumours. We studied paired constitutional and tumour DNA samples from 100 patients with sporadic glial and glioneuronal tumours for loss of heterozygosity (LOH) at the *TSC1* and *TSC2* loci using a combination of seven previously reported and seven novel polymorphic markers. LOH was seen in 1/16 astrocytomas, 3/15 ependymomas, 5/16 gangliogliomas, 2/14 glioblastoma multiforme, 0/7 oligodendrogliomas, 0/7 tumours of mixed oligodendrocytic/astrocytic histology, 2/11 pilocytic astrocytomas and 0/1 subependymal giant cell astrocytomas informative at both loci. However, SSCP screening of all coding exons of the *TSC1* or *TSC2* genes in the tumours displaying LOH, and of both genes in 21 gangliogliomas, revealed no intragenic mutations. The lack of demonstrable inactivation of both alleles of either *TSC* gene in any of the tumours investigated suggests that they do not play a frequent role in the aetiology of sporadic glial or glioneuronal tumours.

Introduction

Malignant brain tumours occur in more than 40,000 individuals each year in the United States. Primary brain tumours represent approximately half of these and approximately 2.3% of all human cancers (American Cancer Society 1998); the remainder are metastases from tumours originating outside the central nervous system (Hill et al. 1999). Tumours of the supporting glial cells are known as gliomas, and are WHO graded I, II, III and IV (Kleihues and Cavenee 2000). Astrocytomas are the most common type of glioma and astrocytomas of grade IV are also termed glioblastoma multiforme. Ependymomas are believed to develop from precursor cells which line the ventricles of the brain and the central canal of the spinal cord. Oligodendrogliomas develop from oligodendroglial precursor cells, while oligoastrocytomas share the morphological features of both astrocytoma and oligodendroglioma. Gangliogliomas are composed of neoplastic glial and neuronal elements.

Research into the stepwise genetic changes required for transformation of a normal glial cell to a malignant one has already identified some of the pathways involved. Low-resolution studies performed using techniques such as comparative genomic hybridisation have revealed common regions of deletion or amplification. Regions on 1p and 19q were frequently lost in a variety of primary gliomas: 68% at both loci in oligodendrogliomas, 19% and 37% in astrocytomas, and 22% and 33% in tumours with mixed oligodendroglioma/astrocytic histology (Smith et al. 1999). Other regions commonly deleted are found on 17p13.3 and 9p21, the sites of the *p53* and *p16* (CDKN2) genes, which are also commonly mutated in gliomas (Steilen-Gimbel et al. 1999). *p53* is mutated or lost early in astrocytomas, whereas submicroscopic deletions of *p16* arise later in glioblastomas (von Deimling et al. 1992; Louis et al. 1993; Ueki et al. 1994). Hill et al. (1999) proposed a possible model for progressive genetic alterations. Early glial cell aberrations involve deletion of *p53* for progression to a WHO grade 2 astrocytoma; losses of chromosomes 10, 19q and 13q (the *RB1* locus) are then required for progression to stage 3, and finally losses of *Mmac*, *PTEN*, *TEP1* on chromosome 10 and *p16*, *p15*, *p14* on 9p as well as amplifications of 9q, 1q, 7q (the location of *EGFR*) and 12q (the *CDK4*, *SAS* and *MDM2* loci) are required for the final transformation to a

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glioblastoma multiforme. Many of these regions contain tumour suppressor genes that are also constitutionally mutated in rare Mendelian cancer or hamartoma predisposition syndromes. Examples include *p53*, which is mutated in the germline in Li-Fraumeni syndrome and somatically mutated in many cancers (Kleihues et al. 1997), *VHL*, which is mutated in the germline in von Hippel-Lindau disease and somatically mutated in sporadic cerebellar haemangioblastoma (Olschwang et al. 1998), and *PTEN*, which is mutated in the germline in Cowden syndrome and somatically mutated in sporadic glioma (Rasheed et al. 1997; Duerr et al. 1998).

The autosomal dominant disorder tuberous sclerosis (TSC) predisposes to hamartomatous growths in many organs (Gomez et al. 1999). Low-grade glial tumours classified as subependymal giant cell astrocytomas occur in approximately 5% of patients with TSC (Sheperd et al. 1991). *TSC1* (van Slegtenhorst et al. 1997) and *TSC2* (The European Chromosome 16 Tuberous Sclerosis Consortium 1993), the genes responsible for TSC, appear to function as tumour suppressor genes since somatic second hit mutations at the *TSC1* and *TSC2* loci have been identified as loss of heterozygosity (LOH) at 9q34 and 16p13.3 in hamartomas and cancers from patients with TSC (Green et al. 1994; Henske et al. 1995; Bjornsson et al. 1996; Carbonara et al. 1996; Henske et al. 1996; Sepp et al. 1996; van Slegtenhorst et al. 1997). The tumour suppressor properties of *TSC2* have been formally demonstrated by transgenic expression in the Eker rat, a naturally occurring animal model in which the homologous *Tsc2* gene is inactivated (Kobayashi et al. 1997). Sporadic astrocytomas have been reported to show reduced levels of expression of *TSC2* and tuberin, the *TSC2* gene product (Gutmann et al. 1997; Wienecke et al. 1997), and tuberin appears to play a role in neuronal differentiation (Soucek et al. 1998). However, studies seeking direct molecular genetic evidence for involvement of the *TSC1* and/or *TSC2* genes in the development of sporadic glial and glioneuronal tumours have not been reported.

We have therefore undertaken a systematic molecular genetic study of the *TSC1* and *TSC2* genes in 100 sporadic glial and glioneuronal tumours. We identified several novel simple sequence repeat polymorphisms at the *TSC1* and *TSC2* loci and used these and previously described markers to assay for LOH in paired tumour and constitutional DNA samples. In cases exhibiting LOH at the *TSC1* or *TSC2* locus, we used single-stranded conformation polymorphism (SSCP) analysis to screen all coding exons for evidence of intragenic somatic mutations in the retained allele. We also screened all coding exons of both *TSC1* and *TSC2* in each of 21 gangliogliomas that were studied.

Materials and methods

Tumour and constitutional DNA samples. One hundred paired sporadic primary brain tumour and constitutional DNA samples were studied. The primary brain tumours comprised 17 astrocytomas (9 WHO grade II and 8 grade III), 16 ependymomas (4 WHO grade I, 8 grade II and 4 grade III), 21 gangliogliomas (20 WHO grade I and 1 grade III), 16 glioblastoma multiforme, 7 oligodendrogliomas (4 grade II and 3 grade III), 9 tumours with mixed oligodendrogliocytic/astrocytic histology (3 grade II and 6 grade III), 13 pilocytic astrocytomas grade I and 1 subependymal giant cell astrocytoma WHO grade I (TSC status unknown). All tumours were classified and graded according to the latest WHO classification (Kleihues and Cavenee 2000) and represented a subset of the 466 tumour DNA samples that have been extensively studied for LOH at other loci, including *TP53*, and reported by von Deimling et al. (2000). All patients' samples were obtained with consent for molecular genetic analysis.

Identification of novel polymorphisms. Genomic sequence data containing and flanking the *TSC1* and *TSC2* genes was obtained from Genbank (www.ncbi.nlm.nih.gov/) and from Dr. N. Doggett (HGMP, Los Alamos National Laboratory). Stretches of simple repetitive DNA sequence were identified using the Xgrail software package via the HGMP-RC Website (www.hgmp.menu.mrc.uk). The Oligo V4.0 program was used to design primers to amplify simple sequence repeats with characteristics suggesting that they were likely to be polymorphic (Charmley et al. 1995). These were assessed for polymorphic variation by genotyping seven unrelated Caucasians.

Loss of heterozygosity analysis. Novel and previously reported polymorphisms at the *TSC1* and *TSC2* loci were used to assay for LOH. At the *TSC1* locus the markers tested were: exon 14 (1556 A/G); intron 21 (2847–4 $\{A\}_{17–21}$), exon 22 (3050 C/T) (Jones et al. 1997) and PM1, PM2, PM4 and PM5 (this study). The *TSC2* locus markers were Kg8 (Snarey et al. 1994); exon 40 (1734 T/C) (van Bakel et al. 1997); IVS8 29 bp VNTR (Migone 1998), EJ1 (Qian et al. 1996) and LP1, LP7 and LP10 (this study). PCR amplification of tumour and constitutional DNA samples was carried out in parallel in 96 well microtitre plates (Hybaid). Each 50 µl reaction contained 100 ng DNA, 25 pmol primer (supplied by Oswel DNA Services, Southampton, UK.), 0.2 m*M* dNTP (Roche, Sussex, U.K.), 5 µl reaction buffer (100 m*M* Tris pH 8.3, 500 m*M* KCl, 15 m*M* $MgCl_2$, 0.01% gelatin) and 1 U AmpliTaq Gold Polymerase (Perkin Elmer, UK). Cycling conditions were 94°C 10 min, followed by 37 cycles of 52–60°C 1 min, 72°C 1 min, 94°C 30 s, and a final step of 72°C 10 min. For autoradiography, reverse primers were end-labelled with γ^{33}P-dATP using T4 polynucleotide kinase (Life Technologies, Paisley, UK) according to the manufacturer's instructions and the products were electrophoresed on 6% polyacrylamide gels (National Diagnostics). The *TSC1* 1556 A/G polymorphism, the *TSC1* exon 22 3050 C/T polymorphism and the *TSC2* exon 40 1734 T/C polymorphism were assayed by digestion of 10 µl amplified product with the enzymes *Nla*IV, *Hae*III and *Eco*RV, respectively. Digested products were visualised on 2.5% agarose gels stained with ethidium bromide. LOH was determined by visual inspection of alleles in normal and tumour DNA samples by three independent observers and all results scored positive by all observers were repeated in at least one further assay.

PCR-SSCP analysis and direct sequencing. Primer sequences and annealing temperatures for amplification of all *TSC1* and *TSC2* coding exons are available at the Cardiff-Rotterdam Tuberous Sclerosis Mutation Database Website (www.uwcm.ac.uk/uwcm/mg/tsc_db/pcrpub.html). Reaction conditions have been previously described (Jones et al. 1999). SSCP was performed on 4 µl PCR product diluted 1:10 with gel loading buffer (95% formamide, 20 m*M* EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol). Samples were denatured at 94°C 2 min and immediately loaded (5-h intervals) on a 0.8 mm MDE gel (Flowgen, Staffs, UK). Electrophoresis was performed in 0.6% TBE at 20 W for 18 h at room temperature. Products were visualised by standard silver staining (Jones et al. 1997). PCR products of samples displaying variant band patterns were sequenced using either the Sequenase PCR Product Sequencing kit (Amersham Pharmacia Biotech, Amersham, UK) or the Thermosequenase cycle sequencing kit (Amersham Pharmacia Biotech, Amersham, UK).

Table 1 Polymorphic markers at the *TSC1* and *TSC2* loci used for LOH analysis

Locus	Marker[a]	Genbank accession no.	Position in sequence	Proximity to gene	Polymorphism	% HZ[b]	Primers	Assay	Temperature (°C)	Reference
TSC1	PM4	AC000394	3238–3310	50 kb telomeric	$(TAA)_{8–18}$	71	PM4F – 5' TTTTAATGGAAACAACTTGACC 3' PM4R – 5' TAAATGGTGCAGCCACTGT 3'	End labelled PCR	60	This study
TSC1	PM2	AC002318	38067–38183	5 kb telomeric	$(CA)_{18–20}$	42	PM2F – 5' TCTTCAGCCCAGGCTACTCA 3' PM2R – 5' GAAGGTTTTCAGCCCTGA 3'	End labelled PCR	60	This study
TSC1	PM1	AC002096	38258–38289	Intron 9	$(A)_{27–30}$	42	PM1F – 5' AGAACACAGGGGGTTAGTG 3' PM1R – 5' ATTATCAAATGGTCTAGAGGAC 3'	End labelled PCR	60	This study
TSC1	Exon 14	AC002096	33366	Exon 14	E445 (1556 A>G)	14	AJ22 – 5' TGTCCAGCCTTCTCTGTTCA 3' AJ21 – 5' GAGCGAGGGTCAGGTTTTAT 3'	Restriction digest	55	Jones et al. (1997)
TSC1	Intron 21	AC002096	24244–24263	Intron 21	2847–4 $(A)_{17–21}$	14	AJ10 – 5' AAAATGGATACAGCATGTTTA 3' AJ40 – 5' TTCTAGCTCTTTCCGATAGG 3'	End labelled PCR	55	Jones et al. (1997)
TSC1	Exon 22	AC002096	23958	Exon 22	A943 (3050 C>T)	10	AJ8 – 5' TCAAACTCCAGGCAAGGTAA 3' AJ7 – 5' CAGCTTAGTCCCAAGGTCAT 3'	Restriction digest	55	Jones et al. (1997)
TSC1	PM5	AC000392	11113–11143	50 kb	$(T)_{18–28}$ centromeric	57	PM5F – 5' CGGTTGGCTTTTCATAACT 3' PM5R – 5' TGACAGATTGATACCCTGTCTC 3'	End labelled PCR	57	This study
TSC2	LP1	AC005363	21654–21698	95 kb telomeric	$(AAT)_{10–18}$	71	LP1F – 5' AAATCTTCCCTGGCAGTAAG 3' LP1R – 5' TCTAACCCTCAGCAGAGTTCT 3'	End labelled PCR	55	This study
TSC2	IVS8	AC005600	34874–35134	Intron 8	29 bp VNTR	37	IT659F – 5' TGGGATGTAGGCTTCTCCTATG 3' IT1484R – 5' GGTTCCAAGGATGGAGGGAAG 3'	End labelled PCR	55	Migone (1998)
TSC2	LP10	AC005600	38455	Intron 10	C>T	50	AKH10 – 5' GAGGATCTGGGGGGTGTCTCA 3' 1.1#2 – 5' CCCGTGGAACTCGTTCTGGT 3'	SSCP	55	This study
TSC2	Exon 40	AC005600	64940	Exon 40	D1711 (5151 T>C)	10	40F – 5' AGGCGTGACGTGGTGGGACAG 3' 40R – 5' CAGAGGCGGCTTGGGCAGTAA 3'	Restriction digest	57	van Bakel et al. (1997)
TSC2	Kg8	AC005600	65562–65580	260 bp centromeric	$(CA)_{5–14}$	25	BP1A – 5' CACAGCCAGCTCCGAGGG 3' BP1B – 5' TCCTCCTGGGGGCTGGCTC 3'	End labelled PCR	57	Snarey et al. (1994)
TSC2	EJ1	L33243/ L39891	12617/51325	1.5 kb centromeric	C>T/insG	25/10	FQF28 – 5' CACGCCTTGCGTGGAGAG 3' FQR35 – 5' ATGGGCCACGGGAAGATCC 3'	End labelled PCR	62	Qian et al. (1996)
TSC2	LP7	AC005212	40296–40338	150 kb centromeric	$(CA)_{32–35}$	42	LP7F – 5' GCTGGGATTACAGGCATGAA 3' LP7R – 5' TGCAGCCTCAGTTGTGTTTC 3'	End labelled PCR	55	This study

[a] Markers descend telomeric to centromeric
[b] HZ, Heterozygosity (estimated by typing seven unrelated Caucasians)

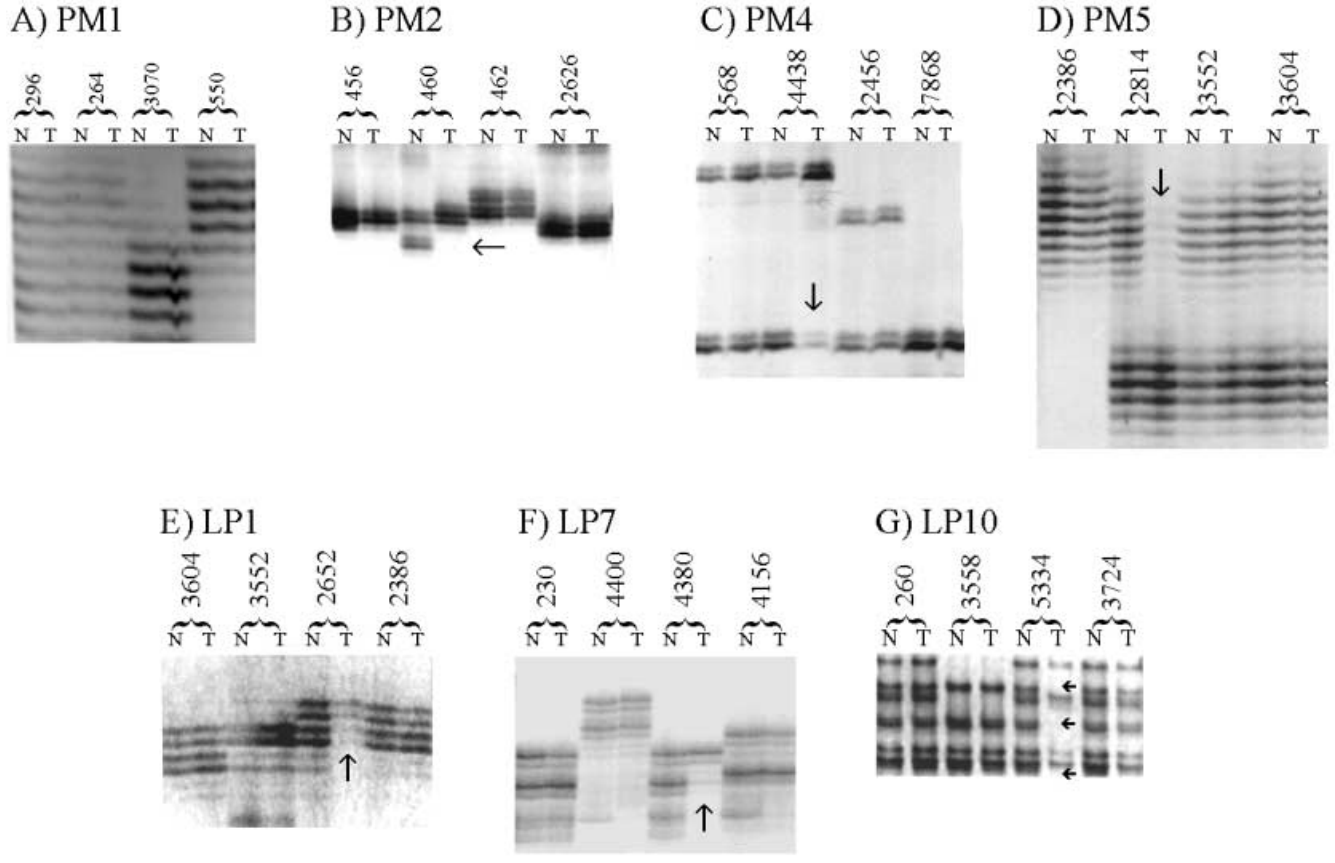

Fig. 1A–G Representative genotypes for the novel polymorphic markers identified in this study. *Arrows* indicate loss of an allele: *N*, constitutional blood DNA; *T*, corresponding tumour DNA. **A–D** *TSC1* markers; **E–G** *TSC2* markers. Markers **A–F** were assayed by radioactive end-labelled PCR and PAGE; marker **G** was assayed by SSCP analysis of PCR product

Results

Novel markers at the *TSC1* and *TSC2* loci

Four simple sequence repeats examined from the *TSC1* region and two from the *TSC2* region, as well as one intronic single nucleotide polymorphism in *TSC2*, were found to represent novel highly polymorphic markers. These were used together with three previously reported markers from the *TSC1* region, and four from the *TSC2* region, to assay for LOH by parallel genotyping of tumour and constitutional DNA samples (Table 1, Fig. 1).

LOH in glial and glioneuronal tumours

Of 100 tumours analysed, 89 were informative for one or more of the markers in the *TSC1* region and nine showed LOH (Tables 2, 3; Fig. 1). Ninety-eight of the 100 tumours were informative for one or more markers in the *TSC2* region and six showed LOH (Tables 2, 4; Fig. 1). One ganglioglioma, from patient 2,652, and one ependymoma, from patient 5,334, showed LOH in both regions. Of the 87 cases informative for markers at both loci, 13 showed LOH in at least one region.

SSCP and sequence analysis

SSCP analysis of all known coding exons of *TSC1* or *TSC2* was performed on the constitutional and tumour DNA samples in cases showing LOH at the corresponding locus. In addition, all 21 gangliogliomas samples were analysed by SSCP for all coding exons of both genes. An aberrant conformer was detected in the constitutional but not the tumour DNA sample from one patient (5,334) with an ependymoma. Sequencing confirmed the single nucleotide polymorphism 1556 A>G in exon 14 of *TSC1*, a marker used in the LOH analysis. This polymorphism had therefore been lost in the tumour, consistent with the LOH already defined. Similarly, the LOH in ependymoma 5,334 and ganglioglioma 3,724 detected in the *TSC2* exon 40 marker (D1711, 5151 T>C), was also detected in the SSCP pattern of the exon 40 PCR product. Sequencing confirmed the loss of the T allele in 5,334 and C allele in 3,724 in the tumour but not the constitutional DNA. The previously reported *TSC2* polymorphisms S526 (C to T at nucleotide 1596; Jones et al. 1999) and G1787S (G to A at nucleotide 5377; Niida et al. 1999) were detected in both tumour and constitutional DNA during comprehensive exon screening of ganglioglioma cases 884 and 2,244 respectively. No other aberrant conformers were identified.

Table 2 Informativity and LOH of glial and glioneuronal tumours

Tumour type and number (*n*)	WHO grade	Number	No. informative and no. showing LOH (parentheses)		
			TSC1	*TSC2*	*TSC1* and *TSC2*
Astrocytoma (*n*=17)	II	9	9 (0)	9 (0)	9 (0)
	Anaplastic III	8	8 (1)	7 (0)	7 (1)
Ependymoma (*n*=16)	I	4	3 (1)	4 (1)	3 (1)
	II	8	8 (1)	8 (0)	8 (1)
	Anaplastic III	4	4 (1)	4 (0)	4 (1)
Ganglioglioma (*n*=21)	I	20	15 (1)	20 (4)	15 (4)
	Anaplastic III	1	1 (1)	1 (0)	1 (1)
Glioblastoma multiforme (*n*=16)	IV	16	15 (1)	15 (1)	14 (2)
Oligodendroglioma (*n*=7)	II	4	4 (0)	4 (0)	4 (0)
	Anaplastic III	3	3 (0)	3 (0)	3 (0)
Oligodendrogliocytic/astrocytic histology (*n*=9)	II	3	1 (0)	3 (0)	1 (0)
	Anaplastic III	6	6 (0)	6 (0)	6 (0)
Pilocytic astrocytoma (*n*=13)	I	13	11 (2)	13 (0)	11 (2)
Subependymal giant cell astrocytoma (*n*=1)[a]	I	1	1 (0)	1 (0)	1 (0)
Total		100	89 (9)	98 (6)	87 (13)

[a] *TSC* status unknown

Table 3 Primary brain tumours showing LOH in the *TSC1* region

Tumour type	Patient no.	Markers (telomeric ⇒ centromeric)						
		PM4	PM2	*PM1*	*EXON14*	*INTRON21*	*EXON22*	PM5
A III	2,814	NI	+	*NI*	*NI*	+	*NI*	+
E I	5,334	NI	NI	*NI*	+	+	*NI*	NI
E II	7,326	NI	+	*NI*	*NI*	+	*NI*	–
E III	4,438	+	NI	*NI*	*NI*	+	*NI*	NI
GG I	2,652	NI	+	*NI*	*NI*	*NI*	*NI*	NI
GG III	592	+	NI	*NI*	*NI*	*NI*	*NI*	NI
GBM	3,708	+	NI	*NI*	*NI*	*NI*	*NI*	NI
PA I	314	NI	+	*NI*	*NI*	*NI*	*NI*	NI
PA 1	460	NI	+	*NI*	*NI*	*NI*	*NI*	NI

A III, astrocytoma grade III; E, ependymoma (grades I, II and III); GG, ganglioglioma; GBM, glioblastoma multiforme; PA, pilocytic astrocytoma; **+**, LOH detected; –, no LOH; NI, not informative. *Italicised boxes* indicate intragenic markers

Table 4 Primary brain tumours showing LOH in the *TSC2* region

Tumour type	Patient no.	Markers (telomeric ⇒ centromeric)						
		LP1	*IVS8*	*LP10*	*EXON 40*	Kg8	EJ1	LP7
E I	5,334	+	+	+	+	+	NI	+
GG I	3,724	NI	*NI*	+	+	+	NI	+
GG1	800	–	*NI*	+	*NI*	NI	NI	+
GG I	2,652	+	*NI*	+	*NI*	NI	NI	NI
GG I	3,382	–	*NI*	*NI*	*NI*	NI	NI	+
GBM	4,380	–	*NI*	–	*NI*	–	NI	+

E, ependymoma (grade I); GG, ganglioglioma; GBM, glioblastoma multiforme; +, LOH detected; –, no LOH; NI, not informative. *Italicised boxes* indicate intragenic markers

Discussion

Assay for loss of alleles at constitutionally heterozygous loci is a convenient and widely used approach for the identification of somatic deletions or chromosome loss in tumours. Where one allele at a particular locus is known, or implied to be mutated in the germline (as is the case in many Mendelian cancer or hamartoma predisposition syndromes), demonstration of loss of the corresponding wild type allele suggests a tumour suppressor function. However, widespread loss of alleles is an extremely common phenomenon in malignant tumours. Carcinomas of the colon, breast, pancreas or prostate may lose heterozygosity at approximately 25% of alleles (Vogelstein et al. 1989; Radford et al. 1995; Seymour et al. 1994; Boige et al. 1997). Unless there is evidence for inactivation of the second allele at a particular locus, the biological relevance

of LOH across relatively large genomic regions that may include many genes of potential importance in tumourigenesis is impossible to assess. Conversely, LOH of biological significance may be restricted to small genomic regions (Colman et al. 1995) and escape detection if the genetic markers studied map hundreds of kilobases or more from the gene(s) actually involved. For these reasons we developed several novel polymorphic markers that map within or very close to the *TSC1* and *TSC2* genes and also assayed for intragenic mutations affecting the corresponding retained *TSC1* or *TSC2* allele in all tumours that showed LOH. However, none of the tumours showing LOH contained a second inactivating mutation that could be detected by SSCP of all coding exons of the retained *TSC1* or *TSC2* allele. These data suggest that homozygous inactivation of *TSC1* or *TSC2* by mutation is not a frequent event in the genesis of primary brain tumours. It is possible that the retained *TSC1* or *TSC2* allele in some tumours had been inactivated by mutations that escaped detection, such as whole exon deletions, or that epigenetic phenomena such as promoter methylation (Merlo et al. 1995) could play a role, but it is unlikely that these mechanisms would account for all inactivating somatic "second hits".

Previous studies have reported reduced or absent tuberin expression in >30% of sporadic astrocytomas, and increased rap1 expression in a further 20%–30%, suggesting that, since modest GAP activity of tuberin has been reported for rap1, alterations in this signalling may be common in human glial tumours (Gutman et al. 1997; Wienecke et al. 1997). If these observations are correct, our data suggest that they may reflect a secondary change in gene expression or a consequence of post-transcriptional or post-translational changes, rather than mutation or loss of the *TSC2* gene itself.

One glioblastoma multiforme (4,380) in which LOH was detected with the marker LP7 that maps ~150 kb centromeric to *TSC2*, was not deleted at Kg8, a marker that lies in the *PKD1* 3' UTR, 260 bp proximal to *TSC2*. The deletion in this tumour therefore did not involve *TSC2* and may indicate the presence of a more proximally located tumour suppressor gene or the random loss of chromosomal material.

LOH at *TSC1* or *TSC2* was detected in 5 of 16 gangliogliomas informative at both loci (Tables 2, 3, 4). Gangliogliomas contain both glial and neuronal elements and giant cells, characteristics shared by the brain lesions of TSC (Gomez et al. 1999). A previous association study has reported a *TSC2* polymorphism that may predispose to sporadic ganglioglioma (Platten et al. 1997). For these reasons we used SSCP to screen all coding exons of both *TSC1* and *TSC2* in all 21 gangliogliomas and paired constitutional DNA samples. No variant banding patterns were observed.

Different histological types of glial and glioneuronal tumours evolve through mutational changes affecting specific and different pathways. This study comprehensively analysed many different tumour types to assess evidence for involvement of the *TSC* genes in a wide range of glial and glioneuronal tumours. We conclude that a primary role for the mutation of the *TSC* genes in the aetiology of these types of sporadic neoplasm is not significant.

Acknowledgements We thank Miss Sally-Ann Price, Miss Stephanie Whittaker-Axon and Dr. Janet Young for their technical assistance and Dr. Norman Doggett for genomic sequence data from the region surrounding the *TSC2* locus. This work was supported by Cancer Research Wales, The Tuberous Sclerosis Association (UK), The National Tuberous Sclerosis Association and the Deutsche Forschungsgemeinschaft (SFB 507).

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