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Phenotype versus genotype correlation in oligodendrogliomas and low-grade diffuse astrocytomas

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Abstract Oligodendrogliomas typically show loss of heterozygosity (LOH) on chromosomes 1p and 19q, which correlates with their response to chemotherapy, whereas low-grade astrocytomas are characterized by frequent *TP53* mutations and lack of sensitivity to alkylating therapeutic agents. Unequivocal histological distinction of low-grade diffuse astrocytomas from oligodendrogliomas and oligoastrocytomas is often difficult. To elucidate the relationships between morphological phenotype and genetic profile, we screened 19 oligodendrogliomas (WHO grade II) and 23 low-grade diffuse astrocytomas (WHO grade II) for *TP53* mutations and LOH on 1p and 19q. In oligodendrogliomas, LOH on chromosomes 1p and/or 19q was found in 15 cases (79%) and *TP53* mutation was detected in 4 cases (21%). The presence of a typical perinuclear halo in >50% of tumour cells and a chicken-wire vascular pattern were significantly associated with LOH on 1p or 19q (93% of cases). This suggests that oligodendrogliomas with classical histologic features are likely to have a better prognosis. In low-grade diffuse astrocytomas, LOH on chromosomes 1p and/or 19q was found in three cases (13%) and *TP53* mutation was detected in ten cases (43%). Histologically, five low-grade astrocytomas (22%) contained small areas with oligodendroglial differentiation, but this did not correlate with the presence of *TP53* mutations or LOH on 1p and 19q. In both oligodendrogliomas and astrocytomas, LOH on chromosomes 1p or 19q and *TP53* mutation were mutually exclusive. Methylation of the promoter of the gene for O^6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein, which confers resistance to chemotherapy with alkylating agents, was detected in 47% of oligodendrogliomas and 48% of low-grade diffuse astrocytomas. There was no correlation with LOH on chromosomes 1p/19q, suggesting that MGMT may not be a prognostic marker for oligodendrogliomas.

Keywords Oligodendroglioma · Astrocytoma · Chromosome arm 1p and 19q · Loss of heterozygosity · *TP53* mutation · MGMT · Methylation

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Introduction

Oligodendrogliomas (WHO grade II) are well-differentiated and diffusely infiltrating gliomas manifesting in adults and composed predominantly of cells resembling oligodendroglia. They are genetically characterized by LOH on chromosomes 1p and 19q in 40–90% of cases [2, 3, 19, 25, 36, 42, 45]. *TP53* mutations occur in 5–15% of oligodendrogliomas [3, 25, 31, 45]. Approximately two-thirds of oligodendrogliomas respond to procarbazine, CCNU, and vincristine (PCV) treatment, resulting in longer survival of patients [13, 26, 44].

Low-grade diffuse astrocytomas (WHO grade II) are well-differentiated tumours that typically develop in young adults. They grow slowly, diffusely infiltrate the surrounding normal brain, and show an intrinsic tendency to progress to more malignant histologic types, i.e., anaplastic astrocytoma (WHO grade III) and, eventually, glioblastoma (WHO grade IV) [16]. In contrast to oligodendroglial tumours, astrocytic tumours are highly resistant to chemotherapy [10]. Low-grade diffuse astrocytomas are genetically characterized by *TP53* mutations that occur in approximately two-thirds of cases [37, 47,

49]. Some tumours with histopathological features of low-grade diffuse astrocytoma may show loss of heterozygosity (LOH) on 1p [1, 19, 25, 45] and on 19q [19, 25, 38, 45, 46, 53].

LOH on chromosomes 1p and 19q is associated with sensitivity to PCV chemotherapy (procarbazine, CCNU, vincristine) and with longer survival of patients with anaplastic oligodendrogliomas (WHO grade III) [4]. Similarly, combined LOH on 1p and 19q has been identified as a predictor of prolonged overall survival of patients with oligodendrogliomas (WHO grade II) [43].

Chemoresistance may result from saturation [18] or lack of expression of the DNA repair protein O^6-methylguanine-DNA methyltransferase (MGMT), which removes alkyl groups from the O^6-position of guanine, a critical site of alkylation by monofunctional (procarbazine) and bifunctional cross-linking (BCNU, CCNU) nitrosoureas [33]. Loss of MGMT expression may be caused by methylation of promoter CpG islands [34, 51] and has been observed in a variety of human cancers, including gliomas [9].

In a substantial fraction of cases, histological distinction of low-grade diffuse astrocytomas from oligoastrocytomas and oligodendrogliomas shows high inter-observer variability, even among pathologists applying the criteria of the new WHO classification [16]. This is particularly true for tumours that contain small areas of oligodendroglial differentiation. These tumours are variably diagnosed as low-grade diffuse astrocytomas, oligoastrocytomas, or even oligodendrogliomas.

The objective of the present study was to correlate the histopathologic features of oligodendrogliomas and low-grade diffuse astrocytomas with their respective genetic profiles. We screened 19 oligodendrogliomas and 23 low-grade diffuse astrocytomas for LOH on 1p and 19q by PCR-based microsatellite analysis, *TP53* mutations by SSCP and DNA sequencing, and MGMT promoter methylation by methylation-specific PCR.

Materials and methods

Tumour samples and DNA extraction

Supratentorial low-grade gliomas (42 cases) were obtained from the Department of Neurosurgery, University Hospital, Zürich, Switzerland, between 1979 and 1995. Tumours were fixed in formalin, embedded in paraffin for routine histopathological examination, and classified according to the WHO grading system [16]. They included 19 oligodendrogliomas (WHO grade II, Table 1) and 23 low-grade diffuse fibrillary astrocytomas (WHO grade II, Table 2). Criteria for the diagnosis of oligodendrogliomas were isomorphic round tumour cell nuclei and areas of honeycomb appearance, i.e., tumour cells with clear cytoplasm, well-defined plasma membrane, and a dense network of branching capillaries resembling chicken wire [16]. Low-grade diffuse astrocytomas were identified by the presence of isomorphous, well-differenti-

Table 1 Phenotype versus genotype correlation in oligodendrogliomas

Patient ID	Age/sex	Perinuclear halo	Chicken-wire vascular pattern	Microcalcification	Transitional GFAP-positive cells	Mitosis/10 HPF	LOH 1p	LOH 19q	*TP53* mutation	MGMT methylation
89	52/F	+++	++	–	–	2	+	+	–	+
225	30/F	++	–	–	+	1	–	–	Codon 162, ATC→AGC, IIe→Ser	–
229	53/M	+++	+	++	+ (focal)	0	+	–	–	–
357	32/M	+++	+	–	+++	1	–	–	Codon 246, ATG→ATA, Met→Ile	+
392	57/M	+++	++	–	++	1	+	+	–	+
393	32/F	++	–	–	±	4	–	–	Codon 193, CAT→CTT, His→Leu	–
394	64/F	+++	+	–	+++ (focal)	3	+	+	–	–
395	40/M	+++	+	–	±	2	+	+	–	–
397	33/F	+++	++	++	±	0	+	+	–	+
398	43/M	+++	–	–	++ (focal)	0	+	+	–	–
399	49/M	+++	++	–	++ (focal)	1	+	+	–	+
400	29/F	+++	+	–	+	2	+	+	–	–
401	27/F	+++	++	+	++ (focal)	2	+	+	–	–
403	37/M	++	–	+	–	1	–	–	Codon 175, CGC→CAC, Arg→His	+
404	52/M	++	++	++	+++	2	+	+	–	+
405	29/F	+++	++	+	+ (focal)	0	–	+	–	–
406	47/F	+++	++	++	+ (diffuse)	4	+	+	–	+
407	33/M	+++	+	+	+++ (focal)	1	+	+	–	–
408	44/M	+++	++	++	±	1	+	+	–	+

Perinuclear halo was recorded as –, presence in <5% of tumour cells; +, presence in 5–25% of the tumour cells; ++, presence in 25–50% of the tumour cells; +++, presence in more than 50% of tumour cells. Chicken-wire vascular pattern was recorded as –, not obvious; +, focally present; ++, diffusely present. Microcalcification was recorded as -, absent; +, one or very few present; ++, present. Transitional cells were recorded as –, absent; ±, very few present; +, few present; ++, present; +++, many present

Table 2 Phenotype versus genotype correlation in low-grade diffuse astrocytomas

Patient ID	Age/ sex	Oligo-dendroglial component	LOH 1p	LOH 19q	*TP53* mutations	MGMT methylation
11	32/F	–	–	–	–	–
72	23/F	–	–	–	exon 8, codon 301, 2 bp deletion	+
92	29/M	–	–	–	–	–
357	32/F	–	–	–	exon 8, codon 273, CGT→TGT, Arg→Cys	+
372	36/M	–	–	–	exon 7, codon 237, ATG→ATA, Met→Ile	+
373	23/F	–	–	+	–	–
374	26/M	–	–	–	–	–
375	36/F	–	–	–	exon 5, codon 179, CAT→CGT, His→Arg	+
376	28/F	–	+	+	–	–
378	26/F	–	–	–	exon 5, codon 135, TGC→CGC, Cys→Arg	+
380	26/F	–	–	–	–	–
381	23/M	–	–	–	exon 5, codon 175, CGC→CAC, Arg→His	+
385	33/F	–	–	–	–	–
386	59/M	–	–	–	–	–
387	37/M	–	–	–	exon 8, codon 273, CGT→TGT, Arg→Cys	+
388	47/F	–	–	–	exon 6, codon 223, 2 bp deletion	+
389	30/M	–	–	–	exon 8, codon 273, 2 bp insertion	–
390	62/M	–	–	–	–	+
83	26/M	+	–	–	–	–
379	33/F	+	+	+	–	+
383	38/F	+	–	–	exon 5, codon 179, CAT→CGT, His→Arg	+
384	41/M	+	–	–	–	–
391	39 M	+	–	–	–	–

ated fibrillary neoplastic astrocytes on a loosely structured background, with diffuse infiltration of neighbouring brain structures [16].

None of the patients with low-grade astrocytomas and oligodendrogliomas underwent chemotherapy. Five patients with low-grade astrocytoma (cases 92, 380, 381, 386, 387) and one patient with oligodendroglioma (case 399) were given postoperative radiotherapy. Only one patient with low-grade astrocytoma received preoperative radiotherapy (case 390; 56 Gy).

Tumour areas were manually microdissected from paraffin sections and DNA was extracted as previously described [12]. In low-grade astrocytomas with an oligodendroglial component, DNA was extracted from such areas. For LOH analyses, control DNA was extracted from peripheral blood DNA using QIAamp DNA Blood Kit (QIAGEN, Courtaboeuf, France) or from adjacent non-tumourous brain tissues.

Histopathological examination

All tumours were histologically examined using both H&E- and GFAP-stained sections. In oligodendrogliomas, the following histologic features were carefully assessed: extent of perinuclear halo, chicken-wire vascular pattern, microcalcification, and transitional cells. The frequency of tumour cells with a perinuclear halo was recorded as: -, presence in <5% of tumour cells; +, presence in 5–25% of tumour cells; ++, presence in 25–50% of tumour cells; +++, presence in >50% (majority) of tumour cells. The results for chicken-wire vascular pattern were recorded as: -, not obvious; +, focally present; ++, diffusely present. The degrees of microcalcification were assessed as: -, absent; +, one or very few present; ++, significantly present. Transitional cells (minigemistocytes or gliofibrillary oligodendrocytes) were defined as small gemistocytes typically with eccentric nuclei and with cytoplasm positive for GFAP. The results for transitional cells were recorded as: -, absent; ±, very few present; +, few present; ++, present; +++, many present. The number of mitoses was assessed per 10 high-power fields.

An oligodendroglial component in low-grade diffuse astrocytomas was defined by the focal presence of oligodendroglial features such as regular rounded nuclei, scanty cellular processes, and an eccentric rim of eosinophilic cytoplasm.

PCR–single-strand conformational polymorphism (SSCP) analysis and direct DNA sequencing for *TP53* mutations

TP53 mutations in 12 astrocytomas (cases 11, 72, 83, 357, 372, 373, 385–390) have been previously reported [28, 47]. *TP53* mutations in 15 oligodendrogliomas (cases 89, 229, 392, 394, 395, 397–401, 404–408) have been previously reported [50]. For other tumours, prescreening for mutations in exons 5–8 of the *TP53* gene by PCR–SSCP analysis was carried out as previously described [32, 48]. Sequencing primers used were as follows: 5′-TCT GTC TCC TTC CTC TTC CTA C-3′ (sense) and 5′-AAC CAG CCC TGT CGT CTC TCC A-3′ (antisense) for exon 5; 5′-CTG GGG CTG GAG AGA CGA CA-3′ (sense) and 5′-GCC ACT GAC AAC CAC CCT TA-3′ (antisense) for exon 6; 5′-TGC CAC AGG TCT CCC CAA GG-3′ (sense) and 5′-GGG TCA GAG GCA AGC AGA GG-3′ (antisense) for exon 7; 5′-TCC TTA CTG CCT CTT GCT TC-3′ (sense) and 5′-TCT CCT CCA CCG CTT CTT GT-3′ (antisense) for exon 8. Samples that showed mobility shifts in SSCP analysis were further analysed by direct DNA sequencing. After PCR amplification with the same set of primers, the PCR products were sequenced on an automated sequencing system (ABI PRISM 310 Genetic Analyzer, Perkin-Elmer Biosystems) using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems).

LOH analyses on 1p and 19q

LOH on chromosomes 1p and 19q was assessed by PCR-based microsatellite analysis as previously described [29]. For all oligodendrogliomas and one astrocytoma (case 391), four (D1S548, D1S508, D1S552, and D1S2734) and five (D19S219, D19S412,

D19S902, D19S246, and D19S601) microsatellite markers were used for analyses on chromosomes 1p and 19q, respectively. These microsatellite markers span the regions on 1p35–36 and 19q13.3 that are commonly lost in oligodendrogliomas [15, 38, 42]. For 22 astrocytomas, 2 additional markers (D1S2667 and D1S228) on chromosome 1p and 4 markers (APOC2, D19S606, D19S596, and D19S180) on chromosome 19q were used. All microsatellite markers were purchased from Research Genetics (Huntsville, AL, USA).

Allelic losses for each marker were determined by comparing the electrophoretic patterns of DNA from tumours with that of reference DNA. PCR was performed using a Genius DNA Thermal Cycler (Techne, Cambridge, UK) in a total volume of 10 µl, consisting of 1 µl genomic DNA, 2 µl of 5× PCR buffer, 6 pmol of each sense and antisense primer, 1.5 mM $MgCl_2$, 1.25 mM dNTPs, 0.225 U Taq polymerase (Sigma, St. Louis, MO, USA), and 0.5 µCi of [α-^{33}P]dCTP (ICN Biomedicals, specific activity 3000 Ci/mmol). Initial denaturation for 2 min at 95°C was followed by 28–38 cycles with denaturation at 94°C for 45 s, annealing at 55–61°C for 45 s, and extension at 72°C for 1 min. A final extension step for 7 min at 72°C was added. PCR products (10 µl) were mixed with an equal amount of loading dye (95% formamide, 20 mM EDTA, 0.05% xylene cyanol, and 0.05% bromophenol blue), denatured at 95°C for 5 min, and separated on 7% denaturing polyacrylamide gels at 70 W for 3.5–6.0 h. Dried gels were exposed to X-ray film (Kodak, BioMax, NY, USA). LOH was scored when signal intensity was reduced in a tumour sample by more than 50% from that of reference DNA measured by densitometry (Bio-Rad model GS-670).

Methylation-specific PCR

MGMT methylation in 12 astrocytomas (cases 11, 72, 83, 357, 372, 373, 385–390) has been previously reported [28]. For the other 11 astrocytomas and all oligodendrogliomas, promoter methylation of the MGMT gene was determined by methylation-specific PCR [14]. Sodium bisulfite modification was performed using the CpGenome DNA Modification Kit (Intergen, Oxford, UK) as described previously [27]. Control methylated DNA (Intergen, Oxford, UK) and unmethylated DNA (normal blood) were treated with bisulfite by the same method.

Primer sequences of MGMT for the methylated and unmethylated reactions have been previously reported [9]. PCR was carried out in 10 µl of mixture containing PCR buffer (10 mM Tris pH 8.3, 50 mM KCl), 2.5 mM $MgCl_2$, dNTPs (250 µM each), sense and antisense primers (0.75 µM each), 0.5 unit of PLTINUM Taq DNA polymerase (GIBCO BRL, Cergy-Pontoise, France) and approximately 40 ng bisulfite-modified DNA. Amplification was carried out in a Robocycler (Stratagene) with initial denaturing at 95°C for 5 min followed by 35 cycles of denaturing at 95°C for 50 s, annealing for 50 s at 59°C, and extension for 50 s at 72°C, followed by a final extension for 2 min at 72°C. Control methylated and unmethylated DNA (Intergen Company, Oxford, UK) were treated with bisulfite as sample DNAs. Amplified products were electrophoresed on a 3% agarose gel and visualized with ethidium bromide.

Statistical analysis

Fisher's exact test was applied for statistical analysis of the correlation for two independent variables.

Results

Oligodendrogliomas

Genetic alterations

In analyses of LOH on chromosome 1p, a total of 76 loci were analysed and 48 (63%) were found to be informative. LOH on chromosome 1p was observed in 14 of 19 (74%) oligodendrogliomas. In 12 oligodendrogliomas, LOH was found at all informative loci on 1p, suggesting the loss of the entire chromosome arm, while two tumours showed partial LOH. All oligodendrogliomas with allelic loss on 1p had deletion of at least one marker on the 1p36 region.

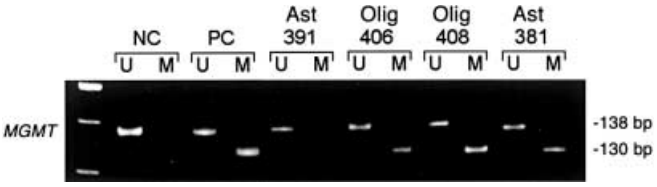

Fig. 1 Methylation-specific PCR showing MGMT promoter methylation in oligodendrogliomas (*Olig*) and low-grade diffuse astrocytomas (*Ast*). Cases 406, 408, and 381 show MGMT promoter methylation, while case 391 shows only the unmethylated sequence. (*NC*, normal control; *PC*, positive control for unmethylated and methylated DNA)

In analyses of LOH on chromosome 19q, a total of 95 polymorphic loci on chromosome 19q were examined and 66 (69%) were found to be informative. Overall, 14 of 19 (74%) oligodendrogliomas exhibited LOH for at least one marker of the 19q loci. In all these 14 cases, LOH was found at all informative loci on 19q, suggesting the loss of the entire chromosome arm. In 15 of 19 (79%) oligodendrogliomas, LOH on 1p and/or 19q was detected, while in 13 of 19 (68%) oligodendrogliomas, simultaneous LOH on 1p and 19q was observed.

TP53 mutations were found in 4 of 19 (21%) oligodendrogliomas (Table 1). LOH on 1p/19q and *TP53* mutations were mutually exclusive. All four oligodendrogliomas lacking LOH on 1p or 19q contained a *TP53* mutation (Table 1).

Methylation-specific PCR revealed MGMT methylation in 9 of 19 (47%) oligodendrogliomas (Fig. 1). There was no significant correlation between MGMT methylation and LOH on 1p and/or 19q or *TP53* mutation.

Correlation between histologic features and genetic alterations in oligodendrogliomas

Of 15 oligodendrogliomas with a typical perinuclear halo in >50% of neoplastic cells and a chicken-wire vascular pattern, 14 cases (93%) showed LOH on 1p and/or 19q, whereas only one case (7%) contained a *TP53* mutation. The presence of perinuclear halos in >50% of tumour cells was significantly associated with LOH on 1p ($P<0.05$) and LOH on 19q ($P<0.05$) (Table 1, Fig. 2 A). In contrast, the presence of a perinuclear halo in <50% of tumour cells was associated with *TP53* mutations ($P<0.05$) (Table 1, Fig. 2B). The presence of chicken-wire vascular pattern was significantly associated with LOH on 1p ($P<0.05$) and on 19q ($P<0.05$) and was inversely correlated with *TP53* mutation ($P<0.05$). Extensive presence of chicken-wire vascular pattern was significantly correlated

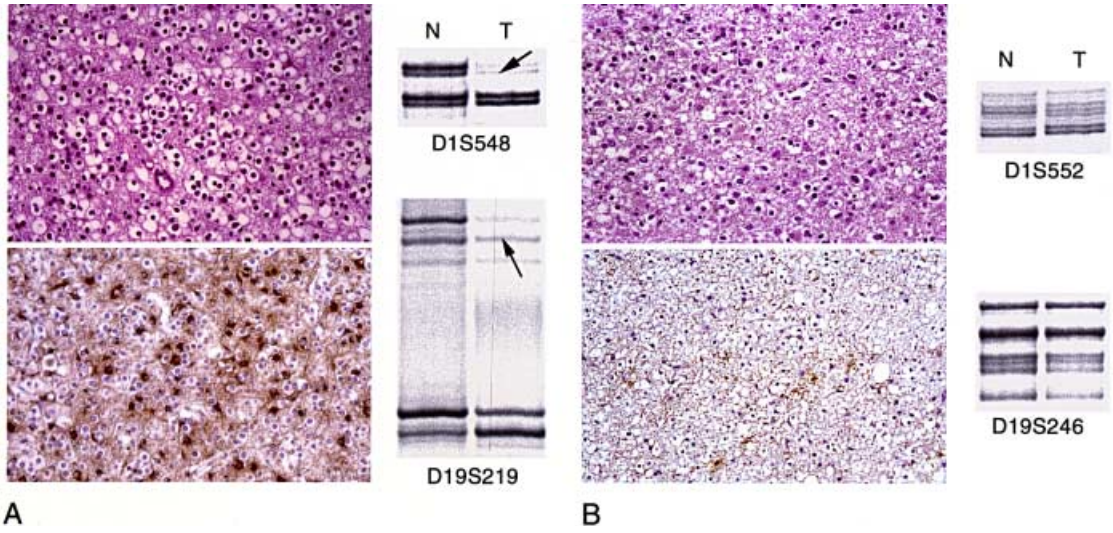

Fig. 2 **A** Oligodendroglioma with typical honeycomb appearance, with GFAP-expressing transitional cells (case 394, Table 1). Microsatellite analyses show LOH on both chromosome 1p (*marker D1S548*) and chromosome 19q (*marker D19S219*). **B** Oligodendroglioma showing scattered neoplastic cells with perinuclear halo (case 403, Table 1). The tumour cells show clear cytoplasm and well-defined plasma membrane. Note retention of heterozygosity on chromosome 1p (*marker D1S552*) and chromosome 19q (*marker D19S246*). (*N*, normal DNA; *T*, tumour DNA)

with typical perinuclear halo in >50% of tumour cells ($P<0.05$). The other histologic features, including the occurrence of transitional cells, were not associated with *TP53* mutations or LOH on 1p/19q. Further, there was no significant correlation between MGMT methylation and histopathological features.

Low-grade diffuse astrocytomas

Genetic alterations

In analyses of LOH on chromosome 1p, a total of 136 loci were tested and 116 (85%) were found to be informative. LOH on chromosome 1p was observed in 2 of 23 (9%) cases. In one case (case 376), LOH was found at all informative loci on 1p, indicating loss of the entire chromosome arm. Another case (case 379) retained allelic balance at the 1p36 region and showed loss at the marker D1S2734 on the 1p35.1 region.

In analyses of LOH on chromosome 19q, a total of 203 polymorphic loci on chromosome 19q were examined and 147 (72%) were found to be informative. LOH for at least 1 of 9 of the 19q loci was found in 3 of 23 (13%) low-grade astrocytomas. Of these, two cases (cases 373 and 379) showed interstitial deletions and another (case 376) displayed allelic loss of the entire long arm. All tumours with allelic loss on 19q had deletion of at least one marker on the 19q13.3 region. Two tumours (cases 376 and 379) exhibited both LOH on 1p and 19q, while one tumour (case 373) had LOH on 19q and maintained heterozygosity on 1p.

SSCP followed by DNA sequencing revealed a *TP53* mutation in 10 (43%) of 23 astrocytomas (Table 2). LOH on 1p/19q and *TP53* mutations were mutually exclusive. MGMT promoter methylation was detected in 11 out of 23 (48%) astrocytomas (Table 2, Fig. 1) and was significantly associated with *TP53* mutation ($P<0.01$). There was no significant correlation between MGMT methylation and LOH on 1p or 19q.

Correlation between histologic features and genetic alterations in low-grade diffuse astrocytomas

The majority of low-grade diffuse astrocytomas (18 of 23, 78%) showed the typical morphologic features of fibrillary astrocytomas such as naked nuclei, scanty cytoplasm, and neoplastic processes forming a loosely structured tumour matrix. There was no sign of oligodendroglial differentiation in any of the tumour areas examined. In 5 of 23 (22%) low-grade diffuse astrocytomas, small areas containing neoplastic cells with regular rounded nuclei, scanty cellular process, and an eccentric rim of eosinophilic cytoplasm were observed and considered as oligodendroglial differentiation (Table 2, Fig. 3, cases 379 and 383).

LOH on 1p and/or 19q was found in 1 of 5 (20%) low-grade astrocytomas with an oligodendroglial component, and in 2 of 18 (11%) tumours without an oligodendroglial component (Table 2). There was no significant difference in frequency of LOH on 1p and/or 19q between tumours with or without an oligodendroglial component ($P=0.5392$). In one low-grade astrocytoma with a small oligodendroglial component (case 379), DNA was extracted separately from the astrocytoma and oligodendroglioma areas; both areas showed LOH on chromosomes 1p and 19q (Fig. 3).

TP53 mutations were found in 9 of 18 (50%) tumours lacking oligodendroglial components, and in 1 of 5 (20%) tumours with areas with oligodendroglial components (Table 2). There was no significant difference in the fre-

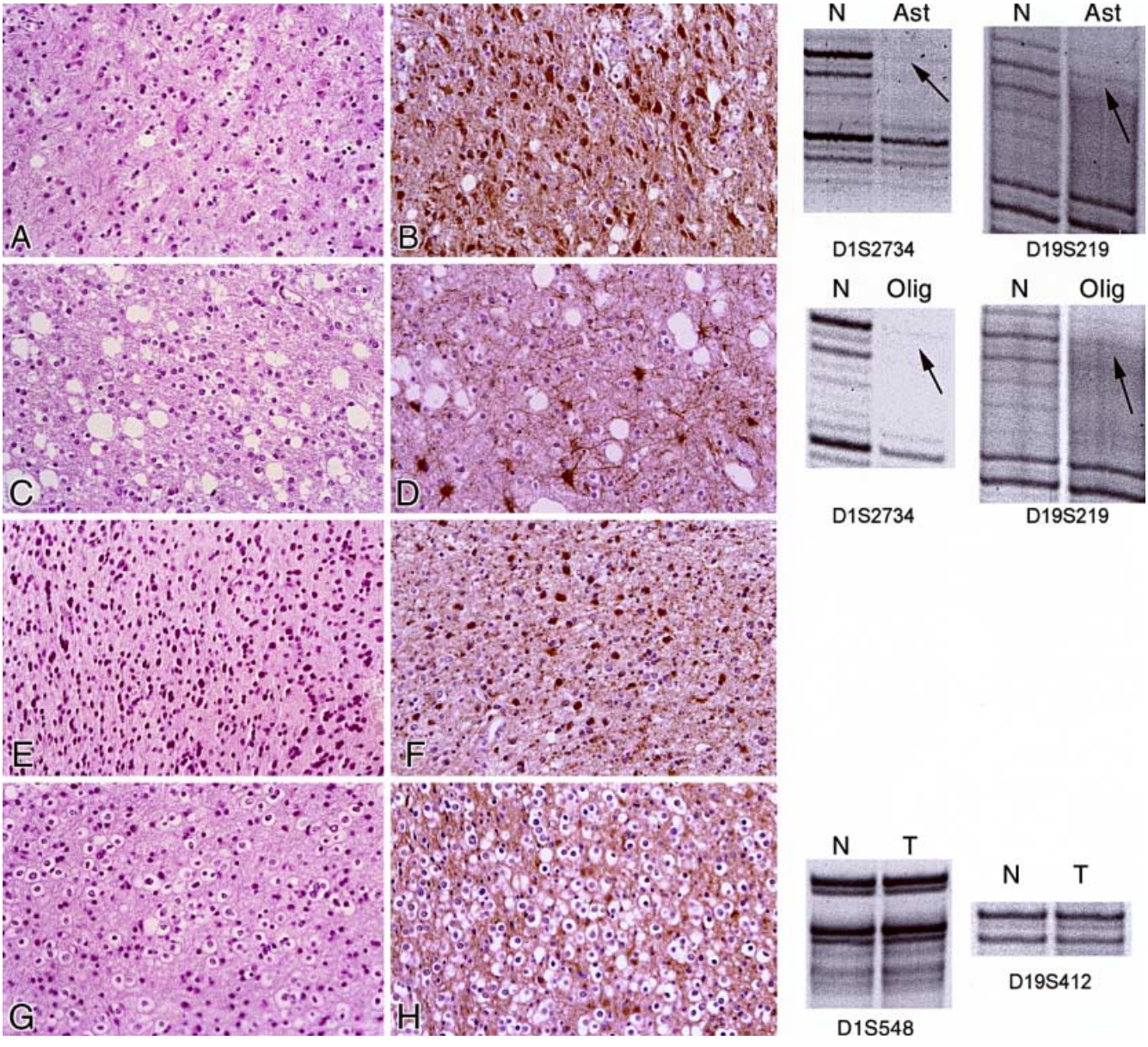

Fig. 3A–H Histologic features and LOH on 1p/19q in low-grade diffuse astrocytomas. Figures 3B, D, F, H show GFAP immunohistochemistry.Case 379 (Fig. 3A–D): most tumour areas show histologic features typical of low-grade diffuse astrocytoma (*Ast*, Fig. 3A, B). In this tumour, a small area with an oligodendroglial component (*Olig*) was observed (Fig. 3C, D). Note that DNA extracted from both areas showed LOH on chromosomes 1p and 19q (*right*).Case 383 (Fig. 3E–H): most tumour areas show histologic features typical for low-grade diffuse astrocytoma (Fig. 3E, F). In this tumour, a small area with an oligodendroglial component was observed (Fig. 3G, H). DNA was extracted from the area containing an oligodendroglial component. LOH on 1p/19q was not detected (*right*), but a *TP53* mutation was detected in this tumour (Table 2)

quency of *TP53* mutations between tumours with or without oligodendroglial components (P=0.3394, Table 2, Fig. 3). There was no significant association between MGMT methylation and the presence of oligodendroglial differentiation (Table 2).

Discussion

Histologic hallmarks of oligodendrogliomas include a perinuclear halo, which may produce a honeycomb/fried-egg appearance, and a dense network of branching capillaries resembling chicken wire. Based on these classical histologic criteria, the frequency of oligodendrogliomas has been estimated as amounting to 5–18% of all gliomas [39, 40]. Since the diagnosis of oligodendroglioma has favourable prognostic implications, there is a recent tendency to expand the histological criteria of oligodendrogliomas to include features such as nuclear regularity and roundness, often accompanied by a thin, eccentric rim of eosinophilic cytoplasm and apparent lack of cell processes [5, 6, 11]. Using these less stringent criteria, Coons et al. [5] found that a significant fraction of tumours previously diagnosed as low-grade diffuse astrocytomas were in fact oligodendrogliomas [5], and that oligo-

dendrogliomas diagnosed based on such criteria may represent 25–33% of all glial tumours [5, 7, 11].

This study shows a clear correlation between the phenotype and genotype. The majority of oligodendrogliomas (13 of 14, 93%) with chicken-wire vascular pattern and typical perinuclear halo in >50% of tumour cells showed LOH on 1p and/or 19q. Since these genetic alterations, independently or in combination, are significant predictors of chemosensitivity in anaplastic oligodendrogliomas [4] and increased survival of patients with oligodendrogliomas and anaplastic oligodendrogliomas [4, 43], the present results suggest that oligodendrogliomas with classical histologic features are likely to be chemosensitive. Thus, expanding the diagnostic criteria may be misleading with regard to chemosensitivity and prognosis. *TP53* mutations, a hallmark of low-grade diffuse astrocytomas, were identified less frequently (21%) and typically in lesions lacking chicken-wire vascular pattern and having occasional but not extensive perinuclear halo.

Oligoastrocytomas are defined as neoplasms with a conspicuous mixture of two distinct neoplastic cell types resembling neoplastic oligodendrocytes and astrocytes [35]. Approximately 30–50% of oligoastrocytomas show LOH on 1p/19q [19, 25, 36, 45], and about 30% contain *TP53* mutations and/or LOH on chromosome 17p [25, 36, 45]. Oligoastrocytomas with *TP53* mutations and/or LOH on chromosome 17p do not show LOH on 1p/19q, and vice versa [25, 36]. Oligoastrocytomas with LOH on 1p/19q typically have predominant features of oligodendroglioma, whereas those with *TP53* mutations are more often astrocytoma-predominant [25]. In this study, we showed that a subset (22%) of tumours diagnosed as low-grade diffuse astrocytomas contained small areas with oligodendroglial features, but that this was not predictive regarding the presence of either *TP53* mutations or LOH on chromosomes 1p/19q. Thus, the presence of small oligodendroglial foci in low-grade diffuse astrocytomas does not necessarily reflect the presence of an oligodendroglial genotype.

In borderline cases, histologic distinction of oligodendrogliomas from astrocytomas can be highly subjective. This may be due to a lack of reliable oligodendroglial markers and the hypothetical origin of oligodendrogliomas from O-2A precursor cells with bipotential capacity to differentiate into either oligodendrocytes or type-2 astrocytes [24, 30]. In contrast to the occasional histologic ambiguity, *TP53* mutations and LOH 1p/19q were mutually exclusive in both oligodendrogliomas and astrocytomas, as was previously observed in mixed oligoastrocytomas [25]. In one astrocytoma with a small oligodendroglial component (case 379), DNA was extracted separately from astrocytoma and oligodendroglioma areas; both samples showed LOH on chromosomes 1p and 19q. These results suggest that LOH on 1p/19q is frequent and that *TP53* mutations are infrequent genetic alterations in the evolution of oligodendrogliomas. The GFAP-positive cells in oligodendrogliomas have been classified as gliofibrillary oligodendrocytes, minigemistocytes, classic large gemistocytes, and entrapped non-neoplastic astrocytes. Oligodendroglial minigemistocytes resemble a smaller version of the gemistocytic astrocytes and apparently lack cell processes [5]. Electron microscopic studies suggest that minigemistocytes and gliofibrillary oligodendrocytes represent transitional forms between oligodendroglial and astrocytic phenotypes [20, 52]. The presence of numerous (>20%) gemistocytes in astrocytomas is considered an unfavorable prognostic sign [17, 23], while GFAP-positive transitional cells in oligodendrogliomas appear to have no prognostic significance [21, 22]. Genetically, classical gemistocytes in astrocytomas are characterized by frequent *TP53* mutations [49]. In this study, the occurrence of transitional cells was not associated with *TP53* mutations or LOH on 1p/19q.

MGMT plays an important role in modulating the chemosensitivity of tumour cells to alkylating agents [33], since it removes alkyl groups from the O^6 position of guanine; O^6-alkylguanine represents a crucial DNA adduct produced by chemotherapeutic agents. A previous study showed slightly lower MGMT activity in oligodendroglial than in astrocytic tumours [41]. MGMT methylation was found in 31% of astrocytoma/oligodendroglioma, 50% of anaplastic astrocytomas, and 41% of glioblastomas [9]. We recently observed MGMT methylation in 48% of low-grade diffuse astrocytomas, in 75% of secondary glioblastomas derived therefrom, and in 36% of primary (de novo) glioblastomas [28]. Esteller et al. [8] assessed MGMT promoter methylation in anaplastic astrocytomas and glioblastomas that were subsequently treated with BCNU and found that MGMT promoter methylation was significantly associated with longer overall survival and time till progression. In this study, we have shown that MGMT methylation is present at similar frequency in oligodendrogliomas (47%) and low-grade diffuse astrocytomas (48%). Furthermore, MGMT methylation did not correlate with LOH on 1p/19q, suggesting that MGMT may not be a prognostic marker for low-grade oligodendrogliomas. A similar lack of correlation in anaplastic oligodendrogliomas (WHO grade III) would suggest that loss of methyltransferase activity is not involved in their response to PCV chemotherapy.

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