Supplementary Online Content

Bichsel CA, Goss J, Alomari M, et al. Association of somatic *GNAQ* mutation with capillary malformations in a case of choroidal hemangioma. *JAMA Ophthalmol*. Published online October 25, 2018. doi:10.1001/jamaophthalmol.2018.5141

This supplementary material has been provided by the authors to give readers additional information about their work.

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Supplemental methods

Choroidal tissue isolation, genomic DNA extraction and droplet digital PCR

Immunofluorescent Staining of FFPE sections

Supplemental list of ddPCR primers and probes

Primer sequences

Probe sequences

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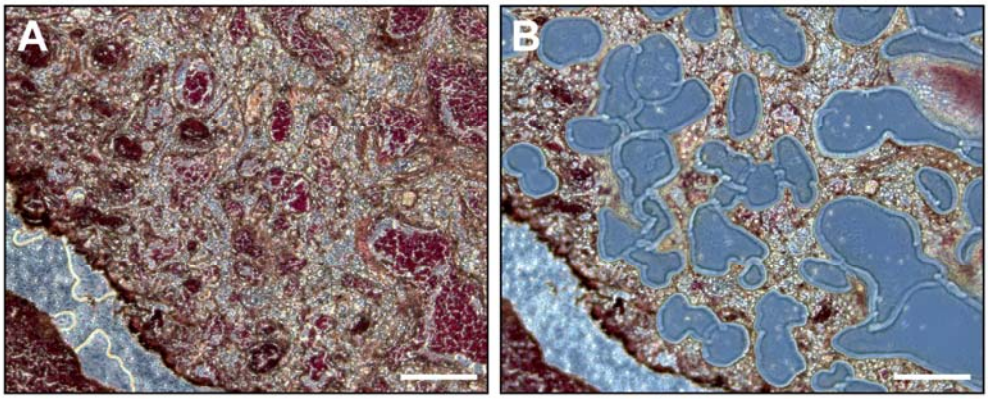
Supplemental Figures



eFigure 1. The patient's choroidal tissue section before and after laser-capture microdissection.
H&E stained choroidal tissue section of the patient showing a plethora of abnormal blood vessels in the choroid (A). Same section after blood vessel isolation by laser-capture microdissection (B). Scale bars: 200 µm.

eFigure 2. Macroscopic view of the patient's eye and the retinoblastoma-affected eye.
H&E stained ocular tissue section of the patient's eye (bottom) and a retinoblastoma-affected eye (top). Scale bar: 10 mm.

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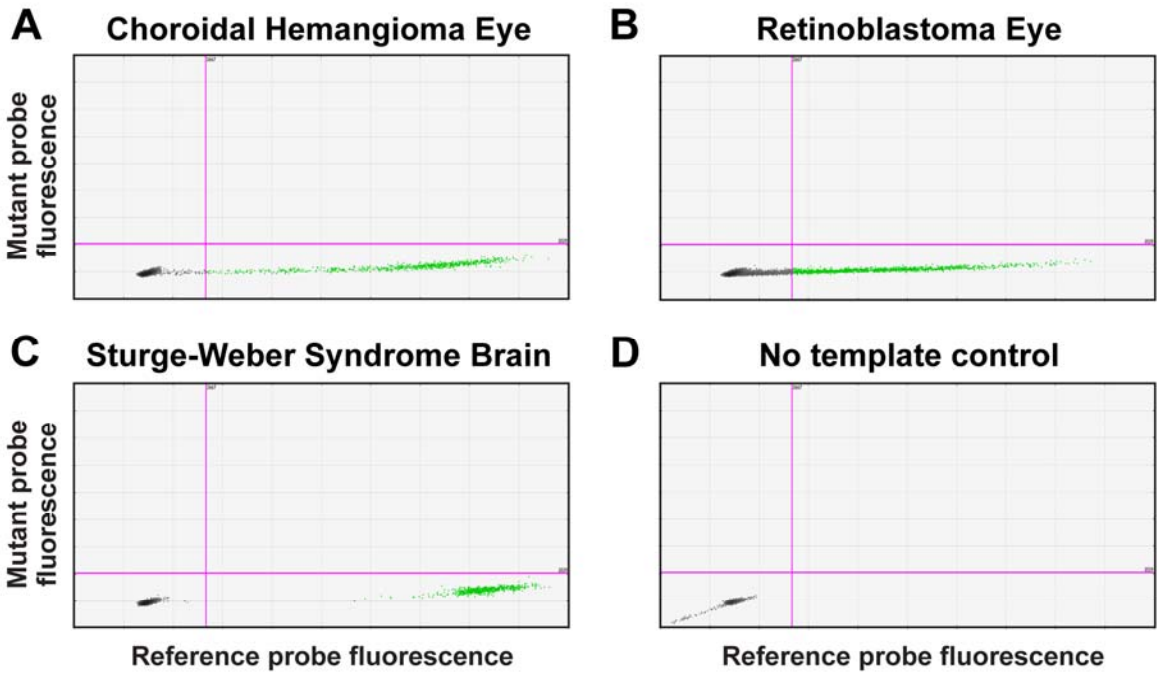

eFigure 3. The patient's ocular tissue is negative for *GNAQ* Q209L, Q209P and Q209H.
DDPCR with pooled probes detecting the *GNAQ* Q209L, Q209P and Q209H mutations in samples from choroidal hemangioma eye (A), retinoblastoma eye (B) and Sturge-Weber brain tissue (C). Water was used as no template control (D). All samples were negative for the three *GNAQ* Q209 mutations.

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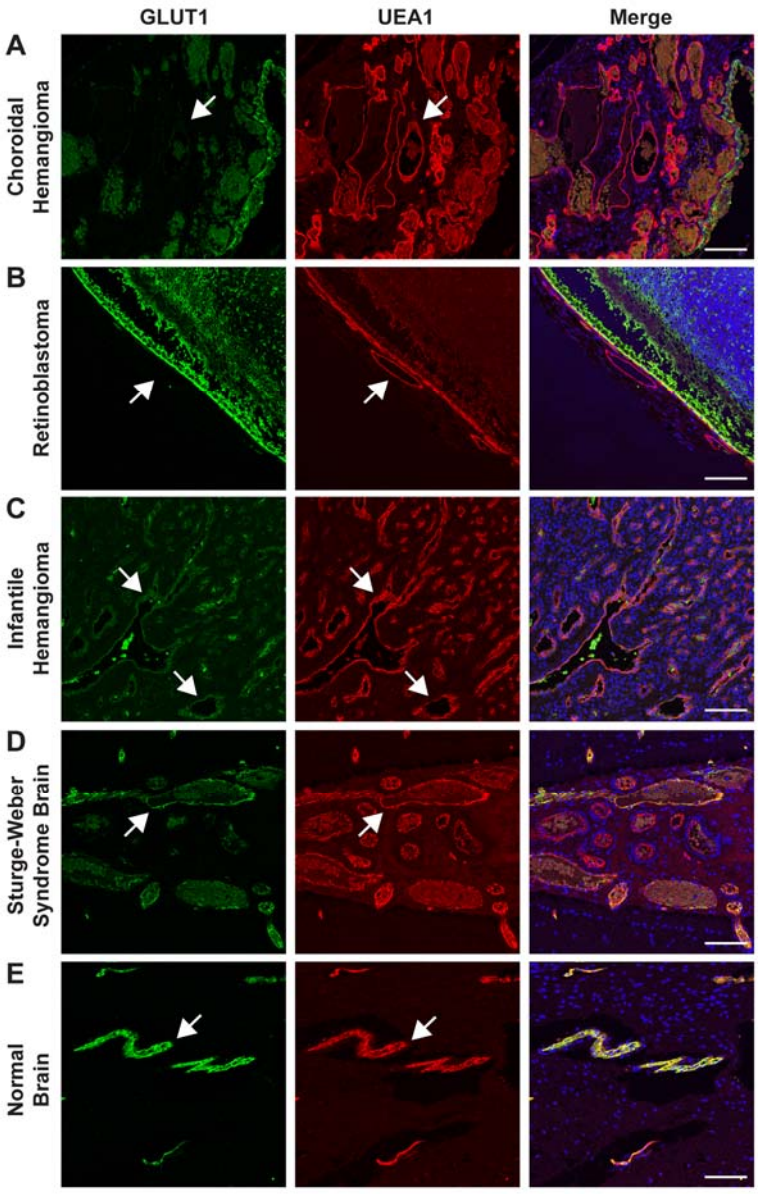

eFigure 4. Tissue sections stained for the infantile hemangioma marker GLUT1.
FFPE sections stained for GLUT1 (green), the endothelial marker Ulex Europaeus Agglutinin I (red), the nuclear stain Hoechst (blue) in the SWS patient's choroidal vessels (A), retinoblastoma choroidal vessels (B), infantile hemangioma (C), SWS-affected leptomeninges (D), normal brain leptomeninges (E). Scale bars: 100 µm.

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Supplemental methods

Choroidal tissue isolation, genomic DNA extraction and droplet digital PCR

Formalin fixed paraffin-embedded (FFPE) tissue from this patient's enucleation was obtained and 15 µm sections were cut and placed onto PEN-membrane slides (Leica Biosystems). Slides were stained with hematoxylin and eosin, and laser-capture microdissection (Leica) was used to isolate choroidal vessels (eFigure 1). Similarly, choroidal vessels were obtained from FFPE tissue from an eye with a normal choroid that was enucleated because of retinoblastoma (eFigure 2). Genomic DNA isolated from a SWS brain specimen and normal lung tissue from different individuals were used as controls.

Choroidal vessels laser-captured from two to five tissue slides were pooled. For whole eye tissue, the entire section was scraped from a slide. Genomic DNA was extracted with the Agencourt FormaPure DNA kit (Beckman-Coulter). 30-90 ng of genomic DNA was then used for droplet digital PCR (ddPCR). Briefly, the DNA was mixed with ddPCR SuperMix (BioRad), primers for a 74bp amplicon in GNAQ Exon4, FAM-labeled quenched mutant probes and HEX-labeled quenched wild-type probes (supplemental list), divided into droplets (QX200 AutoDG) and amplified for 40 polymerase chain reaction cycles using 60°C as annealing temperature. The droplet fluorescence was read in a QX200 droplet reader (BioRad) and analyzed in QuantaSoft (BioRad).

Immunofluorescent Staining of FFPE sections

The FFPE sections were de-paraffinized, and antigen retrieval was performed in citrate buffer at pH 6.0 for 20 minutes at 95°C. The sections were incubated with primary antibodies (40 µg/ml (1:50) anti-GLUT1 (ab40084 Abcam), 40 µg/ml (1:5000) mouse-IgG (I-2000 Vector) for control, and 20 µg/ml (1:100) rhodamine-labeled Ulex Europaeus Agglutinin I, (RL-1062 Vector)) for one hour at room temperature in Tris-NaCl buffer with 0.5% blocking reagent (FP1012 Perkin Elmer), washed in Tris-NaCl buffer with 0.05% Tween-20 (BioRad), and incubated with the secondary antibody (1:200), Alexa647 goat anti-mouse IgG (A21236 Thermo Fisher) and Hoechst (1:1000) (33342 Thermo Fisher) for one hour at room temperature, washed and mounted (P36965 Thermo Fisher). Images were taken with a Zeiss LSM 880 confocal microscope.

Supplemental list of ddPCR primers and probes

Primer sequences

GNAQ R183 fwd: 5'-CCTGCCTACGCAACAAGAT-3'
rev: 5'- GTAAGTCAAAGGGGTATTCGAT-3'
GNAQ Q209 fwd: 5'-GTTAACCTTGCAGAATGGTCG-3'
rev: 5'- GACATTTTCAAAGCAGTGTATCC-3'

Probe sequences

GNAQ R183 reference:
5'-/5HEX/TGCTTAGAG/ZEN/TTCGAGTCCCCACC/3IABkFQ/-3'
GNAQ R183Q mutant:
5'-/56-FAM/TGCTTAGAG/ZEN/TTCAAGTCCCCACC/3IABkFQ/-3'
GNAQ Q209 reference:
5'-/5HEX/CTTCTCTCT/ZEN/GACCTTTGGCCCCCTA/3IABkFQ/-3'
GNAQ Q209L mutant:
5'-/56-FAM/TCTCTCT/ZEN/GACCTTAGGCCCCCTAC/3IABkFQ/-3'
GNAQ Q209P mutant:
5'-/56-FAM/TCTCTCT/ZEN/GACCTTGGGCCCCCTAC/3IABkFQ/-3'
GNAQ Q209H mutant:
5'-/56-FAM/CTCTCTGAC/ZEN/CTGTGGCCCCCT/3IABkFQ/-3'

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