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RESEARCH ARTICLE

Molecular Analysis of Pediatric Oligodendrogliomas Highlights Genetic Differences with Adult Counterparts and Other Pediatric Gliomas

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Abstract

Oligodendroglioma represents a distinctive neoplasm in adults but similar neoplasms occur rarely in children. We studied 20 cases of pediatric oligodendroglioma by SNP array (median age 9 years, range 1–19; 15 grade II and 5 grade III). Cytogenetic abnormalities were present in 8 (53%) grade II and all five anaplastic oligodendrogliomas. Most changes were in the form of deletion and copy neutral loss of heterozygosity (LOH). The most common abnormality was 1p deletion (n = 5). Whole arm 1p19q co-deletion was present in three cases from adolescent patients and 9p loss in 3, including one low-grade oligodendroglioma with *CDKN2A* homozygous deletion. Common losses were largely limited to the anaplastic subset (n = 5) and included 3q29 (n = 3), 11p (n = 3), 17q (n = 3), 4q (n = 2), 6p (n = 2), 13q (n = 2), 14q (n = 2), 17p (n = 2) and whole Ch 18 loss (n = 2). Gains were non-recurrent except for whole Ch 7 (n = 2) and gain on 12q (n = 2) including the *MDM2* locus. Possible germ line LOH (or uniparental disomy) was present in seven cases (35%), with one focal abnormality (22q13.1-13.2) in two. *BRAF-KIAA1549* fusions and *BRAF* p.V600E mutations were absent (n = 13 and 8). In summary, cytogenetic alterations in pediatric oligodendrogliomas are characterized mostly by genomic losses, particularly in anaplastic tumors.

INTRODUCTION

Oligodendrogliomas represent an important clinicopathologic and molecular subset of infiltrating gliomas that have been well studied in adults. These tumors are characterized at the molecular level by the presence of whole-arm 1p19q co-deletion, a cytogenetic abnormality mediated by a t(1;19) translocation (11, 16). Whole-exome sequencing studies have identified mutations in putative suppressor genes located in these regions, particularly *FUBP1* and *CIC* (4, 43) in some, but not all, cases. Like other diffuse gliomas, these tumors also have frequent mutations in *IDH1* or *IDH2* (42). Oligodendrogliomas with 1p19q co-deletion and *IDH1* or *IDH2* mutation demonstrate the best prognosis in the diffuse glioma category, and are particularly responsive to chemotherapy, as highlighted by long-term follow-up in clinical trials (6, 41).

Tumors with all the histologic features of oligodendroglioma also occur in children. These tumors are usually low grade, and often recur but have a low rate of histologic progression to anaplasia (35). They usually lack the molecular changes typical of adult oligodendroglioma, that is, 1p19q co-deletion and *IDH1* or 2 mutations, particularly when developing in patients less than 15 years old (22, 31, 35). The differential diagnosis of oligodendroglioma in the pediatric population encompasses a variety of close mimics, including dysembryoplastic neuroepithelial tumor, neurocytoma, pilocytic astrocytoma and more recently, disseminated oligodendroglioma-like neoplasm (DOLN) (1, 30, 34, 36, 38). However, cytogenetic abnormalities other than 1p19q have not been adequately examined in a systematic fashion in these tumors. In the current study, we assessed single nucleotide polymorphism microarray data from pediatric oligodendrogliomas to characterize cytogenetic changes in these tumors.

MATERIALS AND METHODS

Patients

We studied 28 cases of pediatric oligodendroglioma examined at Johns Hopkins Hospital with sufficient formalin-fixed paraffin-embedded tissue for molecular analysis. The clinicopathologic features of 26 of these cases have been previously published (35). Two additional cases were included and reviewed by two of the authors (PCB and FJR) to confirm the diagnosis of pediatric oligodendroglioma. Patient ages ranged from <1 to 19 years of age at diagnosis, with nine females and 19 males. There were 20 WHO

grade II and 8 WHO grade III (anaplastic) oligodendrogliomas. The study was performed under Institutional Review (IRB) Board approval at respective sites.

Single nucleotide polymorphism (SNP) array analysis

Areas containing tumor were identified and isolated using Pinpoint reagents (ZymoResearch, Orange, CA, USA). Extraction of DNA from recovered tissue was done with QIAmp DNA MiniKit (Qiagen, Valencia, CA, USA) and quantified by optical density. Extracted DNA was treated with the Infinium HD FFPE DNA Restore Kit (Illumina, San Diego, CA, USA). SNP analysis was performed on the CytoSNP-12 BeadChip platform (Illumina). The platform provides information on 298,563 SNPs. The data were visualized in KaryoStudio software (Illumina), with all determinations of LOH performed manually. Single nucleotide polymorphism (SNP) analysis produced interpretable data in 20 cases. We evaluated B-allele frequency (BAF) and log-R ratio (LRR) for chromosomes 1–22 (q arm only for acrocentric chromosomes 13, 14, 15, 21 and 22), as well as the X chromosome. Some alterations were interpreted as likely representing germ line change. Briefly, in areas of increased spread of B-allele frequency (BAF) caused by LOH in the tumor, normal log-R ratio (LRR) would suggest copy-neutral change. However, the presence of genetically normal cells such as lymphocytes and endothelia in the sample results in a "thickening" of BAF plots at the edges, whereas in some instances of widespread BAF with normal LRR such thickening was not seen. This suggests that all cells in the sample show the same LOH, and hence that it is likely present in the germ line. We have interpreted these regions as representing possible germ line LOH (or uniparental disomy).

BRAF:KIAA1549 FISH

In situ hybridization studies were performed on formalin-fixed paraffin-embedded sections using a dual color fusion strategy as previously reported (36), with probe RP11-355D18 (fluorescein isothiocyanate labeled) targeting KIAA1549 and probe RP4-726N20 (rhodamine labeled) targeting *BRAF*.

Immunohistochemistry for BRAF p.V600E

Formalin-fixed paraffin embedded sections were stained with a mouse monoclonal antibody specific for the human BRAF p.V600E mutant protein (Clone VE1, 1:100 dilution). Detailed methods have been previously published (15).

RESULTS

Somatic chromosomal alterations

Somatic cytogenetic abnormalities were present in 13 (of 20) cases (Figure 1 and Table 1). The predominant alterations were deletion, copy neutral loss of heterozygosity (LOH) or LOH indeterminate, particularly 1p deletion (n = 5). Other recurrent losses (involving

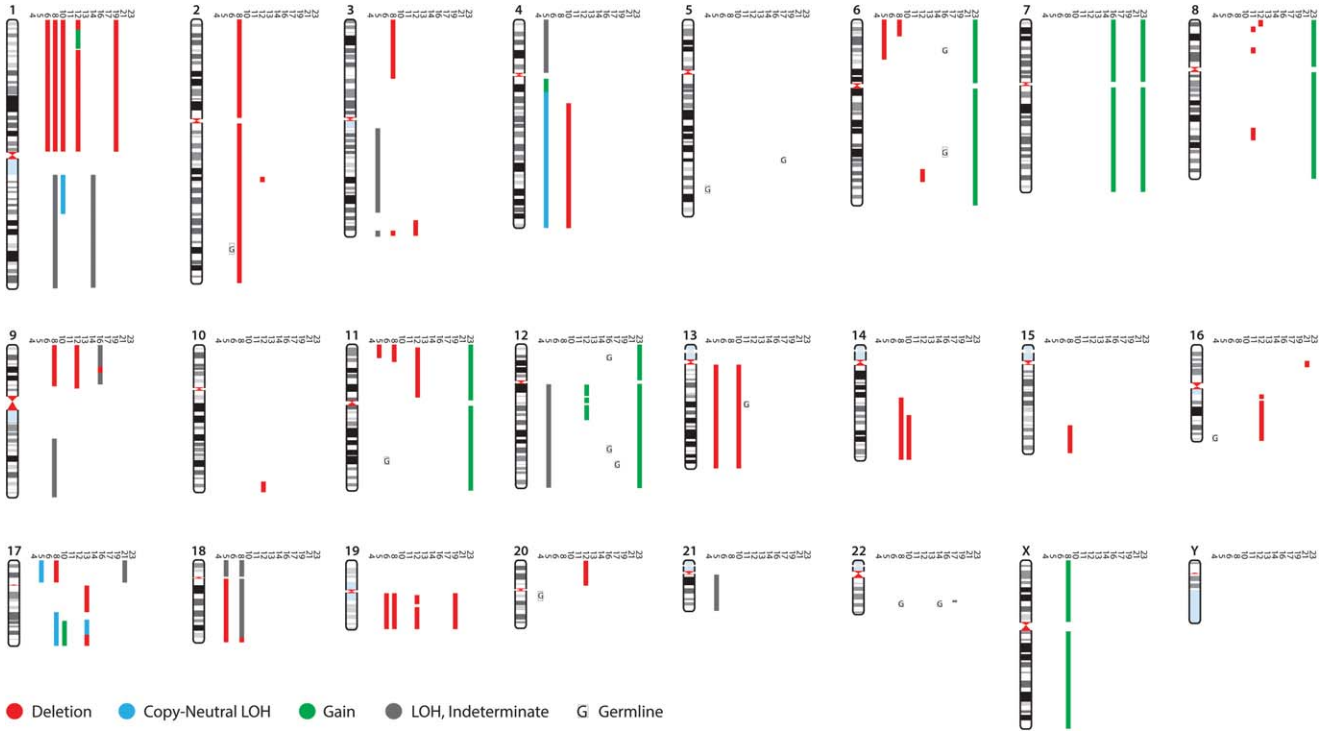

Figure 1. Molecular genetic alterations detected in pediatric oligodendrogliomas by SNP array profiling. 14 (of 20) cases showed loss of heterozygosity, which could be attributed in many cases to deletion, gain or copy neutral change. However, in some cases, definitive determination could not be made. Possible germ line LOH (or uniparental disomy) were present in seven cases.

Table 1. Clinicopathologic and genetic features of pedicatric oligodendrogliomas.

Age/sex	Location	Grade	Prior therapy	Somatic alterations by SNP array	Possible germ line alterations by SNP array	Genes	*BRAF-KIAA1549* FISH	BRAF V600E	IDH1 (R132H) IHC	Molecular other
2/M	Frontal lobe	II	0	Normal karyotype		NA	Negative		Negative	
1/M	Frontal lobe	II	0	Normal karyotype		NA	Negative	Negative (IHC)	Negative	MGMT not methylated
10/F	Frontal lobe	II	0	Normal karyotype		NA	Negative	Negative (IHC)		
13/M	Frontal lobe	II	0	No somatic alterations	5q33.3, 16q24.1qtel(CNN), 20qcenq11.23(CNN)	NA			Negative	
19/F	Temporal lobe	II	0	del 1p (whole arm), del 19q (whole arm)	CN-LOH 2q33.3q35, CN-LOH 11q22.3q22.3(CNN),	Chr 1p (FUBP1), 19q (CIC)			Positive	
1/M	Parietal lobe	II	0	Normal karyotype		NA				
1/M	Frontal lobe	II	0	Normal karyotype		NA				
8/F	Temporal lobe	II	0	del8p23.1p23.1, del8p21.2p12, del8q13.2q13.3, del8q22.1q23.1	13q		Negative	Negative (IHC)	Negative	
15/M	Temporal lobe	II	0	LOH 1q (whole arm)	22q13.1q13.2	22q (germ line XRCC6, RANGAP1, SGSM3,MKL1, ST13,DNAJB7, TEF, TOB2, NHP2L1, SREBF2, CENPM, TNFRSF13C, CYP2D6, TCF20, CYP2D7P1, RBX1, MIR4766, MIR1281, and MCHR1.)			Negative	
9/F	Brain	II	0	Gain whole Chr7, LOH 9ptelp21.3, del9p21.3p21.3 (Homozygous), LOH 9p21.3p13.3	6p22.1p22.1, 6q22.2q22.31; 12p13.2p13.1, 12q23.1q23.3	Ch 7 (EGFR, MET, HGF, BRAF), 9p (CDKN2A, CDKN2B, CDKN2BAS, C9orf53, MTAP)				
1/M	brainstem	II	0	chr22q13.1	5q23.3q31.2; 12q24.11q24.13	DDX17, CSNK1E, DNC1, CBY1, SUN2, CBX6, CBX7, PDGFB			Negative	
2/F	Parietal lobe	II	0	Normal karyotype		NA	Negative	Negative (IHC)	Negative	
16/M	Frontal lobe	II	0	del 1p (whole arm), del 19q (whole arm)		FUBP1, CIC			Negative	
4/M	Medial temporal	II	0	ND		NA			Negative	
14/M	Frontal lobe	II	0	del 16p13.11p12.3, LOH 17p (whole arm)		Chr 17p (TP53)				
12/M	Temporal lobe	II	0	ND		NA	Negative	Negative (IHC)	Negative	Negative for 1p19q LOH (STR)
2/M	Frontal lobe	II	0	ND		NA	Negative		Negative	Negative for 1p19q LOH (STR)
16/M	Parietal lobe	II	0	ND		NA	Negative	Negative (IHC)	Positive	
11/M	Posterior fossa	II	0	Gain whole Chr6, Chr7, Chr8, Chr11, Chr12		Ch 7 (EGFR, MET, HGF, BRAF), FGFR1	Negative	Negative (PCR)		Negative for 1p19q LOH (STR)
19/M	Frontal lobe	II	0	ND		NA			Negative	1p19q loss (STR)
16/M	Frontal lobe	III	0	LOH 3q12.1q26.31, LOH 3q29qtel, LOH 4p (whole arm), dup4q12q13.1, CN-LOH 4q13.1qtel, del6ptelp21.3, del6p21.3p21.31 (small population subset), del11ptelp15.3, LOH 12q (whole arm), del13q (whole arm), CN-LOH 17ptelp11.2, LOH 18p (whole arm), del18q (near whole arm), LOH 21q (whole arm)		Chr 11p (CDKN1C, NUP98, SLC22A18), 13q (RB1), 17p (TP53), Chr 18 (DCC)		Negative (PCR)	Negative	MGMT methylated

Table 1. *Continued*

Age/sex	Location	Grade	Prior therapy	Somatic alterations by SNP array	Possible germ line alterations by SNP array	Genes	*BRAF-KIAA1549* FISH	BRAF V600E	IDH1 (R132H) IHC	Molecular other
11/F	Frontal lobe	III	0	del 1p (whole arm), LOH 1q (whole arm), del 2 (whole Chr deletion), del 3ptelp21.1, del 3q29qtel, del6ptelp22.3, del9ptelp13.2, LOH 9q21.33qtel, del 11ptelp15.1, del 14q21.3qtel, del 15q24.3qtel, del 17ptelp11.2, CN-LOH 17q22qtel, heterogeneous whole Ch18 loss, del 19q (whole arm), gain X (whole Chr)	22q13.1q13.2	Chr 1p (FUBP1), 11p (CDKN1C, NUP98, SLC22A18), 19q (CIC), 17p(TP53), Chr 18 (DCC), 22q(germ line XRCC6, RANGAP1, SGSM3,MKL1, ST13,DNAJB7, TEF, TOB2, NHP2L1, SREBF2, CENPM, TNFRSF13C, CYP2D6, TCF20, CYP2D7P1, RBX1, MIR4766, MIR1281, and MCHR1)				
17/F	Frontal lobe	III	Chemotherapy and radiation 10 years prior for leukemia with CNS involvement	del 1p (whole arm), CN-LOH 1qcentq25.2, del 4q21.1qtel, del 13q (whole arm), del14q23.3qtel, gain 17q23.2qtel		Chr 1p (FUBP1), Chr 13 (RB1)				
3/M	cerebellum	III	RT+Chemo	del 1ptelp36.23, gain 1p36.23p36.13, gain 1p36.12p36.11, del 1p36.11cent, del 2q22.3p23.1, del 3q26.32qtel, del 6q23.3q25.1, del 8ptelp23.2, del 9ptelp13.1, del 10q26.3qtel, del 11p15.4p11.2, gain 12q12q13.11, gain 12q13.13q13.2, gain 12q13.2q13.3, gain 12q14.1q15, del 16q12.1qtel, del 19q12q13.11, del 19q13.13qtel, del 20p (whole arm)		12q (CDK4, MDM2)			Negative	
10/F	Parietal lobe	III	0	del17qcentq21.33, CN-LOH 17q22q24.2(CNN), del17q24.3qtel		NF1	Negative		Negative	
18/F	Frontal lobe	III	0	ND		NA	Negative		Positive	1p19q loss (STR)
7/M	Frontal lobe	III	0	ND		NA	Negative		Negative	Negative for 1p19q LOH (STR)
11/M	Occipital lobe	III	0	ND		NA	Negative		Negative	Negative for 1p19q LOH (STR)

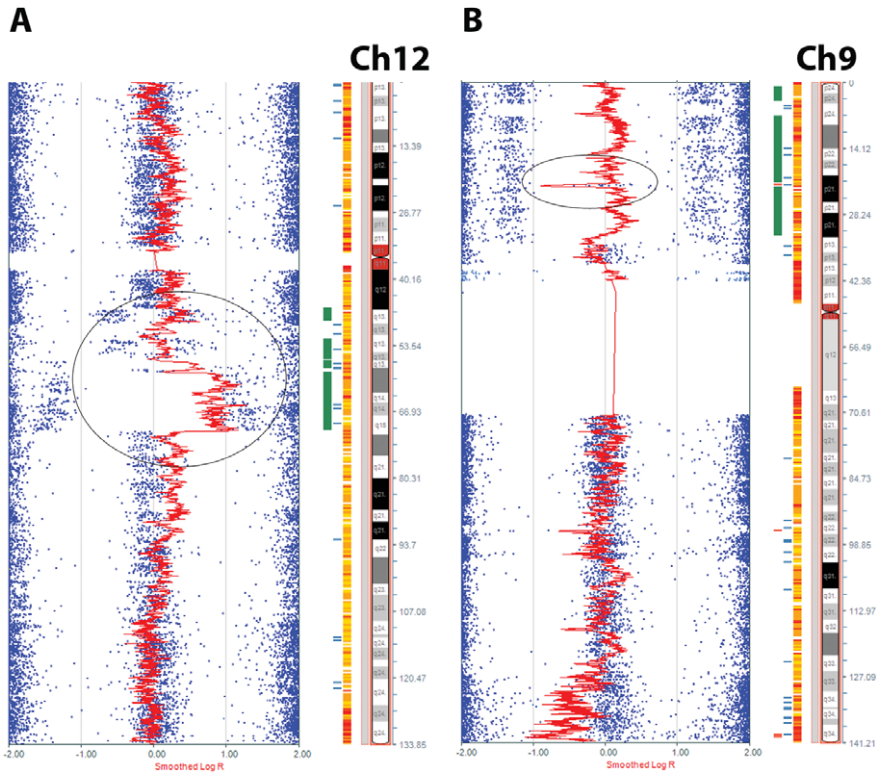

Figure 2. Recurrent genetic alterations in pediatric oligodendrogliomas. Common gains were very rare with two cases showing chromosome 12 gain, including the whole chromosome or a central portion of 12q (circle) in the region containing the known oncogene *MDM2* (A). Recurrent alterations in these tumors were predominantly in the form of losses, such as 9p deletion, which in one case was homozygous and included the *CDKN2A* gene region (circle), with most of the remaining 9p arm showing LOH (B).

two or more tumors) included 1q (n = 3), 3q (n = 3), 4q (n = 2), distal 6p (n = 2), 9p (n = 3), distal 11p (n = 3), 13q (n = 2), 17p (n = 3), 17q (n = 2), whole 18 (n = 2) and 19q (n = 4). Recurrent gains were rare and involved the whole Ch 7 (n = 2) and 12q (n = 2, whole chromosome in one and central/proximal region in one, the latter including the *MDM2* oncogene but probably not *CDK4*)(Figure 2A). One low-grade oligodendroglioma had a unique karyotype characterized by a combination of whole chromosome gains (triploidy) of 6, 7, 8, 11 and 12, with no other abnormalities. This case also lacked BRAF alterations (*BRAF : KIAA1549* and *BRAF* p.V600E). Some of the alterations appeared to involve a subpopulation rather than the whole tumor. Homozygous deletion, frequent in diffuse gliomas, was identified in one low-grade oligodendroglioma involving 9p.21.3 (Figure 2B). Genes in this region include *CDKN2A*, *CDKN2B*, *CDKN2BAS*, *C9orf53* and *MTAP*.

1p19q co-deletion

The classic whole-arm co-deletions of 1p and 19q were present in three cases, patients aged 11, 16 and 19 years (Figure 3A,B). Two of these cases were classified as low grade, and the only other defects were possible germ line alterations on 2q and 11q in one of the two. The third case was diagnosed as anaplastic and demonstrated numerous other abnormalities similar to those previously reported as common in anaplastic ODG, including hemizygosity of 9p and LOH on 14q and 15q (28), as well as gain of X. Two other cases with noteworthy alterations included one low-grade oligodendroglioma with whole-arm deletion of 1p but intact 19q, and one anaplastic oligodendroglioma with complex cytogenetic changes on 1p characterized by deletion of most of the arm, and gain of the portion from 1p36.23 to 1p36.11 along with complex changes in 19q (Figure 3C,D).

Increased number of cytogenetic alterations by grade

Next, we stratified somatic cytogenetic alterations by grade. These were present in 8 of 15 (53%) grade II oligodendrogliomas and all five anaplastic oligodendrogliomas studied. Common losses, other than 1p19q co-deletion, were absent in low-grade pediatric oligodendrogliomas. Gain in chromosome 7 was present in two cases as the only recurrent abnormality in this group.

Among the anaplastic cases (n = 5), the number of somatic alterations was higher (mean 11.6, range 3–19), compared with grade II oligodendrogliomas (mean 1.4, range 0–5), a difference that was statistically significant ($P = 0.002$, Wilcoxon rank-sum; Figure 4). 1p19q co-deletion was present in two cases, and isolated 1p deletion in one. Alterations in anaplastic tumors are outlined in Table 1 by case, but encompassed all common losses other than 1p and 19q, including 3q29 (n = 3), 4q (n = 2), 6p (n = 2), 11p(n = 3), 9p (n = 2), 13q (n = 2), 14q (n = 2), 17p(n = 2), 17q (n = 3) and chromosome 18 loss (n = 2).

Possible germ line cytogenetic alterations

Possible germ line alterations were present in seven cases (35%) (Table 1). One focal possible germ line LOH (or uniparental disomy) on 22q13.1-13.2 overlapped in two tumors (Figure 5), one anaplastic oligodendroglioma with whole arm 1p19q co-deletion

in an 11-year-old girl and the second in a low-grade oligodendroglioma lacking 1p19q alterations in a 15-year-old boy. This region of interest included several genes: *XRCC6*, *RANGAP1*, *SGSM3*, *MKL1*, *ST13*, *DNAJB7*, *TEF*, *TOB2*, *NHP2L1*, *SREBF2*, *CENPM*, *TNFRSF13C*, *CYP2D6*, *TCF20*, *CYP2D7P1*, *RBX1*, *MIR4766*, *MIR1281* and *MCHR1*.

Pediatric oligodendrogliomas usually lack BRAF alterations

Fluorescence *in situ* hybridization studies were negative for *BRAF-KIAA1549* fusion in all cases tested (n = 13). *BRAF* p.V600E mutation tested by immunohistochemistry (n = 6) or sequencing (n = 2) was also absent.

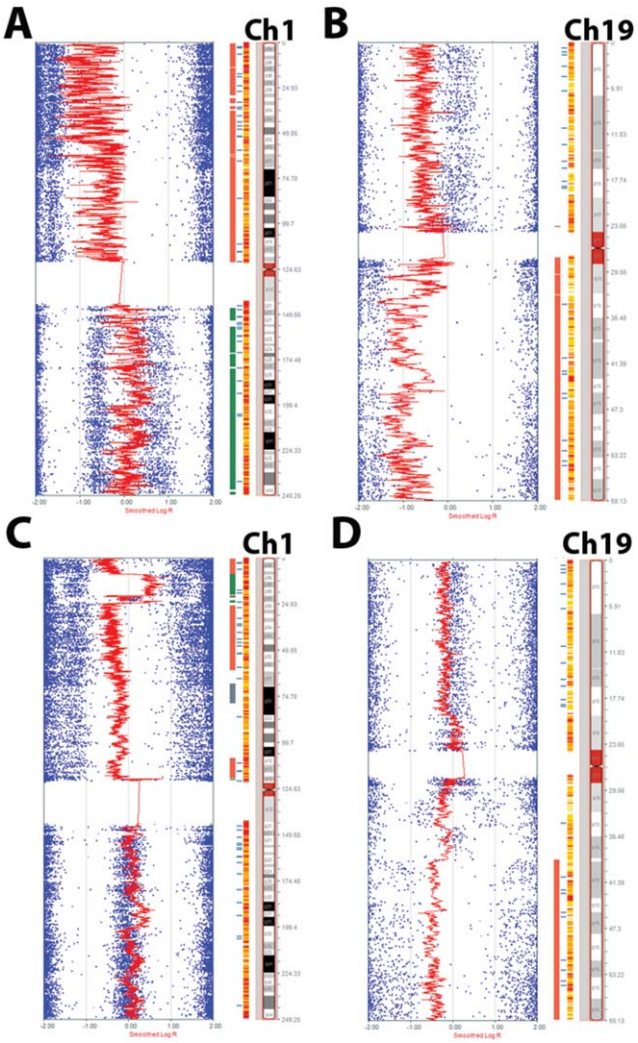

Figure 3. 1p19q co-deletion occurs in a small subset of pediatric oligodendrogliomas. Classic whole arm co-deletion of 1p19q occurred in three cases (A, B). One unique case had a noteworthy complex abnormality of 1p characterized by a large area of loss and a more focal area of gain near the telomere end (C), as well as LOH in areas of 19q (D).

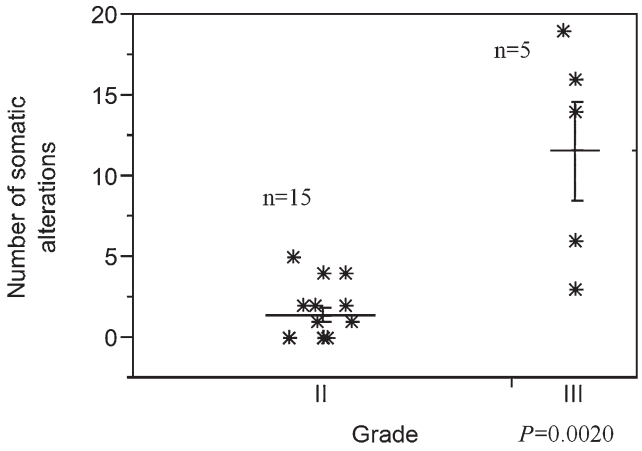

Figure 4. Anaplasia in pediatric oligodendrogliomas is associated with an increased number of cytogenetic abnormalities. The mean number of cytogenetic abnormalities was higher in anaplastic pediatric oligodendrogliomas compared with low-grade (II) tumors (Wilcoxon rank-sum test).

DISCUSSION

Pediatric oligodendroglioma is emerging as a neoplasm with distinct biology from its adult counterpart. Numerous studies have demonstrated a lack of 1p19q co-deletion in most pediatric oligodendrogliomas (22, 31, 35), and more recently, a lack of IDH1 (R132H) mutations (35, 39). In the largest clinicopathologic series to date focusing on pediatric oligodendrogliomas and critically excluding morphologic mimics as well as tumors with ambiguous histology, 1p19q co-deletion was present in 10 (25%), and isolated 1p loss in one (2%), while the majority lacked these alterations (35).

Array-based methods such as SNP arrays and array CGH are powerful techniques, with excellent performance in formalin-fixed paraffin-embedded tissues (12, 26), that are increasingly finding clinical applications in the study of diffuse gliomas. We searched for global cytogenetic alterations in pediatric oligodendroglioma, which emerged as molecularly distinct from adult oligodendroglioma. Our current study of pediatric tumors using SNP array profiling in formalin-fixed paraffin-embedded tissue showed molecular cytogenetic alterations in 8 of 15 (53%) grade II and all 5 (100%) grade III tumors. In contrast, a prior study of 35 adult oligodendroglial tumors by Kitange *et al* using array comparative genomic hybridization in frozen tissue demonstrated genetic alterations in 18 of 20 (90%) grade II and 9 of 9 (100%) grade III oligodendrogliomas (18). Recurrent alterations present in this and other CGH studies included losses involving 4q, 9p, 11p and 13q (5, 21, 23, 37, 40).

Of interest, almost half of low-grade oligodendrogliomas in our group lacked gross somatic cytogenetic alterations (47%), a much higher frequency compared with adult oligodendrogliomas. This suggests that smaller genetic alterations are the genetic drivers in a significant subset of these tumors, as has been reported in other low-grade pediatric gliomas. For example, in a recent whole-genome sequencing study, Zhang *et al* found *FGFR1* tyrosine

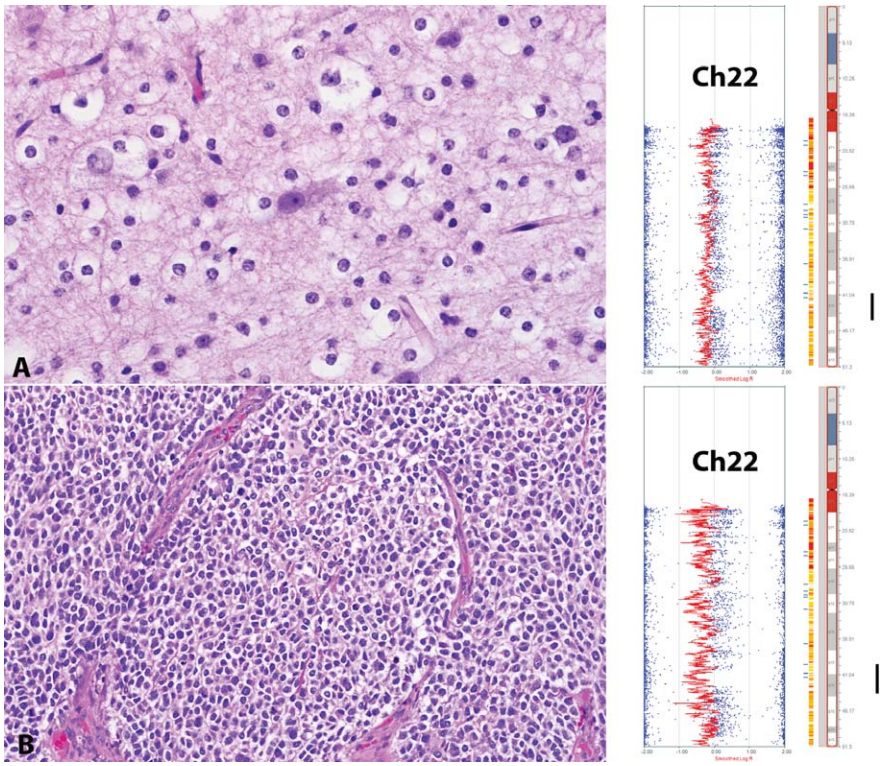

Figure 5. Possible germ line alterations in pediatric oligodendrogliomas. Possible germ line alterations (or uniparental disomy) were present in seven cases. However, in two unrelated cases (A and B), the germ line alteration involved a focal, overlapping region in 22q. This raises the possibility of a pediatric oligodendroglioma-predisposing gene (s) in this region.

kinase duplications in three, combined 1p19q co-deletion, *CIC* and *IDH1* mutation in one, and a *MYB-MAML* fusion in one of their five pediatric oligodendrogliomas (44). Conversely, all anaplastic tumors in our cohort had identifiable cytogenetic alterations, consistent with a higher degree of chromosome instability in these tumors.

In our cohort, 9p loss was present in three cases (two anaplastic and one low grade). The low-grade oligodendroglioma showed a homozygous *CDKN2A* deletion. In a recent study, *CDKN2A* deletions were frequent in a subset of pediatric low-grade gliomas that progressed to high grade over time and this appeared to be an early event (25). Unfortunately, this patient was lost to follow-up and it is therefore unclear if malignant progression developed over time. RB1 pathway alterations, including *RB1* and *CDKN2A*, have been associated with worse outcome in adult low-grade diffuse gliomas (33), and appear to be frequent in those tumors lacking more common diffuse glioma alterations (e.g. 1p19q co-deletion, *IDH1/2* and *TP53* mutations) (19).

Another recent study reported a high frequency of 1q gain and 6q loss in diffuse high-grade astrocytomas of infancy (9), alterations that were in general absent in our group. Loss of 10q is a frequent event in adult diffuse gliomas, may be associated with worse prognosis in oligodendrogliomas (14), and occurs in a subset of pediatric gliomas (27). This alteration was absent in our cases, except for a small distal 10q26.3qtel deletion in one case, not involving *DMBT1*, *PTEN* or *MGMT*, relevant genes in adult gliomas. These findings suggest that pediatric oligodendrogliomas have a distinct genetic cytogenetic profile from other adult and pediatric gliomas.

Chromosomal gains were infrequent in the current cohort, although small gains involving putative oncogenes may be potentially missed given the increased noise involved in karyotyping FFPE tissue. Other studies of low-grade adult oligodendrogliomas have also found a predominance of deletions, with common gains in the study by Rossi *et al* involving mostly chromosome 7 (37). Whole chromosome 7 gains were present in two cases in our study, a relatively frequent alteration in a variety of pediatric gliomas, including pilocytic astrocytomas (3). Chromosome 12 gains were present in two cases, which has also been previously reported in adult gliomas, gains including the known oncogene *MDM2* (32). Additionally, 8q gains, previously associated with a subset of diffuse astrocytomas and oligodendroglial tumors with worse outcome (10, 20), were absent.

In addition, we identified possible germ line LOH (or uniparental disomy) in approximately one-third of this group, which appears to be higher than in adult cases using the same platform (28), and raises the possibility that these contain pediatric oligodendroglioma-predisposition loci. In adult patients, specific SNPs in 8q24.21 have been associated with an increased risk of *IDH1* or *2* mutant glioma development, including oligodendroglioma, in genotyping studies (17). Interestingly, in our cohort, two patients had a focal overlapping area of possible germ line alteration at 22q13.1-13.2, which included a number of interesting genes, involved in cell biology, including DNA repair (*XRCC6*), nuclear transport and other nuclear proteins (*RANGAP1*, *NHP2L1*, *CENPM*), cell signaling (*SGSM3*, *MKL1*, *TOB2*, *TNFRSF13C*, *RBX1*, *MCHR1*), transcription (*TEF*, *SREBF2*, *TCF20*), molecular chaperones associating with heat shock proteins (*ST13*, *DNAJB7*), cytochrome P450 (*CYP2D6*, *CYP2D7P1*) and microRNAs (*MiR-4766*, *MiR-1281*). *ST13* is also a candidate tumor suppressor in colorectal carcinoma (2). LOH in these and nearby 22q regions are not uncommon in glial tumors

(13). *CYP2D6* abnormalities/polymorphisms been associated with the development of glial tumors, including oligodendrogliomas (8, 18). However, these preliminary observations using only neoplastic tissue should be validated in future studies using matched non-neoplastic tissues.

The main differential diagnosis with pediatric oligodendroglioma is dysembryoplastic neuroepithelial tumor (DNT), a tumor that may be difficult if not impossible to distinguish from, particularly in small biopsies. Recurrent genetic alterations have not been described in these tumors, other than *BRAF* p.V600E in a subset (7). Interestingly, in the whole-genome sequencing study of Zhang *et al*, the single DNT studied had an *FGFR1* tyrosine kinase duplication, as did 3 of 5 pediatric oligodendrogliomas, suggesting that these two lesions may be difficult to distinguish even at the molecular level (44). However, no alterations were present by array CGH in a recent study of six DNT and grade II pediatric gliomas (29).

A primarily low-grade, pediatric neoplasm with oligodendroglioma-like cytology but extensive and disproportionate superficial parenchymal and leptomeningeal involvement has been studied in several series and case reports. These disseminated oligodendroglioma-like leptomeningeal neoplasms (DOLN) have been recently found to be characterized by a high frequency of combined 1p deletion and *BRAF : KIAA1549* fusion (36), a molecular signature that separates them both from adult and pediatric oligodendrogliomas. Our current data reinforce this molecular distinction in the pediatric setting, as isolated 1p loss was relatively rare and *BRAF : KIAA1549* fusion was absent in all cases tested (n = 13). It must be noted, however, that rare case reports have also described *BRAF : KIAA1549* fusions in pediatric oligodendroglioma (24).

In summary, we report global molecular cytogenetic alterations in pediatric oligodendrogliomas present in approximately half of low-grade tumors but in all anaplastic oligodendrogliomas studied. A subset of these tumors is characterized by predominantly genomic losses. Deletion of 1p, usually combined with 19q deletion, is relatively rare in the group overall [5 of 20 (25%)], but represented the most common identifiable abnormality, with the caveat that these alterations are relatively rare compared with their adult counterparts and other pediatric low-grade gliomas. BRAF alterations do not appear to play a prominent role in pediatric oligodendroglioma, and lack of *BRAF* fusions or BRAF p.V600E separate these tumors from several other pediatric low-grade gliomas.

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