

RESEARCH ARTICLE

A novel *PTPRZ1-ETV1* fusion in gliomasAlenka Matjašič ; Andrej Zupan; Emanuela Boštjančič; Jože Pižem; Mara Popović ; Danijela Kolenc

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Abstract

The aggressive nature of malignant gliomas and their genetic and clinical heterogeneity present a major challenge in their diagnosis and treatment. Development of targeted therapy brought attention on detecting novel gene fusions, since they represent promising therapeutic targets (eg, TRK inhibitors in *NTRK* fusion-positive tumors). Using targeted next-generation sequencing, we prospectively analyzed 205 primary brain tumors and detected a novel *PTPRZ1-ETV1* fusion transcript in 11 of 191 (5.8%) gliomas, including nine glioblastomas, one anaplastic oligodendroglioma and one pilocytic astrocytoma. *PTPRZ1-ETV1* fusion was confirmed by RT-PCR followed by Sanger sequencing, and *in-silico* analysis predicted a potential driver role. The newly detected fusion consists of the *PTPRZ1* promoter in frame with the highly conserved DNA-binding domain of *ETV1* transcription factor. The *ETV1* and *PTPRZ1* genes are known oncogenes, involved in processes of tumor development. *ETV1* is a member of the ETS family of transcription factors, already known oncogenic drivers in Ewing sarcoma, prostate cancer and gastrointestinal stromal tumors, but not in gliomas. Its overexpression contributes to tumor growth and more aggressive tumor behavior. *PTPRZ1* is already considered to be a tumor growth promoting oncogene in gliomas. In 8%–16% of gliomas, *PTPRZ1* is fused to the *MET* oncogene, resulting in a *PTPRZ1-MET* fusion, which is associated with poorer prognosis but is also a positive predictive biomarker for treatment with kinase inhibitors. In view of the oncogenic role that the two fusion partners, *PTPRZ1* and *ETV1*, exhibit in other malignancies, *PTPRZ1-ETV1* fusion might present a novel potential therapeutic target in gliomas. Although histopathological examination of *PTPRZ1-ETV1* fusion-positive gliomas did not reveal any specific or unique pathological features, and the follow-up period was too short to assess prognostic value of the fusion, careful monitoring of patients and their response to therapy might provide additional insights into the prognostic and predictive value of this novel fusion.

INTRODUCTION

The formation of fusions between oncogenes is one of the hallmarks of cancer development. With the development and advances in high-throughput sequencing techniques, more and more gene fusions and other genomic rearrangements are being detected, helping us to increase our knowledge of tumor biology and understand the mechanisms and functions of present aberrations. Malignant gliomas, especially glioblastomas, are aggressive primary brain tumors and one of the most clinically and genetically heterogeneous group of neoplasms, presenting major challenge in their treatment. In the majority of cases, gliomas recur due to diffuse growth and incomplete tumor resection, and patient survival is poor (14, 31).

Gene fusions are frequent genomic abnormalities in gliomas, especially in glioblastomas; previous studies have shown that fusions in gliomas most frequently occur at two major specific genomic hotspots; chromosomes 7p and

12q, and chromosomes 1, 4, 6 and 19 (41, 51). Although most fusions appear to be passenger genomic changes, they are also promising treatment targets.

Chromosome arm 7p harbors one of the most characterized and common fusions in high-grade gliomas (detected in a third of glioblastoma cases)—the *EGFRvIII* mutation (deletion of exons 2-7 and fusion of exon 1 with exon 8), which results in truncated, constitutively active EGF receptors and dramatically enhances the malignancy of the glioblastoma cells (30, 48). Fusions of the *EGFR* gene with various fusion partners, such as *SEPT14* (4% of glioblastomas) or *PSHS* (2.2% of glioblastomas), are the most frequent recurrent fusions in glioblastomas (2, 16).

Other potentially targetable fusions in malignant gliomas are the *FGFR-TACC* fusions (*FGFR1* or *FGFR3*, and *TACC1* or *TACC3*, respectively) of two oncogenes at a close chromosome location. *FGFR3-TACC3* fusion protein promotes tumorigenesis and enhances tumor progression through

different signaling pathways (MAPK, ERK, PI3K and STAT3), and is more frequent in glioblastomas (in up to 8%) (29, 44).

Fusions of *NTRK* genes, encoding the tropomyosin receptor kinase (TRK) receptor family, have been reported to occur in 1.2%–1.7% of adult malignant glioma, but are more frequent (around 40%) in high-grade gliomas in the cerebral hemispheres of infants less than 3 years of age (50, 51). *NTRKs* play an important role in nervous system development, regulating cell proliferation, differentiation, apoptosis and survival of neurons in both central and peripheral nervous systems. *NTRK* has several different partner genes, including *NFASC* and *BCAN* (both expressed in neuronal tissue), *EML4*, *TPM3* and also *CHTOP* and *ARHGEF2* (26, 53). Rearrangements of *NTRKs* play an important oncogenic role in many types of tumors, including gliomas, non-small cell lung cancer, colon cancer and papillary thyroid cancer (47), and several drugs targeting cancers harboring the *NTRK* fusion are under clinical trials (51).

Several other gene fusions have been detected, including *FIG-ROSI*, as one of the first fusions detected in glioblastoma cell lines, and also fusions involving non-coding genes such as *RP11-745C15.2-LANCL2* (10, 41). Last in the line of identified fusions in gliomas is the *PTPRZ1-MET* fusion, resulting in the constitutive activation of *MET*. The fusion induces expression and phosphorylation of the *MET* oncoprotein and is associated with a poor prognosis. The *PTPRZ1-MET* fusion is found in around 10% of pediatric and up to 16% of adult high-grade gliomas (anaplastic astrocytoma and secondary glioblastoma) (6, 11, 20, 52). In glioblastomas, activation of *MET* signaling has been proposed as one of the mechanisms of resistance to EGFR-inhibitors (4).

There are several promising ongoing preliminary clinical trials of molecularly targeted treatment for patients with glioma, but there is still a substantial gap and the need for better understanding the mechanisms of gliomas' development. Moreover, there is an active search to identify additional druggable fusions across different glioma subtypes for more personalized treatment (51). Herein, we report the presence of a novel fusion detected in gliomas—the *PTPRZ1-ETV1* fusion.

MATERIAL AND METHODS

Patients and samples

We prospectively analyzed tumor tissue samples from 205 patients with diagnosed primary brain tumor who had undergone tumor resection from March 2017 until March 2019. All tumors were classified according to the WHO 2016 Classification of Tumours of the Central Nervous System (31). The cohort of analyzed tumors consisted of 191 gliomas (8 pilocytic astrocytomas, 11 diffuse astrocytomas, 18 anaplastic astrocytomas, 8 anaplastic oligodendrogliomas, 1 anaplastic oligoastrocytoma, 143 glioblastomas, 2 diffuse midline gliomas—H3 K27 mutant) and 14 other primary

brain tumors (five medulloblastomas, two gangliogliomas, one ganglioneuroblastoma, one atypical teratoid/rhabdoid tumor, one choroid plexus papilloma, one neuroblastoma, one craniopharyngioma, one pineal parenchymal tumor of intermediate differentiation and one embryonal tumor, NOS).

DNA and RNA isolation

Total DNA and RNA were isolated from formalin-fixed, paraffin embedded (FFPE) tumor tissue samples. Most samples were enriched in the region of tumor tissue using a 0.6-mm needle (punch) (Manual Tissue Arrayer MTA, Beecher, Estigen). Isolation of DNA and RNA was carried out on the Promega automated system using a Maxwell RSC FFPE Plus DNA Purification kit and Maxwell RSC RNA FFPE kit (Promega), or the manual magnetic beads isolation approach with a MagMAX DNA/RNA Ultra FFPE kit (Thermo Fisher Scientific). The quality and concentration of isolated DNAs and RNAs were assessed both spectrophotometrically on NanoDrop-1000 and fluorometrically on Qubit 3.0 (both from Thermo Fisher Scientific).

NGS analysis

We performed targeted, amplicon-based DNA and RNA-sequencing analysis using the Oncomine Focus Assay test (Thermo Fisher Scientific), which focuses on detection of hotspots and other single nucleotide variants, small indels, copy number variations, and gene fusions across 52 genes relevant to solid tumors and with clinical significance. All steps of library preparation were performed according to the manufacturer's instructions. Briefly, we used 30 ng of total RNA for the reverse transcription (RT) reaction (SuperScript VILO cDNA Synthesis kit, Thermo Fisher Scientific) and further cDNA amplification. Thermal cycling was performed according to the manufacturer's recommendations and consisted of 30 cycles of amplification for the cDNA reactions (RNA libraries). For DNA library preparation, we used 30 ng of DNA and 20 cycles for target amplification. The prepared DNA and RNA libraries were quantified using an Ion Library Quantitation kit (Thermo Fisher Scientific). After quantification, libraries were mixed proportionally in 33-pM concentrations. Preparation of template ion sphere particles, enrichment PCR (emulsion PCR) and chip loading was carried out on the automated Ion Chef system, sequencing analysis and further raw data processing of DNA/RNA libraries were performed on the Ion S5 system (Thermo Fisher Scientific). Output files were uploaded onto Ion Reporter software (Thermo Fisher Scientific) for variant annotation and bioinformatical analysis.

Validation of fusion by RT-PCR and Sanger sequencing

We used 100 ng of total RNA for a 10- μ L RT reaction (SuperScript VILO cDNA Synthesis kit, Thermo Fisher Scientific) following the manufacturer's protocol. We used

an RNA-seq-negative FFPE tumor sample as *PTPRZ1-ETV1* fusion negative control and a non-template control for RT-PCR and Sanger sequencing validation control.

For PCR amplification, we added cDNA to 10% of the reaction's volume and one of the two primer pairs (location of primer annealing in parenthesis):

- (i) pair 1 (88 bp): *PTPRZ1-ETV1* forward 5'-GTGTTT GCCGCCTGGTG-3' (annealing on the junction) and *ETV1* reverse 5'-AAAGGGCTGTTCTTGGACTG-3' (exon 5);
- (ii) pair 2 (121 bp): *PTPRZ1* forward primer 5'-CAT TCAGCTCCTCTGTGTTT-3' (exon 1) and *ETV1* reverse primer 5'-AAAGGGCTGTTCTTGGACTG-3' (exon 5). We also performed a control PCR for verifying the success of the RT reaction, by amplifying the *GAPDH* gene.

PCR products were then purified of all excess dNTPs and primers using the ExoSAP-IT enzymatic approach (Applied Biosystems) according to the manufacturer's protocol. We used 2 μL of purified PCR product, 1 μL of designated primer (5-μM concentration) and a BigDye Terminator Cycle

Sequencing kit (v1.1, Thermo Fisher Scientific) for the sequencing reaction. The ethanol precipitation approach was used for purification of the sequencing reactions. Sanger sequencing analysis was carried out on the SeqStudio system (Thermo Fisher Scientific), and sequences were analyzed using SeqScanner software (Thermo Fisher Scientific) and the Ensembl genome browser. The obtained sequences were uploaded onto the BLASTN tool for homology search.

Prediction of the oncogenic potential of gene fusions

In order to predict the oncogenic potential of the novel gene fusion, we performed *in-silico* analysis using a Bayesian classifier that provides accurate classification of driver and passenger fusion genes, as described by Shugay and colleagues and Panigrahi and colleagues (36, 43). Briefly, the Oncofuse bioinformatical pipeline predicts the oncogenic potential of fusion genes by calculating the Bayesian probability that a fusion sequence behaves as an oncogenic driver (*P*-values <0.5 are classified as "driver"), based on features present in known oncogenic fusions.

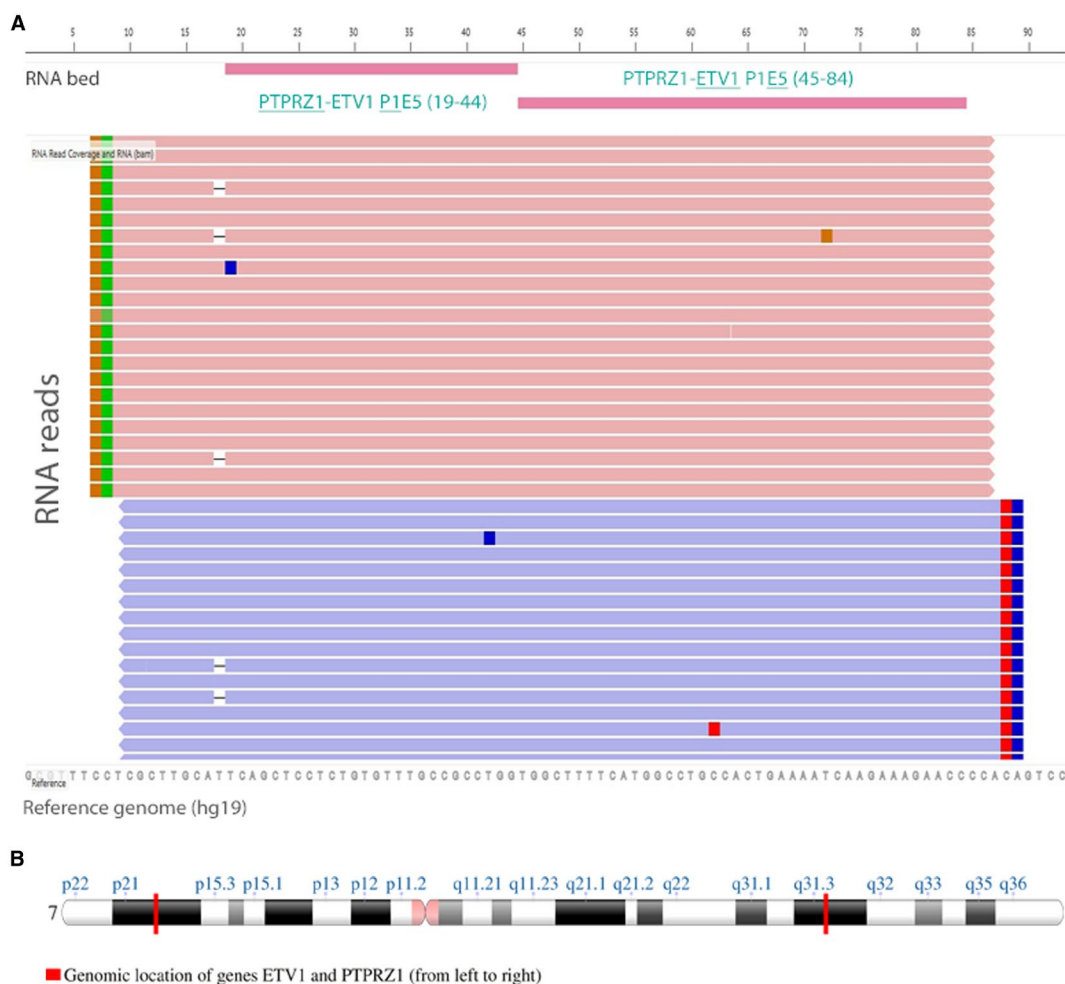


Figure 1. Novel fusion gene *PTPRZ1-ETV1* detected by amplicon-based RNA-seq analysis. **A.** IGV-Light visualization of sequence read pileup for the fusion detected. **B.** Genomic location of genes *ETV1* and *PTPRZ1* on chromosome 7.

Table 1. Patient demographics and histopathological characteristics of gliomas harboring the *PTPRZ1-ETV1* fusion.

Case number	Diagnosis	Tumor location	Gender	Age at diagnosis (years)	Primary or recurrent tumor	Multifocal tumor	Other neoplasms
1	Pilocytic astrocytoma	Cerebellum	Male	7	Primary	Yes	Neurofibromatosis type 1
2	Glioblastoma	Left frontal lobe	Male	61	Primary	Yes	None
3	Glioblastoma	Left temporal lobe	Male	68	Primary	No	None
4	Anaplastic oligodendroglioma	Left frontotemporal lobe	Male	43	Primary	No	Testicular teratocarcinoma in 2000
5	Glioblastoma	Left cerebellum	Male	41	Recurrent (radiotherapy and Temodal)	No	None
6	Glioblastoma	Right temporal lobe	Female	69	Primary	No	None
7	Glioblastoma	Right parietal lobe	Male	50	Primary	No	None
8	Glioblastoma	Left frontal lobe	Female	84	Primary	No	None
9	Glioblastoma	Left frontal lobe	Male	69	Primary	Yes	Basal cell carcinoma on the cheek and cutaneous squamous cell carcinomas on the neck and forehead in 2017, treated by complete surgical excisions
10	Glioblastoma	Frontal lobe	Male	48	Primary	Yes	None
11	Glioblastoma	Bilateral in parietal lobes	Female	58	Primary	Yes	None

RESULTS

A novel *PTPRZ1-ETV1* fusion detected by targeted RNA-sequencing

Targeted RNA-sequencing analysis of 205 primary brain tumors revealed the presence of a novel *PTPRZ1-ETV1* fusion in 11 of 191 gliomas (5.8%) (Figure 1), of which nine were glioblastomas, one anaplastic oligodendroglioma and one pilocytic astrocytoma (Table 1). Since the fusion was discovered only in gliomas, cases of other primary brain tumor types (fourteen in total) were not considered in further analyses.

RNA-sequencing software detected two distinct in-frame fusion transcripts, with the lowest read count of 258 reads (the default threshold of reads for intergenic fusion is ≥ 20) (Table 2). The two transcripts involved two breaking points within the *ETV1* coding sequence (exon 4 or 5, respectively). In contrast, the breakpoints in the *PTPRZ1* coding sequence were located at the same junction (at the end of exon 1). All other variants detected on DNA and RNA levels are listed in Table 2.

Successful validation of the *PTPRZ1-ETV1* fusion in gliomas

To confirm the presence of the detected fusion, we designed two primer pairs. The forward primer of one pair was designed as a fusion-specific primer, flanking exon 1 of *PTPRZ1* and exon 5 of *ETV1* (Figure 2A). The other pair was designed in such way that one of the primers flanked

exon 1 of the *PTPRZ1* gene and the other exon 5 of the *ETV1* gene (Figure 2A), which also amplifies the exon1-exon4 transcript of the *PTPRZ1-ETV1* fusion.

At the transcription level, we successfully validated both fusion variants using the RT-PCR approach followed by Sanger sequencing. The presence of PCR products was visualized on 2% agarose gel electrophoresis, which showed an individual band of amplified products for the primer pair used, approximately 90 bp (primer pair 1) and 120 bp long (primer pair 2) (Figure 2B, left image). Surprisingly, using primer pair 2, we also observed two fragments (sample B, right image), which corresponded to the two fusion transcripts present, *PTPRZ1*(exon 1)-*ETV1*(exon4) and *PTPRZ1*(exon1)-*ETV1*(exon5). Sequence analysis and further homology search (BLASTN) in the genome database confirmed that the PCR product consisted of the *PTPRZ1* gene fused to the *ETV1* gene (Figure 2D). The presence of fusion was confirmed in 9 of 11 tumor samples; we were not able to obtain a confirmatory PCR product in two samples, which may be due to a lower frequency of fusion present or a poorer quality of the RNA sample.

Demographic, histopathological and molecular features of gliomas harboring the *PTPRZ1-ETV1* fusion

The average age of patients with a glioma harboring the *PTPRZ1-ETV1* fusion at the time of diagnosis was 55 years (median 58.7 years; range 7–84 years), and female to male

Table 2. Molecular data of gliomas harboring the *PTPRZ1-ETV1* fusion, detected by immunohistochemistry and amplicon-based NGS analysis. NA = not available; WT = wild type; MT = mutated; IHC = immunohistochemistry.

Case number	<i>PTPRZ1-ETV1</i> fusion detected by NGS RNA-seq	Read count (threshold >20)	RT-PCR validation of <i>PTPRZ1-ETV1</i> fusion	IDH mutation (NGS)	1p/19 co-deletion	MGMT promoter methylation	Other variants detected with OncoPrint assay	IHC TP53	IHC ATRX
1	Exon 1–exon 5 <i>in-frame</i>	1795	Exon 1–exon 5	WT	NA	Not methylated	None	NA	WT
2	Exon 1–exon 5 <i>in-frame</i>	389	Exon 1–exon 5	WT	NA	Methylated	• <i>EGFRvIII</i>	WT	WT
3	Exon 1–exon 5 <i>in-frame</i>	275	Exon 1–exon 5	WT	NA	Not methylated	• <i>EGFR</i> amplification	Questionable	WT
4	Exon 1–exon 5 <i>in-frame</i>	258	Exon 1–exon 5	R132H	Very likely	Methylated	None	WT	WT
5	Exon 1–exon 5 <i>in-frame</i>	403	Exon 1–exon 5 Exon 1–exon 4	R132H	NA	Methylated	• <i>PTPRZ1-MET</i> fusion • <i>PDGFRA</i> , <i>CDK6</i> and <i>CCND1</i> amplification	MT	MT
6	Exon 1–exon 5 <i>in-frame</i>	266	Exon 1–exon 5	WT	NA	Methylated	• <i>EGFRvIII</i> • <i>EGFR</i> amplification • mutation in exon 20 of <i>EGFR</i>	WT	WT
7	Exon 1–exon 5 <i>in-frame</i>	457	Exon 1–exon 5	WT	NA	Not methylated	• <i>EGFRvIII</i>	WT	WT
8	Exon 1–exon 4 <i>in-frame</i>	480	Exon 1–exon 5 Exon 1–exon 4	WT	NA	Methylated	• <i>EGFR</i> amplification • <i>METΔ14</i> • <i>PTPRZ1-MET</i> fusion • <i>MET</i> and <i>PDGFRA</i> amplification	MT	WT
9	Exon 1–exon 4 <i>in-frame</i>	462	Exon 1–exon 5	WT	NA	Methylated	• <i>PDGFRA</i> mutation in exon 18 • <i>EGFRvIII</i>	WT	WT
10	Exon 1–exon 4 <i>in-frame</i>	389	No PCR amplification	WT	NA	Methylated	• <i>EGFR</i> amplification • <i>ETV6-NTRK3</i> fusion • <i>PDGFRA</i> and <i>KIT</i> amplification	WT	WT
11	Exon 1–exon 4 <i>in-frame</i>	304	No PCR amplification	WT	NA	Not methylated	• <i>EGFRvIII</i> • <i>EGFR</i> amplification	WT	WT

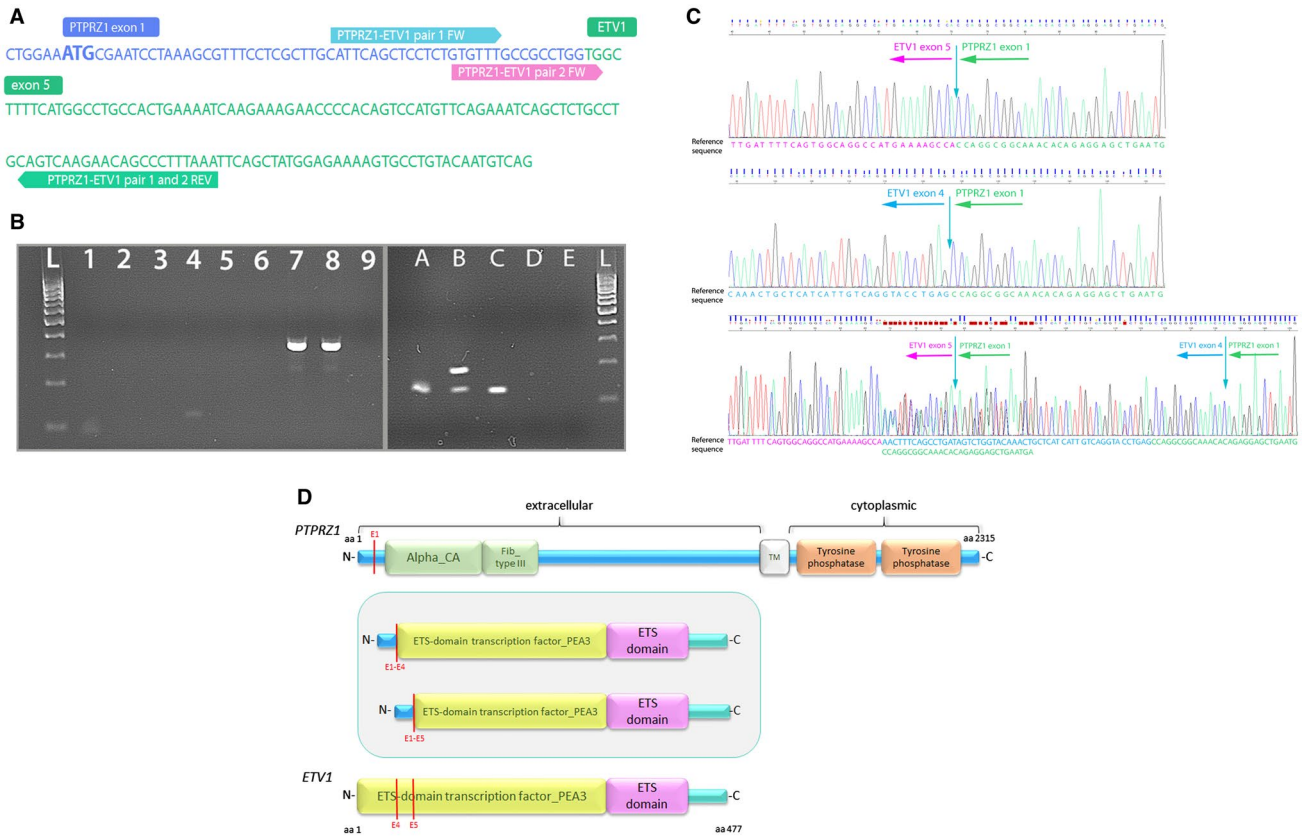


Figure 2. RT-PCR validation of novel *PTPRZ1-ETV1* fusion. **A.** Nucleotide sequence of fused *PTPRZ1* exon 1 and *ETV1* exon 5 with primers designed for RT-PCR and direct sequencing. **B.** RT-PCR for validation of *PTPRZ1-ETV1* fusion present (lane L—DNA 100bp ladder; lanes 1–3—PCR reactions with primer pair 1 (fusion-positive sample (88 bp), fusion-negative sample and no template control (NTC)); lanes 4–6—PCR reactions with primer pair 2 (fusion-positive sample (121 bp), fusion-negative sample and no template control (NTC); lanes A–D—PCR validation with primer pair 2, lane E—NTC). Sample B shows two amplified fragments and Sanger sequencing showed it harbors both

fusion transcripts, P1E4 (175 bp long fragment) and P1E5 (121 bp long fragment). Sample D showed no amplified product. **C.** Chromatogram of the RT-PCR amplified fusion *PTPRZ1*(exon1)-*ETV1*(exon5), *PTPRZ1*(exon1)-*ETV1*(exon4) and both fusion transcripts present (for Sanger sequencing, we used the reverse primer of pair 2). **D.** Schematic depiction of predicted fusion protein (in the frame) involving *PTPRZ1* and *ETV1* genes. Red lines represent exons of the *PTPRZ1* and *ETV1* gene, and also the point of break and fusion. aa = amino acid; Alpha_CA = alpha carbonic anhydrase; Fib_Type III = fibronectin type-III; TM = transmembrane domain.

ratio was 1:2.7 (Table 1). Histopathological assessment of gliomas with the *PTPRZ1-ETV1* fusion did not show any specific or unique pathological features, other than those characteristic of a particular glial tumor. With the exception of Case 1, all were high-grade gliomas. Case 1 was a 7-year-old patient, diagnosed with pilocytic astrocytoma, which showed no variants in the *BRAF* gene (eg, V600E mutation or *KIAA1549-BRAF* fusion) or in other genes of the MAPK signaling pathway, often detected in pilocytic astrocytoma. However, the patient had been previously diagnosed with neurofibromatosis type 1 (NF1), which is associated with an increased risk of the occurrence of gliomas, pilocytic astrocytomas being the most frequent type, which occurs in about 15% of these patients. Pilocytic astrocytoma in patients with neurofibromatosis tend not to harbor *BRAF* mutations (18, 32).

There was no association between the presence of *PTPRZ1-ETV1* fusion and glioma biomarkers, such as *IDH1/2* mutation status or methylation of *MGMT* promoter (Table 2).

The *IDH1/2* mutation was present in two of 11 tumors, one was an anaplastic oligodendroglioma and one glioblastoma, both carrying an R132H mutation in the *IDH1* gene. The *MGMT* promoter was methylated in seven of 11 tumors.

Sanger sequencing analysis showed the presence of both *PTPRZ1-ETV1* fusion transcripts—*PTPRZ1*(exon1)-*ETV1*(exon5) and *PTPRZ1*(exon1)-*ETV1*(exon4)—in two tumors, although RNA-sequencing detected only one of the two transcripts (Table 2, cases 5 and 8). Patocs and colleagues observed multiple splice variants of *EWSR1-ETS* fusion transcripts co-existing within the same tumor. The most probable explanation for the occurrence of multiple variants of one fusion event is alternative mRNA splicing. Nevertheless, there is also a chance of occurrence of two different chromosomal rearrangements in the same patient (39). Interestingly, both tumors also harbored the *PTPRZ1-MET* fusion and mutated *TP53*. *EGFRvIII* and *EGFR* amplification (detected by NGS analysis) were the most common additional genetic alterations, present in

five glioblastomas. In one glioblastoma, RNA-sequencing detected the exon1-exon4 transcript, but we detected only the exon1-exon5 transcript with RT-PCR validation (Table 2, case 9). Such discrepancies could be caused by a mispriming event or amplification bias.

In-silico prediction of *PTPRZ1-ETV1* oncogenic potential

In-silico prediction analysis, using the Oncofuse classifier and the Bayesian probability of oncogenic potential for specific fusion transcripts, revealed a high probability (P -value = 0.0008) that the novel *PTPRZ1-ETV1* fusion acts as a driver in the oncogenic process with a completely retained 3'-Ets domain (Winged helix-turn-helix DNA-binding domain). The complete results of Oncofuse classification are listed in Supplemental Table S1.

DISCUSSION

Over the past decade, molecular profiling of numerous cancer types has been incorporated into cancer diagnostics, including gliomas, allowing for a more personalized, targeted therapy approach (9). To the best of our knowledge, this is the first report of a *PTPRZ1-ETV1* fusion in general, not just in glial tumors. Furthermore, the newly described fusion is predicted to have an oncogenic role, retaining a highly conserved ETS domain, as established by *in-silico* prediction analysis. Both *PTPRZ1* and *ETV1* genes are known fusion partners in other cancer types, for example, *TMPRSS2-ETV1* in prostate cancer and *EWSR1-ETV1* in Ewing sarcoma and, as already mentioned, *PTPRZ1-MET* in gliomas (6).

The *PTPRZ1* gene is located on chromosome 7q31.32 and encodes member Z of the receptor protein tyrosine phosphatase family. It is preferentially expressed in the central nervous system and is one of the most abundant tyrosine phosphatases expressed in oligodendrocyte precursor cells (28). As such, it may be involved in the regulation of specific developmental processes in the central nervous system. Kubojama and colleagues indicated that *PTPRZ1*, as part of the phosphatase family, acts as a negative regulator of oligodendrocyte differentiation and myelination of embryonic mice white matter (27, 28). Protein tyrosine phosphatases are the enzymatic counterparts of protein tyrosine kinases and are important regulators of developmental and disease-related signaling pathways. Multiple members are directly linked to malformation syndromes and tumorigenesis (35, 46). In glioblastoma, *PTPRZ1* receptors were found preferentially expressed in glioma stem cells, predicting a poor prognosis (33, 42). They bound pleiotropin (PTN), an inhibitory ligand with a protective role, which has been suggested to promote myelination by inhibiting *PTPRZ1* (28). PTN is a neurotrophic factor, secreted by infiltrating tumor-associated macrophages, which have multiple functions in either inhibiting or promoting tumor progression, and are correlated with increased intra-tumor heterogeneity (42). In addition, Bao and Wang showed that *PTPRZ1-MET* signaling contributes to malignant progression in gliomas by

recruiting tumor-associated macrophages (7). A study by Shi and colleagues showed that disrupting *PTPRZ1* expression abrogated glioma stem cell maintenance and their tumorigenic potential. Blocking PTN-*PTPRZ1* signaling in animal models by shRNA or anti-*PTPRZ1* antibody potently suppressed glioblastoma tumor growth and prolonged animal survival (42). The selective inhibition of *PTPRZ1* is a promising approach for glioma therapy (17, 38).

The *ETV1* gene is also located on chromosome 7 (7p21.2) and encodes a member of the ETS (E twenty-six) family of transcription factors, which regulate many target genes that modulate biological processes, such as cell growth, angiogenesis, migration, proliferation and differentiation (25). All ETS proteins contain an ETS DNA-binding domain in the C-terminal region that binds to DNA sequences. At the N-terminal region, the *ETV1* protein contains a conserved short acidic transactivation domain, which is a feature of the small PEA3 subfamily of ETS proteins. The PEA3 subfamily consists of three ETS-translocation variants (ETV): *ETV1* (Ets-related protein or ER81), *ETV4* (polyoma enhancer activator 3 or PEA3) and *ETV5* (Ets-related molecule or ERM) (25, 34). PEA3 transcription factors have a role in morphogenesis and neuronal differentiation (1, 13, 24, 37). The involvement of *ETV1* in cancer was initially observed in Ewing sarcoma, in which a chromosomal translocation involving the Ewing sarcoma gene (*EWSR1*) and *ETV1* gene results in the *EWS-ETV1* fusion protein, which exerts oncogenic properties (23, 34). *ETV1* is involved in chromosomal translocations underlying prostate cancer development, having numerous fusion gene partners, including *TMPRSS2* (which is more often fused to the *ERG* gene of the ETS family in prostate cancer), *SLC45A3*, *HERV-K_22q11.23*, *C15orf21* and *HNRPA2B1* (45). *ETV1* drives the androgen receptor transcriptional response associated with aggressive prostate cancer (5). Amplification of *ETV1* is found in approximately 40% of melanomas, and *ETV1* has been shown to promote melanoma cell growth (21). *ETV1* overexpression has been detected in early stages of breast cancer development, and was found to be significantly associated with amplification of *HER2/neu* (15, 49). Amplification of *ETV1* has also been detected in gastrointestinal stromal tumors (12, 22). Heeg and colleagues found that *ETV1* is involved in epithelial-mesenchymal transition during pancreatic development in mice and that tumor cells with elevated *ETV1* expression have increased invasive capacity, contributing to metastatic progression of pancreatic cancer (19).

As observed in cases of childhood high-grade gliomas with *PTPRZ1-MET* fusion, expression of full-length *MET* is driven from the highly active *PTPRZ1* promoter, leading to *MET* overexpression (8). In view of the same *PTPRZ1* exon-1 breakpoint in both *PTPRZ1-MET* and *PTPRZ1-ETV1* fusions, the activated *PTPRZ1* promoter may also affect the expression of *ETV1*. As elevated expression of *ETV1* has been shown to contribute to increased invasiveness of tumor cells in other cancer types, gliomas harboring the *PTPRZ1-ETV1* fusion could potentially exhibit a more aggressive phenotype. In the *PTPRZ1-ETV1* fusion, the ETS DNA-binding domain of *ETV1* gene is retained, which would suggest that the activation of *ETV1* affects the

transcription of its target genes. Regarding the role of *ETV1* as a transcription factor, even a small change in its expression can have a significant biological impact. However, the biological function of the *PTPRZ1-ETV1* fusion and its contribution to tumor development remains undetermined. Since gliomas with *PTPRZ1-ETV1* fusion in our series represent recent cases with a very short follow-up, the prognostic significance of the fusion remains unknown.

Although *ETV1* as a transcription factor lacks enzymatic activity, its increasingly recognized function in oncogenic signaling pathways of various tumor types makes it a possible target for future therapeutic approaches (40). Akgül and colleagues showed that glial tumors are polyclonal tumors, creating a dynamic environment consisting of diverse tumor elements and treatment responses. Designing targeted therapies based on a range of tumor-specific molecular profiles (stratified and precise therapy) may be a more effective strategy for diagnosing and assigning a prognostic subgroup, and minimizing treatment resistance, recurrence and metastasis (3).

CONCLUSIONS

Fusion *PTPRZ1-ETV1* presents in about 6% of gliomas and represents a novel potential therapeutic target due to the fact that both genes exhibit oncogenic effects in the development and progression of various tumor types. The presence of the *PTPRZ1-ETV1* fusion does not seem to be associated with some unique morphologic features of gliomas. A longer follow-up and careful monitoring of patients' response to therapy might provide additional insights into the prognostic and predictive roles of this novel fusion.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web site:

Table S1. Oncofuse *in-silico* prediction analysis of newly described *PTPRZ1ETV1* fusion.