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Constitutional Tandem Duplication of 9q34 that Truncates *EHMT1* in a Child with Ganglioglioma

Hannah C. Cheung, PhD^{1,*}, Svetlana A. Yatsenko, MD^{2,*}, Meena Kadapakkam, BS¹, Hélène Legay³, Jack Su, MD¹, James R. Lupski, MD, PhD², and Sharon E. Plon, MD, PhD¹

¹Texas Children's Cancer Center, Baylor College of Medicine, Houston, TX, USA

²Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX, USA

³Faculté de Pharmacie, Université Claude Bernard, Lyon, France

Abstract

Point mutations of *EHMT1* or deletions and duplications of chromosome 9q34.3 are found in patients with variable neurologic and developmental disorders. Here, we present a child with congenital cataract, developmental and speech delay who developed a metastatic ganglioglioma with progression to anaplastic astrocytoma. Molecular analysis identified a novel constitutional tandem duplication in 9q34.3 with breakpoints in intron 1 of *TRAF2* and intron 16 of *EHMT1* generating a fusion transcript predicted to encode a truncated form of EHMT1. The ganglioglioma showed complex chromosomal aberrations with further duplication of the dup9q34. Thus, this unique tandem 9q34.3 duplication may impact brain tumor formation.

Keywords

9q34; ganglioglioma; EHMT1; histone methyltransferase

INTRODUCTION

An overlapping set of shared clinical features including: hypotonia, dysmorphic features, speech delay, mental retardation and behavioral problems have been noted in pediatric patients with microdeletions (Kleefstra syndrome OMIM 610253), duplications and translocations of the 9q32-qter region resulting from allelic and non-allelic meiotic homologous recombination events [1-3]. Patients heterozygous for truncating point mutations and intragenic deletions of *EHMT1* provide further evidence that the *EHMT1* gene (OMIM 607001), which encodes euchromatic histone-lysine N-methyltransferase within 9q34.3, is the causative gene [2]. Recent findings of somatic loss and gain of 9q34 in two patients, respectively, with ganglioglioma as well as somatic homozygous deletions of *EHMT1* in two cases of medulloblastoma suggest an additional tumor suppressor role for this gene [4-6]. Here, we report a child with a unique germline 9q34.3 duplication that truncates *EHMT1* who developed an aggressive ganglioglioma.

Corresponding author: Sharon E. Plon, 6621 Fannin St., MC 3-3320, Houston, TX 77030, Phone: 832-824-4251, Fax: 832-825-4276, splon@bcm.edu.

*co-first authors

Note: Svetlana Yatsenko is currently at yatsenkosa@mail.magee.edu

CONFLICT OF INTEREST STATEMENT James R. Lupski is a consultant for Athena Diagnostics and is based in the Department of Molecular and Human Genetics at Baylor College of Medicine (BCM), which offers extensive genetic testing, including use of arrays for genomic copy number analysis, and derives revenue from this activity.

METHODS

Patient samples

The family was enrolled in a Baylor College of Medicine Institutional Review Board approved protocol, including collection of medical history, family history, and biologic specimens.

Tissue culture and nucleic acid isolation

Immortalized LCLs were established according to established protocol [7] and grown in RPMI medium 1640 + 25 mM HEPES, 10% bovine growth serum, pen-strep at 37°C, 5% CO₂. Genomic DNA (gDNA) was obtained from lymphoblastoid cells (LCLs) and peripheral blood lymphocytes using the Puregene kit (Gentra Systems, MN, USA). DNA digestion, labeling, and hybridization were performed according to the manufacturer's protocol (Agilent Technologies, Inc, CA, USA). RNA was isolated by Trizol extraction (Invitrogen, Carlsbad, CA) until addition of isopropanol, followed by purification through RNeasy Mini columns (Qiagen Inc., Valencia, CA). 293T cells were grown in Dulbecco's modified Essential Medium with 10% fetal bovine serum.

Fluorescence In Situ Hybridization (FISH)

Interphase FISH analysis was performed as previously described on fixed blood and tumor derived cells [8]. The BAC (bacterial artificial chromosomes) probes RP11-350O14 and RP11-611D20 (BACPAC Resource Center, Oakland, CA) which target the 5' and 3' ends of the duplication region were labeled with Spectrum Orange-dUTP, Spectrum Green-dUTP, or Spectrum Red-dUTP using a commercially available kit (Abbott Molecular Diagnostics, Abbott Park, Illinois).

Breakpoint XL-PCR

Long-range PCR was performed using modified conditions of the Takara kit (Takara Bio Inc., Otsu, Shiga, Japan) from 100 ng of gDNA. Male reference gDNA (G1471) was purchased from Promega (Madison, WI). Reactions (50 µL with 100 ng gDNA) included; 25 µL of 2X LA GC buffer II, 8 µL of 2.5 µM dNTPs, 0.5 µL of 10 uM dGTP and dCTP, 2 µL of each primer (F1 and R1), and 0.4 µL of LA *Taq* polymerase. See Supplemental Table I for primer sequences.

Rapid amplification of cDNA ends and polymerase chain reaction (RACE-PCR)

We used the SMART™ RACE cDNA Amplification kit (Clontech Laboratories Inc., Mountainview, CA) with EHMT1-RACE or transferrin receptor primers as a control. Following cDNA synthesis, nested PCR was done with primers F1 or F2 and R2 using the Platinum© *Taq* DNA polymerase (Invitrogen, Carlsbad, CA)

RESULTS

Case Report

The proband was born to parents who both have histories of mild learning disability (see pedigree in Supplemental Fig. 1A). Multiple maternal relatives have a history of learning disabilities without tumor diagnosis but no molecular analysis has been performed. At two years old, the proband was diagnosed with a congenital cataract, developmental and speech delay and complex partial seizures. MRI of the brain showed abnormal T2/flair signal with restricted diffusion in the left mesial temporal lobe suggestive of a tumor although she was lost to follow-up. Repeat MRI of brain at age six showed an extensive infiltrative left mesial temporal mass. Surgery revealed a firm tumor involving two major intracranial arteries

which was biopsied. Pathology demonstrated an atypical ganglioglioma (infiltrative nature, focally increased proliferative index) without features of anaplasia. A metastatic tumor nodule at thoracic T4-5 was seen on MRI. She received proton radiation therapy 30.6 Gy to the craniospinal axis, boosted to a total of 50.4 Gy to the primary tumor, and another boost to a total of 45 Gy to the metastatic nodule. There was stabilization of the tumor for 8 months, followed by clinical progression with tumor infiltration of the midbrain and pons. Tumor debulking was performed and pathology was most consistent with anaplastic astrocytoma. The patient expired a month later.

Identification of chromosome 9q34 duplication

Based on the combined history of brain tumor, developmental delay and congenital cataract, clinical array Comparative Genomic Hybridization (aCGH) was ordered and revealed a subtelomeric 9q34 duplication (dup9q34) spanning approximately 0.9 MB (see Suppl. Methods). This duplication was confirmed by interphase FISH studies and was also found in the proband's mother and brother. Among 20 recently described patients with 9q34 duplications, this proband's duplication was unique (subject p46 in Yatsenko, personal communication). Using a custom 9q34 high-resolution array (Suppl. Fig. 1B), we further refined the duplication breakpoints to within intron 1 of *TRAF2* and intron 16 of *EHMT1* (Fig. 1A).

Head-to-tail orientation and fusion transcript characterization

Using two-color FISH targeting the ends of the duplication, alternating linear signals delineated a head-to-tail tandem orientation (Fig. 1B). Sequence of the Long-range PCR product revealed at least two recombination events involving intron 16 of *EHMT1* and intron 1 of *TRAF2* with an intravening inverted 21 bp sequence highly homologous to a region just upstream of the breakpoint in *TRAF2* (Fig. 1).

In patient but not in control LCL RNA we detected duplication-derived fusion transcripts containing exons 1 through 16 of *EHMT1* spliced to exon 2 of *TRAF2* (Figure 1D-E). The long isoform of *EHMT1* (NM_024757.4) would be truncated by a nonsense codon in the fused *TRAF2* exon removing part of the EHMT1 ankyrin repeats domain. The short isoform of *EHMT1* (NM_01144527) would be unaltered by the fusion. Translation of *TRAF2* initiates at ATG in exon 2 and would be left intact in this polycistronic mRNA (Fig. 1F).

EHMT1 and TRAF2 protein expression

We probed proband and control LCL whole cell lysates with N-terminal epitope EHMT1 antibody that should detect both long and short isoforms. Only the long isoform was observed without change in expression level (Supplemental Fig. 2). No change in TRAF2 protein was seen. Thus, the fusion transcript may not be translated in LCLs. Protein lysate from the tumor was not available.

Analysis of the chromosome 9q34 duplication in the ganglioglioma

Cytogenetic analysis of the initial biopsy specimen revealed a complex karyotype including trisomy for chromosomes 2, 7 and 9, and 9p deletion. We determined by FISH that cells with trisomy 9 have two copies of chromosome 9 containing the dup9q34.3 (Figure 2A-D). The 9p loss also occurred on the dup9q34.3 chromosome.

DISCUSSION

Fusion transcripts identified in malignancies are most commonly derived from somatic translocations or interstitial deletions with rare examples of duplications such as dup7q34 which confers increased expression of *BRAF1* in pilocytic astrocytomas [9]. More fusions

are expected to be identified with the advent of RNA and whole genome sequencing. Here, we describe a constitutional head-to-tail dup9q34.3 which disrupts *EHMT1* and results in a fusion transcript with *TRAF2*.

In the tumor, if the fusion transcript is translated it would disrupt the long isoform of EHMT1 but retain the short isoform. It is unclear whether translation signals in this transcript are sufficient to upregulate TRAF2 which might promote survival through NF- κ B activation and diminished TNF-induced apoptosis [10-11]. Deficiencies in EHMT1 decrease the levels of interacting protein G9a, which may promote genomic instability by decreasing H3K9 methylation or transcriptionally activating oncogenes such as *C-MYC* [6, 12-15]. Further analysis of EHMT1 phosphorylation and histone methylation activities in ganglioglioma may be informative with regard to the role of this histone methylase in brain tumor development [16].

The relatively mild developmental delay in this patient may relate to the short duplication size as clinical severity of other patients with 9q34 aberrations correlates more with the size of the interval rather than whether it gained or lost [17-18]. This is the first reported 9q34 syndrome patient with cancer although not all of the family members with the duplication developed tumors [2-3]. The effects of both 9p loss (including *CDKNA/B*), which is frequent in sporadic and familial brain tumors as well as the increased dosage of the other 46 genes within the 950 kb duplicated segment could have contributed to tumorigenesis [3, 19-20]. Several of the duplicated genes (*PTGDS*, *ABCA2*, *GRIN1*, and *NOXA1*) have some association with nervous system development or brain tumors (see references in Supplemental Table II). The ganglioglioma had an aggressive course and a large proportion of cells had 9p loss (including *CDKN2A/B*) as well as further duplication of the 9q34 duplicated region suggesting selection for the dup9q34 during tumor evolution (Fig. 2E-F).

In summary, this patient with developmental delay, seizures, congenital cataract and an aggressive ganglioglioma demonstrated a novel 9q34.3 duplication which results in a fusion transcript between the truncated *EHMT1* and *TRAF2* genes. Further studies of *EHMT1* and other genes within the duplicated segment in pediatric brain tumors are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Molecular characterization of the duplication event. A. Interphase FISH using BAC probes RP11-350O14 and RP11611D20 were labeled green and red respectively. Alternating duplicate sets of fluorescent signals for one chromosome with another chromosome having just one set is evidence of head-to-tail duplication event in one chromosome. B. Schema of the breakpoint region. The breakpoint is ~1.1 kb away from the end of *EHMT1* exon 16 and ~13 kb upstream of the *TRAF2* exon 2. Primers were designed based on the high resolution aCGH results to amplify gDNA from *EHMT1* exon 16 to a position 10.2 kb upstream of *TRAF2* exon 2 (F1 and R1). PCR components were modified to account for the high GC content of the region (See Methods). C. The expected product (~3.5 kb) was observed in the proband, but not in the male reference DNA (Promega G1471), confirming that it is from the chromosome containing the duplication. D. Forward primers were designed for *EHMT1* exons 15 and 16 with a reverse primer on *TRAF2* exon 3 producing nested 3' RACE PCR products of approximately 400 bp and 300 bp respectively (F1, F2, R2). These were present in the proband sample and absent in both the normal LCL and the placenta normalization control RNAs. Transferrin receptor was used as a loading control. E. Sequencing results of the RACE-PCR product using primers (F3 and R3) from both ends confirmed a splicing reaction joining *EHMT1* exon 16 to *TRAF2* exon 2 across the breakpoint region to form a novel fusion product.

Figure 2.

Evidence for two copies of chromosome 9 containing the duplication in tumor cells. Four subpopulations of cells were characterized in the ganglioglioma cell pellet using probes to 9p, upstream of the duplication, and within the duplication. A. One copy of each normal chr 9 and dup9q34, which is the constitutional genotype. B. Trisomy chromosome 9 with 2 chromosomes harboring the dup9q34. C. One normal chromosome 9 and another with both del9p and dup9q34. D. One normal chromosome 9 and two 9q34 with one del9p. E-F. Possible evolution of chromosome 9 in the ganglioglioma.