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Letter to the Editor

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Diffuse hemispheric glioma with *H3-3B* G34R mutation: Expanding the spectrum of histone H3 genes in diffuse hemispheric glioma, H3 G34-mutant

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To the Editor:

Diffuse hemispheric glioma, H3 G34-mutant (DHG-H3 G34), a new 2021 WHO central nervous system (CNS) tumor type, is defined as a hemispheric cellular infiltrative glioma with mitotic activity that harbors an H3.3 p.G35R (G34R) or p.G35V (G34V) mutation involving *H3-3A* (previously *H3F3A*), and for unresolved lesions, with a methylation profile matching DHG-H3 G34; OLIG2 immunonegativity, loss of ATRX expression, and diffuse p53 immunopositivity are diagnostically supportive findings (1). H3.3 is a variant of histone 3 that is encoded by 2 genes—*H3-3A* and *H3-3B* (previously *H3F3B*), located in chromosomes 1 and 17, respectively. These 2 genes have distinct sequence structure but encode identical replication-independent replacement histone H3.3 proteins (2, 3). *H3-3B* is also oncogenic and implicated in tumor types that harbor *H3-3A* mutations (4). The *H3-3B* p.K37M (K36M) mutation is a driver event in chondroblastoma (5, 6). In CNS tumors, *H3-3B* mutations are extremely rare, with a single p.K28I (K27I) mutation reported in a spinal cord diffuse glioma with H3 p.K28me3 (K27me3) loss of expression by immunohistochemistry (7). Herein, we describe a tumor that has been comprehensively characterized and fulfills all diagnostic criteria for DHG-H3 G34, but harbors an *H3-3B*, instead of *H3-3A*, p.G35R (G34R) mutation.

A 47-year-old man without significant past medical or family history presented with a 4-week history of progressive cognitive decline, gait instability, and speaking difficulty. Magnetic resonance imaging showed an extensive bifrontal non-enhancing infiltrating tumor (Fig. 1A). A biopsy was performed and showed a mitotically active cellular infiltrating glioma, without evidence of necrosis or microvascular proliferation (Fig. 1B). By immunohistochemistry, the tumor cells were negative for IDH1-R132H, and had loss of OLIG2 (Fig. 1C) and ATRX (Fig. 1D) expression as well as diffuse expression of p53 and mosaic expression of H3.3 G34R (clone RM240; rabbit monoclonal; RevMAb Biosciences) (Fig. 1E). A custom targeted 187-gene neuro-oncology next-generation sequencing (NGS) panel was performed and detected *TP53* (x2), *ATRX*, and *PTPRD* clinically relevant mutations. There was no evidence of *IDH1*, *IDH2*, or *H3-3A* mutations. OncoScan plus chromosomal microarray revealed a complex copy-number pattern including 3q, 9p, and 13q losses (Fig. 2A). Given the non-specific molecular and copy number profile, methylation array profiling was also performed. This tumor matched to methylation class DHG-H3 G34 with high confidence score in the DKFZ/Heidelberg classifier v.11b6 (calibrated score: 0.92) and v.12b6 (calibrated score: 0.98) using the NCI/Bethesda pipeline, and in the NCI-Bethesda classifier v.1 (calibrated score: 0.9), clustered within the DHG-H3 G34 methylation group (Fig. 2B), and was predicted to have *MGMT* promoter methylation. Follow-up TruSight Oncology 500 NGS panel testing detected an additional *H3-3B*:c.103G>A p.G35R (G34R) mutation at 28% variant allele frequency. *H3-3B* was not included in the list of genes evaluated by the initial NGS panel. Postoperatively, the patient received a single radiotherapy session at 4 weeks. His family elected for hospice care without further treatment and he died 7 weeks after surgery.

To our knowledge this is the first report of a molecularly, cytogenetically, and epigenetically well-characterized DHG-H3 G34 harboring an *H3-3B*, instead of *H3-3A*, G34 mutation. Although the *H3-3B*:c.103G>A p.G35R (G34R) mutation has

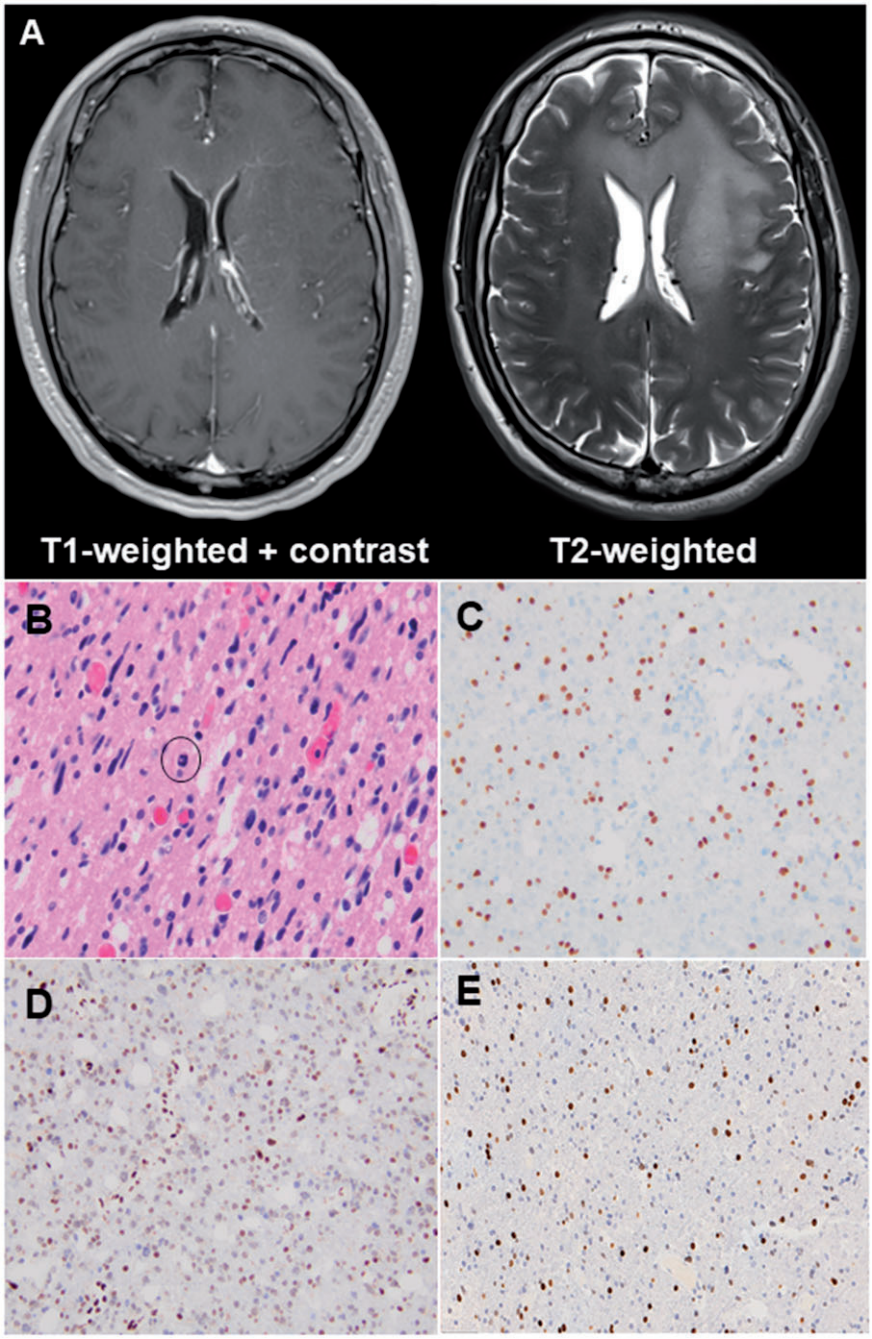

Figure 1. (**A**) Radiological findings. Extensive non-enhancing T1 hypointense, T2 hyperintense infiltrating tumor within the left frontal lobe crossing the genu of the corpus callosum and extending into the deep white matter of anteromedial right frontal lobe. (**B–E**) Histopathological findings (200×). Cellular infiltrating glioma with mitotic activity (circle) and tumor cells showing scant cytoplasm with irregularly shaped hyperchromatic and occasionally pleomorphic nuclei (**B**). By immunohistochemistry, the tumor cells had loss of OLIG2 expression (**C**), loss of ATRX expression with background nonspecific nucleolar staining (**D**), and mosaic expression of H3.3 G34R (**E**).

not been functionally characterized, this mutation translates into identical histone H3.3 G34R mutant protein as shown by immunohistochemistry and is expected to have similar downstream effects. The clinicopathological, genetic, and epigenetic findings are also in keeping with a DHG-H3 G34 and support the impression that the *H3-3B*:c.103G>A p.G35R (G34R) mutation is functionally equivalent to the *H3-3A*:c.103G>A p.G35R (G34R) mutation. Our findings suggest that DHG-H3 G34 is another tumor type driven by functionally convergent molecular mechanisms involving homologous genes as in diffuse midline glioma, H3 K27-altered and astrocytoma, IDH-mutant. Future classification schemes and clinical neuro-

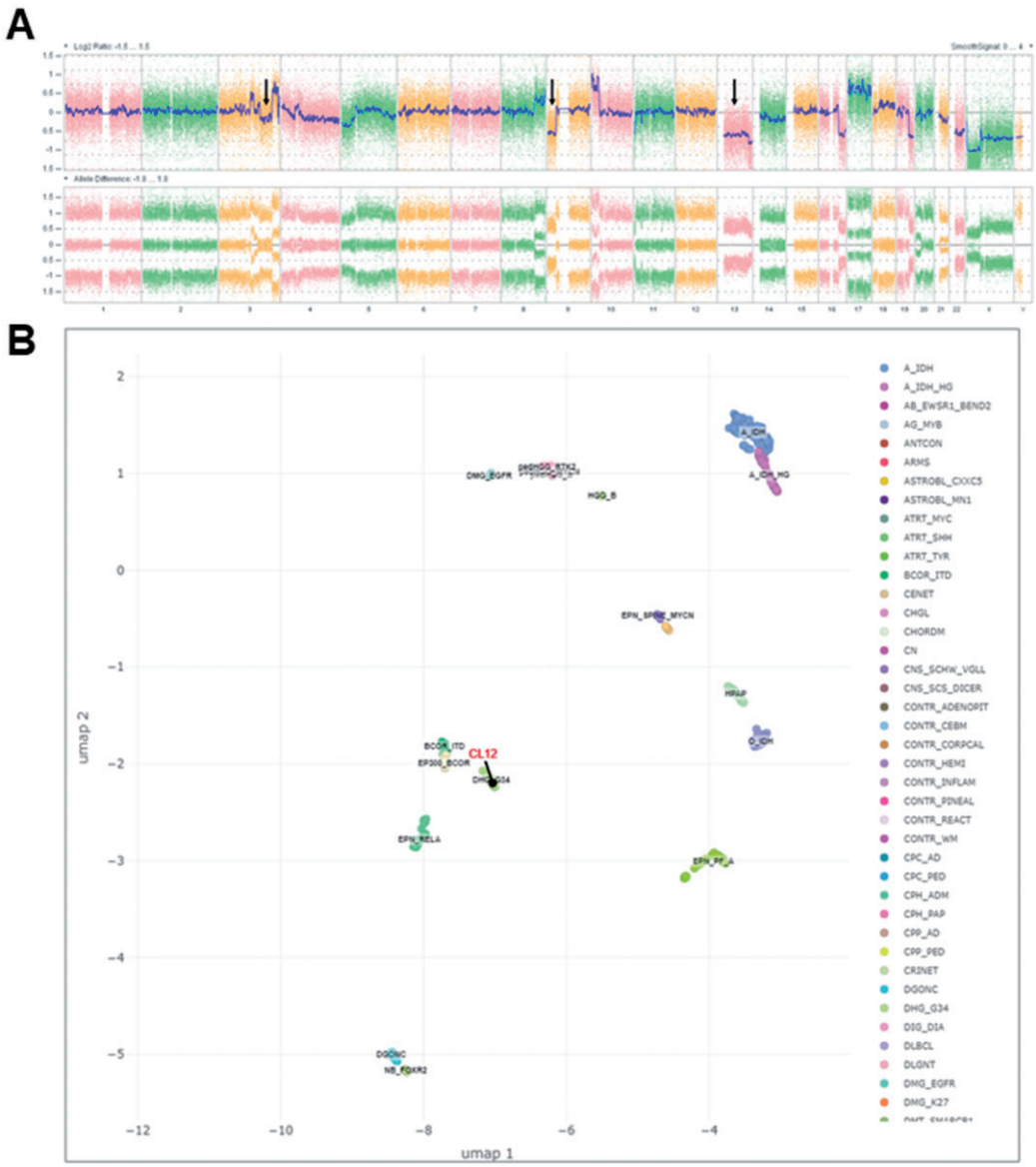

Figure 2. (**A**) Copy number profile by chromosomal microarray. Complex copy number pattern including changes recurrently reported in DHG-H3 G34 such as 3q, 9p, and 13q losses (black arrows) among other partial/whole chromosomal copy number changes. (**B**) Uniform Manifold Approximation and Projection (UMAP) embedding showed that our case (CL12) clustered within the DHG-H3 G34 methylation group.

oncology genetic tests may consider inclusion of *H3-3B* in the spectrum of histone H3 genes associated with DHG-H3 G34.

CONFLICT OF INTEREST

The authors have no duality or conflicts of interest to declare.

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