

# Supporting Information

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## Histopathologic characteristics of non-categorical diffuse PLGGs

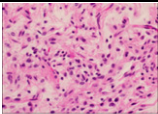
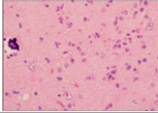
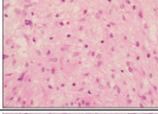
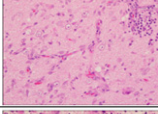
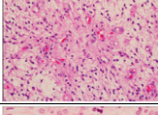
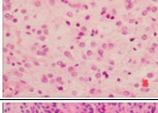
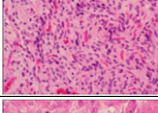
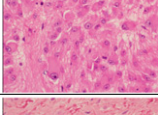
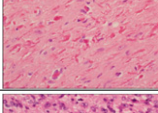

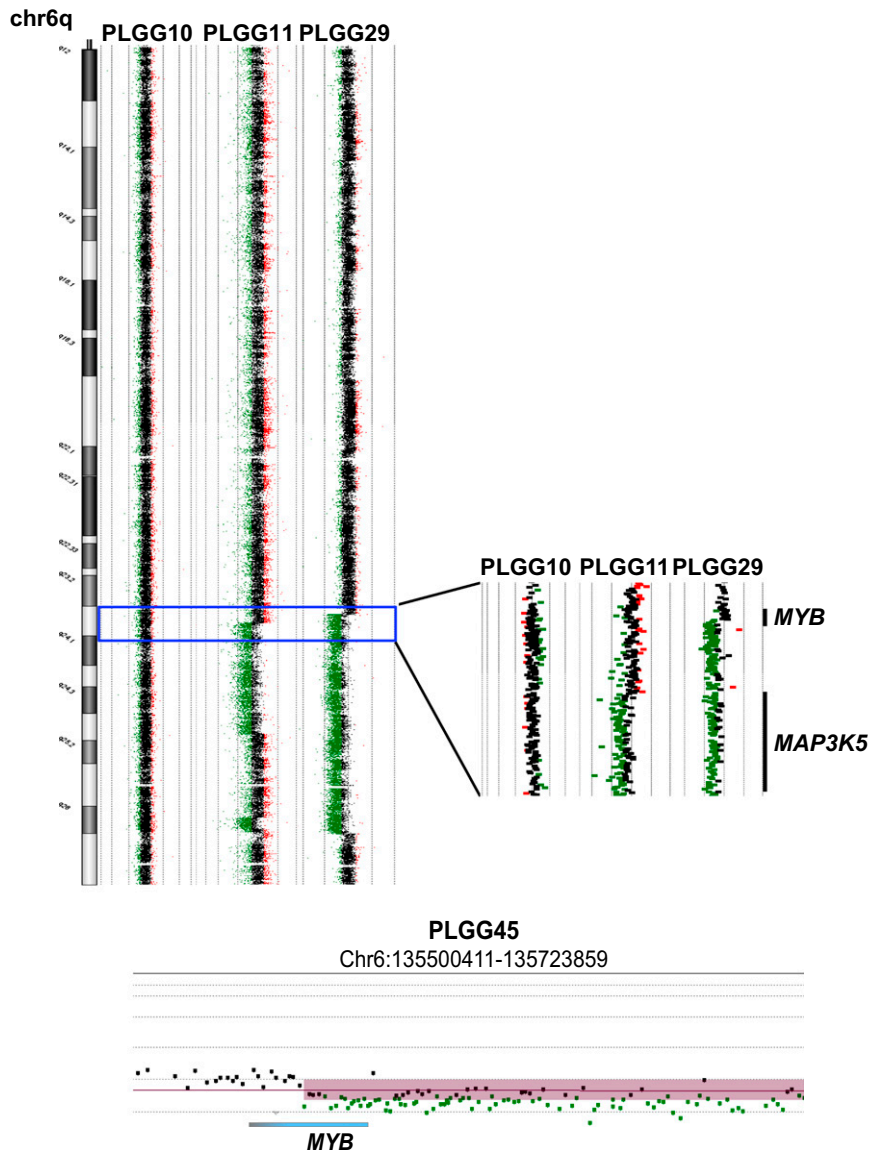
PLGG ID	Dx	Histologic Characteristics	Representative H&E
20	NOS	Low grade glial neoplasm with round and spindle cell morphologies. Areas of low cellularity associated with microcystic changes and areas of increased cellularity with fascicular growth. No evidence of RFs or EGBs.	
21	NOS	Infiltrating glioma cells involving reactive cortex with neuronal dysplasia and scattered microcalcifications. No evidence of RFs, EGBs or mitoses.	
22	NOS	Moderately dense glial neoplasm with mild nuclear pleomorphism and frequent cells with foamy/xanthomatous cytoplasm. No evidence of RFs, EGBs or mitoses.	
23	NOS	Paucicellular infiltrating glial neoplasm involving reactive cortex with prominent perivascular spread along Virchow-Robin spaces and focal perivascular inflammation. No evidence of RFs, EGBs, neuronal dysplasia or mitoses.	
32	NOS	Moderately cellular glioma with round-to-ovoid cells, predominantly loose growth pattern and abnormal blood vessels. No evidence of RFs, EGBs, neuronal dysplasia or mitoses.	
33	NOS	Moderately cellular glioma with 'round cell' morphology and mild nuclear atypia in a background showing prominent microcystic change and scattered EGBs. No evidence of RFs or mitoses.	
34	NOS	Ovoid-to-spindle cell glioma with areas of dense cellularity, frequent calcifications and perivascular spread. No evidence of RFs, EGBs, or mitoses.	
42	NOS	Large polygonal glioma cells with abundant amounts of eosinophilic cytoplasm and eccentric nuclei with prominent nucleoli. No evidence of RFs, EGBs, or mitoses.	
43	NOS	Mild-to-moderately cellular glioma composed of spindle-to-elongated cells with moderate nuclear atypia. Abundant RFs but no EGBs, mitoses, vascular proliferation, or necrosis.	
44	NOS	Moderately cellular glioma with infiltrating and solid components associated with RFs and ganglioid-like cells. No evidence of EGBs, mitoses and anaplastic features.	

Fig. S1. Representative histology of not otherwise specified (NOS) (noncategorical) tumors. RFs, Rosenthal fibers; EGBs, eosinophilic granular bodies.





**Fig. S3.** Array comparative genomic hybridization data for three angiogenic gliomas demonstrating focal 6q23.3 deletions in 2/3 samples. The centromeric breakpoint in PLGG29 truncates *MYB* after exon 10. A fourth angiogenic glioma, diagnosed and obtained after assembly of the PLGG cohort, shares the deletion breakpoint within *MYB*.

**Table S1. Demographic, histologic, and clinical features with selected copy-number alterations (CNAs) in an international cohort of 45 diffuse pediatric low-grade gliomas**

PLGG ID	Sex	Age at diagnosis, y	Research diagnosis	Location	BRAF mutation status	Arm-level CNAs	Selected focal CNAs detected by aCGH		
							Gene	Cytoband	Position
1	M	9.0	DA2	Forebrain	WT	—	—	—	—
2	M	4.4	DA2	Forebrain	ND	—	—	—	—
3	M	2.2	DA2	Forebrain	ND	—	—	(+) 8q24.21	Chr8:130858957–131122982
4	M	2.0	DA2	Forebrain	WT	—	—	(-) 5q11.2	Chr5:58737992–58770177
5	F	1.4	DA2	Forebrain	V600E	—	—	—	—
6	M	5.6	DA2	Forebrain	V600E	—	—	—	—
7	F	13.2	DA2	Cerebellum	WT	—	—	—	—
8	F	6.1	DA2	Cerebellum	ND	—	—	—	—
9	M	20.5	DA2	Forebrain	WT	—	—	—	—
10	F	1.8	AG	Forebrain	V600E	—	—	—	—
11	M	4.0	AG	Forebrain	ND	—	—	(-) 6q23.3-q25.1	Chr6:136910180–151323142
								(-) 6q26	Chr6:162118724–163980938
12	F	8.5	SEGA	Forebrain	ND	—	—	(-) chr7q36.2	Chr7:154006525–154014651
								(+) chr11q14.1	Chr11:83922064–84539665
13	M	0.6	DIGG	Forebrain	ND	—	—	—	—
14	F	0.3	DIGG	Forebrain	ND	—	—	(-) chr6p25.1	Chr6:5203314–5217108
								(-) chr7q21.11	Chr7:80032136–80211482
15	M	0.9	DIGG	Forebrain	ND	—	—	(+) chr17q12	Chr17:34113512–34123428
16	M	6.0	GG	Forebrain	ND	—	—	(-) chr1p33	Chr1:49599467–49607560
17	M	3.0	GG	Thalamus	V600E	—	—	—	—
18	M	12.6	GG	Forebrain	WT	—	—	—	—
19	M	12.0	GG	Forebrain	ND	—	—	(+) chr5p13.1	Chr5:39848680–39919235
20	F	10.2	NOS	Cerebellum	V600E	—	—	(-) chr10q21.3	Chr10:68606529–68669439
								(-) chr13q33.1	Chr13:101729178–101739386
21	M	10.6	NOS	Forebrain	V600E	—	—	(+) chr3q26.2	Chr3:167758620–167767100
22	F	10.2	NOS	Brainstem	V600E	—	—	(-) chr7q21.12	Chr7:86939984–86965358
								(-) chr10q21.3	Chr10:68606529–68669439
								(-) chr13q33.1	Chr13:101729178–101739386
23	M	10.2	NOS	Forebrain	V600E	—	—	—	—
24	F	1.0	DA2	Forebrain	WT	—	MYBL1-Tr gain	(+) 8q13.1-q21.3	Chr8:67492176–89029825
25	F	8.8	DA2	Forebrain	WT	—	MYBL1-Tr gain	(+) 8q13.1-q21.3	Chr8:67492176–89262806
26	F	3.1	DA2	Forebrain	ND	—	MYBL1-Tr gain	(+) 8q11.23	Chr8:53404754–53527412
								(+) 8q13.1-q24.23	Chr8:67492176–136561025
27	M	11.6	DA2	Forebrain	ND	Chr8 chromo-thripsis	MYBL1-Tr gain	200+ gains and losses	Multiple
								(-) 12q23.2	Chr12:102175538–102213968
								(+) 12q24.31	Chr12:125393030–125418249
28	M	3.6	DA2	Forebrain	ND	—	MYBL1-Tr gain	(+) 8q13.1-q21.3	Chr8:67492176–89241741
								(-) 13q31.3	Chr13:92029350–94958981
29	F	6.0	AG	Forebrain	WT	—	MYB-Tr loss	(-) 6q23.3-q26	Chr6:135520027–163974512
30	F	5.7	GG	Cerebellum	ND	—	BRAF duplication	(-) 7q11.21	Chr7:65889335–66085074
								(+) 7q34	Chr7:138526050–140495111
								(-) 14q32.32	Chr14:103858286–103867644
31	F	8.0	GG	Third ventricle	ND	+7, +8	BRAF duplication	(+) 1q32.3	Chr1:212428215–212734560
								(-) 6p25.3	Chr6:2058927–2259041
								(-) 6p21.1	Chr6:42878838–42891682
								(+) 7q34	Chr7:138550998–140492722
								(+) 9q31.3	Chr9:111641238–112081173
								(+) 7q34	Chr7:138541076–140490068
32	M	2.2	NOS	Cerebellum	WT	—	BRAF duplication	(+) 7q34	Chr7:138541076–140490068
33	M	0.9	NOS	Forebrain	WT	—	BRAF duplication	(+) 7q34	Chr7:138556120–140482234
								(-) 12q12	Chr12:44508075–44520159
34	F	15.8	NOS	Forebrain	V600E	—	CDKN2A/B homozygous deletion	(-) 9p21.3-p21.1	Chr9:20261670–30619011

**Table S1. Cont.**

PLGG ID	Sex	Age at diagnosis, y	Research diagnosis	Location	BRAF mutation status	Arm-level CNAs	Selected focal CNAs detected by aCGH		
							Gene	Cytoband	Position
35	F	8.3	DA2	Forebrain	V600E	-13q	CDKN2A/B homozygous deletion	(-/-) 9p21.3 (-) 9p24.3-p21.3	Chr9:21501717-22652621 Chr9:204104-23750707
36	M	12.0	DA2	Forebrain	WT	-9p	—	( <sup>-/-</sup> ) 9p21.3 ( <sup>-/-</sup> ) 9p21.3 (-) 6q24.1-q27 (-) 13q13.3-q32.2 (-) Xp22.22-p11.22 (-) Xq23-q28	Chr9:20812628-21555412 Chr9:21814614-22028332 Chr6:142214794-170178588 Chr13:38135395-98869461 ChrX:2703845-52961273 ChrX:115733379-152689497
37	F	18.6	DA2	Forebrain	ND	-13q	—	(-) 6q16.1-q27 (+) 10p15.3-p11.22	Chr6: 94458948-170160022 Chr10:136361-32316162
38	M	13.0	DA2	Forebrain	V600E	+5, +7, +8, +12, +13q, +15q, +16, +18, +20, +21q, +X, +Y	—	—	—
39	M	2.4	GG	Third ventricle	ND	+7	—	(+) chr4q32.3-q33	Chr4:170098694-170316797
40	F	13.0	GG	Forebrain	V600E	-4, -8, -14, -18, -19, -22, +7, +15	—	(+) chr1p31.1	Chr1:74649280-74661977
41	F	13.0	GG	Forebrain	V600E	-1p, -16q	—	(+) chr10q11.21 (+) chr10p15.3	Chr10:45448181-45503379 Chr10:209746-776607
42	F	14.2	NOS	Forebrain	WT	-2, +7p	—	(+) 7q11.21-q22.2 (+) 7q31.32-q36.3	Chr7:62453156-104540185 Chr7:121516510-158545562
43	F	22.5	NOS	Forebrain	ND	+5, +6, +7, +8, +18	—	—	—
44	M	20.1	NOS	Brainstem	ND	-11p, -17q	—	—	—
45*	F	3.3	AG	Forebrain	ND	—	MYB-Tr loss	(-) 6q23.3-q26	Chr6: 135561520-163886616

Position relative to hg19/b37. aCGH, array comparative genomic hybridization; AG, angiocentric glioma; DA2, diffuse astrocytoma grade II; DIGG, desmoplastic infantile ganglioglioma; GG, ganglioglioma; ND, not determined; NOS, not otherwise specified (noncategorical histology); PLGG, pediatric low-grade glioma; SEGA, subependymal giant-cell astrocytoma.

\*PLGG45 was not included in the initial diffuse PLGG cohort and GISTIC analysis.

**Table S2. All GISTIC focal peaks with significance of  $q < 0.1$  and associated genes**

Rank	Cytoband	Peak boundaries	GISTIC $q$ -value*	No. of genes <sup>†</sup>	Candidate gene(s)	Other gene(s)	PLGG samples with focal or arm-level amplification including peak
<b>Amplifications</b>							
1	8q13.1	Chr8:67487369–67539268	3.37E-06	1	<i>MYBL1</i>	—	24, 25, 26, 27, 28, 31, 38, 43
2 <sup>‡</sup>	7q34	Chr7:138550999–140485435	1.85E-05	18	<i>BRAF</i>	—	14, 30, 31, 32, 33, 38, 39, 49, 42, 43
2 <sup>‡</sup>	5q14.3	Chr5:87950142–87993154	1.85E-05	1	<i>mir-9-2</i>	—	09, 11, 17, 28, 38, 43
2 <sup>‡</sup>	1q42.3	Chr1:234730190–234751251	1.85E-05	1	<i>IRF2BP2</i>	—	11, 17, 40
2 <sup>‡</sup>	22q13.31	Chr22:46449352–46480549	1.85E-05	1	—	<i>C22orf26</i>	11, 17, 27, 28, 29, 30, 40
6	10p12.31	Chr10:21798200–21826851	2.43E-04	2	—	<i>MLLT10, SKIDA1</i>	11, 13, 17, 37, 43
7	2q12.1	Chr2:105450734–105485255	3.45E-04	1	<i>POU3F3</i>	—	09, 40
8	12q24.31	Chr12:125390806–125418189	1.07E-03	1	—	<i>UBC</i>	11, 13, 17, 38, 40
9 <sup>‡</sup>	14q24.3	Chr14:77491586–77518449	0.022863	1	—	<i>C14orf4</i>	17, 29, 40
9 <sup>‡</sup>	19q13.2	Chr19:39883053–39911846	0.022863	2	—	<i>PLEKHG2, ZFP36</i>	17, 40
11	3q26.33	Chr3:181408749–181464146	0.027842	1	<i>SOX2</i>	—	11, 28
12	15q26.1	Chr15:93340550–93467966	0.035057	1	—	<i>CHD2</i>	11, 17, 30, 38
13	7q21.12	Chr7:53184923–104541440	0.042776	257	—	<i>EGFR</i>	29, 31, 38, 39, 40, 42, 43
14	17p13.1	Chr17:8037293–8083879	0.057162	3	—	<i>PER1, VAMP2, TMEM107</i>	09, 17, 28
15 <sup>‡</sup>	7p22.1	Chr7:1–39456778	0.078767	185	—	—	29, 31, 38, 39, 40, 42, 43
15 <sup>‡</sup>	8p23.1	Chr8:12603561–12633539	0.078767	0	—	—	17, 27, 31, 38, 43
17	1q21.3	Chr1:150517902–150557247	0.09669	2	<i>MCL1</i>	<i>ADAMTSL4</i>	17, 28
<b>Deletions</b>							
1	9p21.3	Chr9:20808130–21558353	0.026273	21	—	—	34, 35, 36
2 <sup>‡</sup>	9p21.3	Chr9:21809877–22031472	0.026562	4	<i>CDKN2A/B</i>	<i>MTAP, C9orf53</i>	34, 35, 36
2 <sup>‡</sup>	10q21.3	Chr10:68600931–68672368	0.026562	1	—	<i>CTNNA3</i>	20, 22
2 <sup>‡</sup>	13q31.3	Chr13:73280263–98874025	0.026562	48	—	—	28, 35, 36, 37
5 <sup>‡</sup>	6q26	Chr6:107439997–171115067	0.096062	252	<i>MYB</i>	<i>QKI</i>	11, 29, 36, 37
5 <sup>‡</sup>	8p22	Chr8:14238458–14262853	0.096062	1	—	<i>SGCZ</i>	11, 27

*MYBL1*, v-myb myeloblastosis viral oncogene homolog (avian)-like 1; *BRAF*, v-raf murine sarcoma viral oncogene homolog B1; *mir-9-2*, microRNA 9-2; *IRF2BP2*, interferon regulatory factor 2 binding protein 2; *C22orf26*, chromosome 22 open reading frame 26; *MLLT10*, myeloid/lymphoid or mixed-lineage leukemia; *SKIDA1*, SKI/DACH domain containing 1; *POU3F3*, POU class 3 homeobox 3; *UBC*, ubiquitin C; *C14orf4* (*IRF2BPL*), interferon regulatory factor 2 binding protein-like; *PLEKHG2i*, pleckstrin homology domain containing, family G member 2; *ZFP36*, ZFP36 ring finger protein; *SOX2*, sex determining region Y-box2; *CHD2*, chromodomain helicase DNA binding protein 2; *EGFR*, epidermal growth factor receptor; *PER1*, period circadian clock 1; *VAMP2*, vesicle-associated membrane protein 2; *TMEM107*, transmembrane protein 107; *MCL1*, myeloid cell leukemia sequence 1; *ADAMTSL4*, ADAMTS-like 4; *CDKN2A/B*, cyclin-dependent kinase inhibitor 2A/2B; *MTAP*, methylthio-adenosine phosphorylase; *C9orf53*, chromosome 9 open reading frame 53; *CTNNA3*, catenin (cadherin-associated protein), alpha3; *MYB*, v-myb myeloblastosis viral oncogene homolog (avian); *QKI*, KH domain containing, RNA binding; *SGCZ*, zeta sarcoglycan.

\*Residual  $q$ -value representing significance independent of neighboring peaks. Note that this may differ from the overall  $q$ -values reported for individual genes or sites within a peak.

<sup>†</sup>Genes in peaks are determined with 95% confidence interval. Only RefSeq-validated and -reviewed genes encoding proteins or microRNAs are considered (not provisional, predicted, inferred genes, pseudogenes, or long intergenic noncoding RNA).

<sup>‡</sup>Tied in rank (same  $q$ -value).

**Table S3. Sequencing quality control metrics in PLGG24 tumor and normal samples**

Category	Territory, bp	Sequenced bp (tumor)	Sequenced bp (normal)	Average tumor depth	Average normal depth	Callable*, bp	Callable*, %
IGR	1929696481	179814707141	173862259054	93.2	90.1	1626356779	84.3
Intron	883403538	90675703323	89125720821	102.6	100.9	866518615	98.1
UTR	234744341	22923299883	22846219918	97.7	97.3	223967563	95.4
Exon	32575120	2835292031	3031336888	87.0	93.1	30040020	92.2
Total	3080419480	296249002378	288865536681	96.2	93.8	2746882977	89.2

\*Bases are considered "callable" if  $\geq 14x$  coverage is present in the tumor and  $\geq 8x$  coverage is present in the normal.

**Table S4. All nonsynonymous and synonymous coding region mutations and insertions and deletions in the MYBL1 duplicated-truncated DA2 tumor PLGG24 as determined by 90x whole-genome sequencing**

Gene	Mutation type	AA change	Allelic fraction	Previously reported in gliomas	No. of mutations reported in COSMIC
<i>CRIPAK</i>	Missense	p.W287R	0.214	No	0
<i>RPA2</i>	Missense	p.S11R	0.182	No	1
<i>CEP290</i>	Splice site	p.K314_splice	0.179	No	7
<i>ATAD38</i>	Silent	p.D313D	0.150	—	—
<i>DHX40P1</i>	Silent	p.Q162Q	0.100	—	—
<i>FOXD4L1</i>	Silent	p.I329I	0.160	—	—

COSMIC, Catalogue of Somatic Mutations in Cancer.