Rare BRAF and non-BRAF fusion variants characterize spinal low-grade gliomas in children

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Gliomas are most common CNS tumors in children and adolescents, however intramedullary spinal cord low-grade gliomas (sLGGs) are rare with scarce information about molecular background. Therefore, clinical and genetic single institutional study was performed to reveal sLGG-associated molecular alterations.

Patients characteristics	N= 24
Age at diagnosis	
Mean (interval)	6,7 y (1,1–17,5 y)
Sex	
Male	14 (58 %)
Female	10 (42 %)
Progression after initial surgery	9 (38 %)
Treatment	11 (46 %)
Chemotherapy	8
Radiotherapy	5
Brachytherapy	1
DOD	2 (8 %)

Methods. Demographic data was collected and targeted genomic approach was employed to uncover known and novel alterations associated with sLLGs. Multiplex Ligation Probe Amplification (MLPA) and RT-PCR were used to screen for *KIAA1549-BRAF* fusion and direct sequencing for point mutations (*BRAF, H3F3A, HIST1H3B, FGFR1*). Samples with no detected alteration were subjected to panel RNA-sequencing (FusionPlex Archer Diagnostics). In patients with unusual clinical course of the disease Methylation array was done.

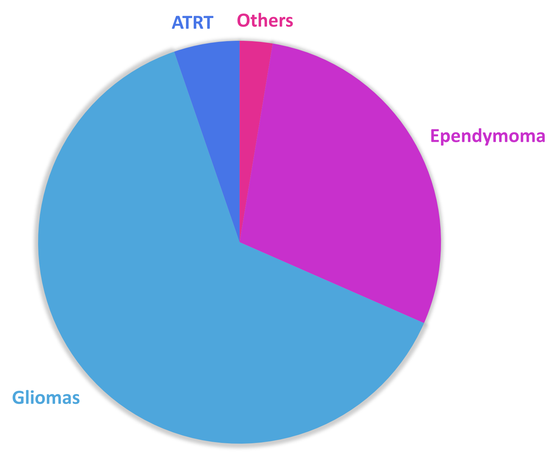


Fig. 2 Spectrum of histology in spinal tumors, excluding NF2 pts

Results. Within 2000-2019 we diagnosed 24 patients with sLGG which represented 5% of all our LGGs. RT-PCR revealed 10 tumours harbouring KIAA1549-BRAF. Interestingly, only 6 cases were identified with common 15-9 and one case with 16-9 fusion variants. Rare KIAA1549-BRAF variants were detected in 4 patients (one 16-11, one 15-11, two 13-11). Additional 5 patients harboured novel variant 10-9 which was specific for spinal gliomas with absence in any other anatomical locations (n=64). Non-BRAF fusions and BRAF fusions with novel or rare fusion partner were detected using RNA-sequencing. Surprisingly, we have not identified any BRAF V600E case in our cohort. Two patients (one with *KIAA1549-BRAF* 10-9 variant, one without known driver alteration) died of the disease.

Diagnosis according to histopathologist	No. pts	Molecular alteration	Biological-clinical diagnosis
Anaplastic astrocytoma	1	*CLIP2-NTRK2* fusion	Anaplastic pilocytic astrocytoma
Diffusion astrocytoma	9	*KIAA-BRAF* ETV6-NTRK3 fusion	LGG
Ependymoma grade 2/3	1	*KANK1-NTRK2* fusion	Pleomorphic xanthoastrocytoma
Clear cell ependymoma	1	*QKI-RAF1* fusion	Glioneuronal tumor
Gangliocytoma grade 1	3	*KIAA-BRAF* fusion	LGG
Pilocytic astrocytoma	6	*KIAA-BRAF* *BCAS1-BRAF* TMEM-*BRAF* GNAI-*BRAF* fusion	LGG
LGG NOS	1	*KIAA-BRAF* fusion	LGG

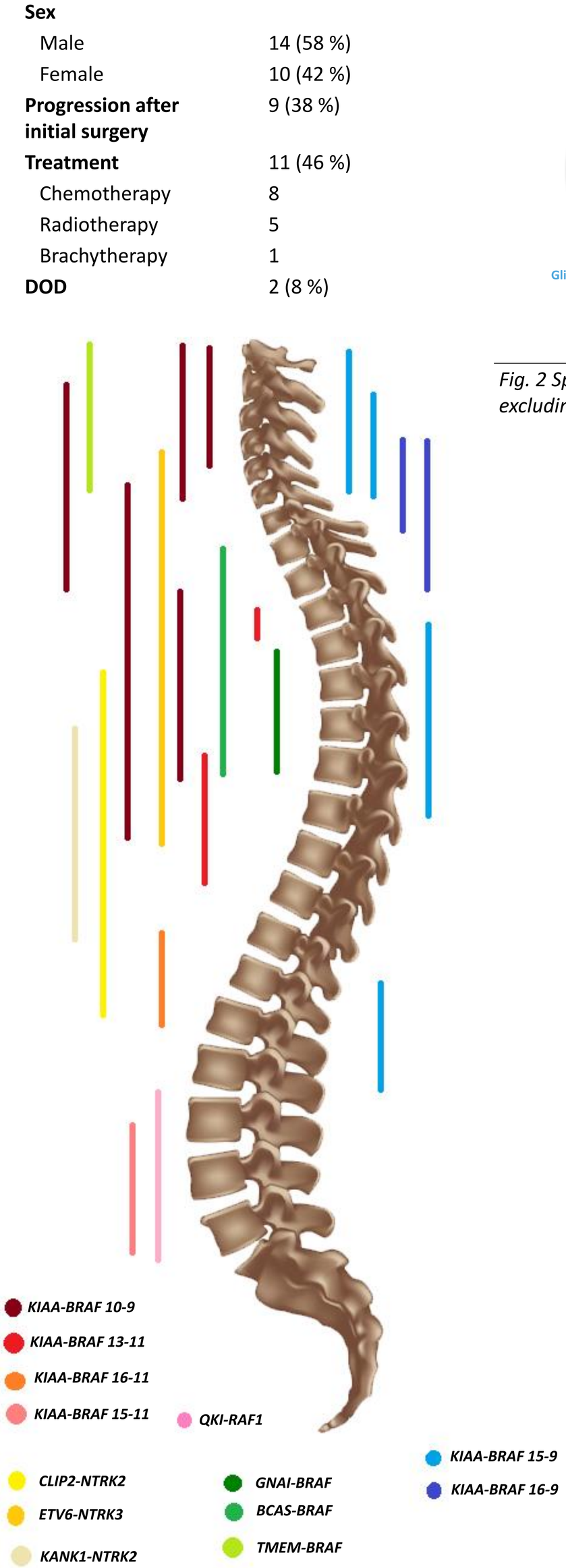


Fig. 1 Distribution of molecular alterations across spine. Majority (73 %) of sLLGs harbour novel and rare alterations (left side of the figure). Notably, generally most common fusions – KIAA-BRAF 15-9, 16-9 – occur in 23 % (right side).

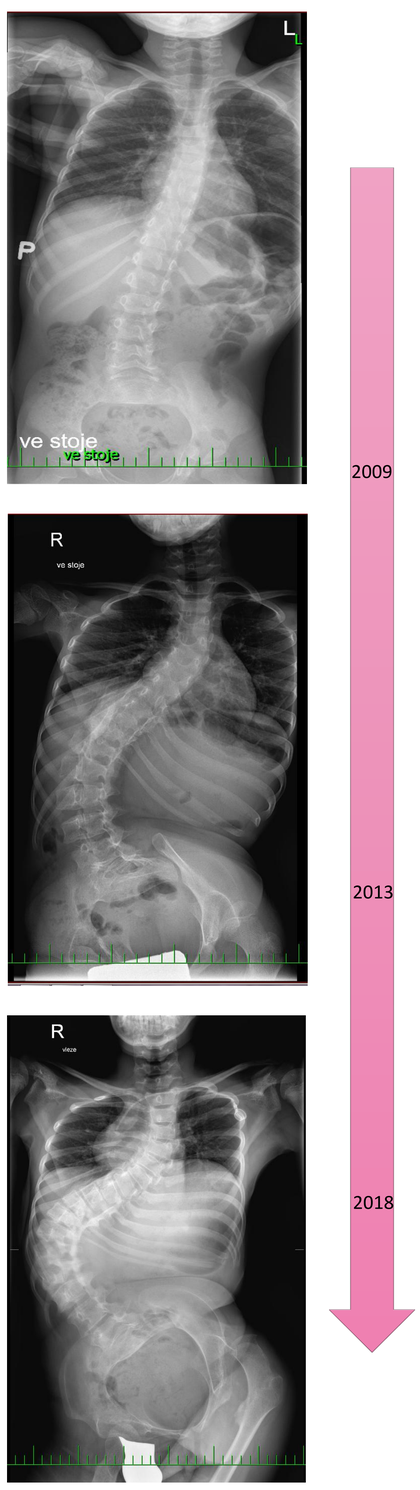
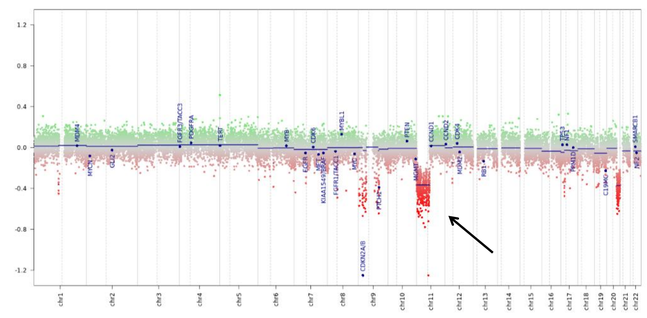
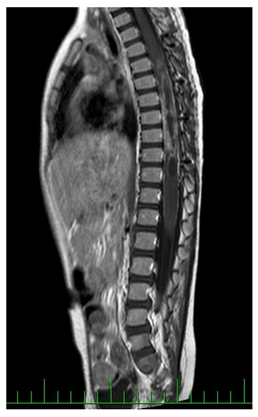


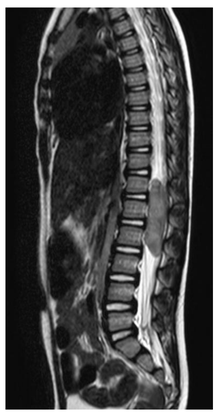
Fig. 3 Aggressive course of disease with multiple progressions in patient with CLIP2-NTRK2 fusion

Pleomorphic xanthoastrocytoma





Glioneuronal tumor



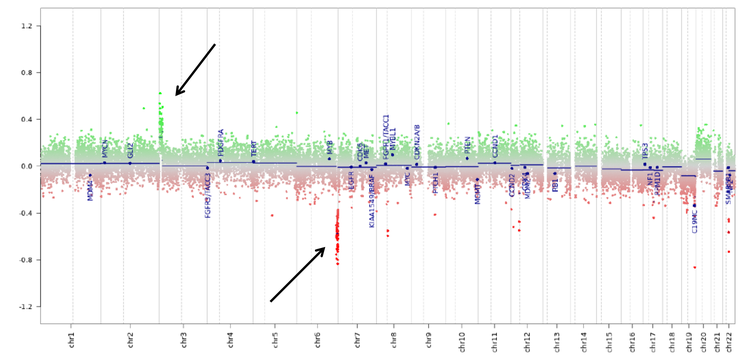


Fig. 4 Two cases of very young children initially diagnosed with spinal ependymoma. Using Methylation array and RNA seq rather glioma biology was confirmed.

This study provides important data on the molecular background of pediatric sLGGs. Rare *KIAA-BRAF* variants including novel variants are more frequent in sLGGs compared to intracranial LGGs. Our data clearly demonstrates that most patients carry drugable targets.