Homozygous pathogenic variant in *BRAT1* associated with non-progressive cerebellar ataxia

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Supplemental Methods

Whole Exome Sequencing (WES)

WES library preparation, exon capture and sequencing were performed for both siblings and parents at the Genome Québec Innovation Center (Montréal, QC, Canada). Genomic DNA was captured using the SureSelect Human 50Mb All Exon kit v5 (Agilent Technologies, Santa Clara, CA, USA). Sequencing was performed on an Illumina HiSeq2000 (Illumina, San Diego, CA, USA) with paired-end 100-bp reads. A mean coverage of 159X was obtained and 99% of the bases were covered at more than 10X. Read alignment, variant calling, and annotation were done with a pipeline based on BWA, SAMtools, GATK, ANNOVAR, and custom annotation scripts, and reads were aligned to the reference human genome (hg19). A homozygous missense c.185T>A (p.Val62Glu) variant in *BRAT1* was identified in both siblings and the parents were heterozygous carriers of the variant.

Cell Culture and treatment

For ATM kinase activation studies:

All cell lines were tested for the absence of mycoplasma. BRAT1-patient primary fibroblasts (Sib1) and parent (Father) primary fibroblasts were generated from a skin biopsy and the LCL cell lines (Sib1, Sib2, Mother and Father) were obtained from fresh blood by Epstein-Barr virus transformation. Primary fibroblasts were immortalized by retroviral-mediated hTERT

expression and selected in a medium containing 1 µg/ml puromycin. All fibroblasts were grown in Minimum Essential Media (MEM; Gibco, Thermo Fisher Scientific, Waltham, MA) containing 15% FBS (Gibco), 2 mM glutamine, and the antibiotics penicillin (100 units/ml) and streptomycin (100 µg/ml). LCLs were cultured in RPMI medium (Gibco) containing 10% FBS, 2 mM glutamine and penicillin/streptomycin, in a humidified atmosphere of 5% CO_2 at 37 °C. Where indicated, cells were treated with 10 µM MG132 (Sigma-Aldrich, St.Louis, MO) for 24 hours, 50 µg/ml cyclohexamide (Sigma-Aldrich, St.Louis, MO) for selected times or irradiated with the dose of 5 Gy using an X-ray instrument T-200 (Wolf-Medizintechnik GmbH, St. Gangloff, DE).

For mitochondrial studies:

Patient and healthy control fibroblasts were cultured in low glucose DMEM (Gibco, Thermo Fisher Scientific, Waltham, MA) supplemented with 10% FBS (Gibco), 1mM sodium pyruvate (Gibco) and 50 µg/ml uridine (Acros Organics, Thermo Fisher Scientific). Cells were seeded at a density of 10^6 per 100 mm plate and treated with 5mM dichloroacetate (Sigma-Aldrich, St.Louis, MO) for 48 hours.

Western Blotting and antibodies

For ATM kinase activation studies:

Cells were lysed in 1x Laemmli loading buffer, heated for 10 min at 95°C, and sonicated for 30 s using a Bioruptor® Pico (Diagenode). Protein concentrations were determined using the BCA assay (Pierce). Samples were subjected to SDS-PAGE, proteins transferred onto nitrocellulose membrane and detected by the relevant primary antibody (e.g. rabbit α-BRAT1, 1:5000, Abcam, #181855; rabbit α-BRAT1, 1:1000, Thermo Scientific, PA530916; mouse

α-α-tubulin, 1:1000, Santa Cruz, sc-23948; mouse α-p53, 1:1000, Millipore, #OP29; rabbit α-actin, 1:1000, Sigma-Aldrich, #A2066; rabbit α-ATM phosphoS1981, 1:5000, Abcam, #81292; rabbit α-Chk2 phosphoT68, 1:1000, Cell Signaling, #2661; rabbit α-p53 phosphoS15, 1:1000, Cell Signaling, #9284 or rabbit α-γH2AX, 1:7000, Abcam, #11174) and horseradish peroxidase-conjugated secondary antibody (goat α-rabbit HRP, 1:10000, Dako, #P0448 or goat α-mouse HRP, 1:10000, BioRad, #170-6516). Peroxidase activity was detected by ECL reagent (GE Healthcare) and Amersham Hyperfilm ECL (GE Healthcare).

For mitochondrial studies:

Protein lysates were fractionated by SDS-PAGE and transferred to 0.2 μM PVDF membrane with Bio-Rad Trans-Blot. Membrane was blocked for 1 hour at room temperature and incubated overnight with primary antibodies for PDH E1α subunit phospho293 (1:600 rabbit polyclonal, Abcam, Cambridge, MA) and β-actin (1:10,000 mouse monoclonal, Abcam, Cambridge, MA) followed by IRDye labelled secondary antibodies (LI-COR Biosciences, Lincoln, NE). Protein levels were detected with the LI-COR Odyssey infrared imager at 680 and 800 nm.

Quantitative Real Time PCR (qPCR)

Total RNA was extracted from LCL cell lines using Rneasy Mini Kit (Qiagen; #74104) according to the manufacturer's protocol. Complementary DNA was synthesized from 2 μg of isolated RNA using Random Hexamer Primers (Thermo Scientific; #SO142) and RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific; # K1622). qRT-PCR was performed on a LightCycler 480 (Roche) using SYBR Green PCR Master Mix (Thermo Scientific; #4309155) with the following set of primers: Brat1 (CCCCACGGTTCTGAAGAAGG and TTTCTGCAGCACAGGGAACA), Actb (CACCATTGGCAATGAGCGGTTC and

AGGTCTTTGCGGATGTCCACGT), Rplp2 (TCTTGGACAGCGTGGGTATCGA and CAGCAGGTACACTGGCAAGCTT). Each sample was run in triplicate and statistical analysis was based on the results of 3 independent experiments. Relative Brat1 mRNA expression was normalized against reference gene Actb or Rplp2 and calculated by Pffafl method as:

$$\mathbf{RQ} = \frac{(\mathbf{E}\ target)^{\mathbf{\Delta Ct}(target)}}{(\mathbf{E}\ reference)^{\mathbf{\Delta Ct}(reference)}}$$

Measurement of oxygen consumption rate (OCR)

OCR of human fibroblasts was measured using the XFe96 Seahorse system (Seahorse Bioscience, Billerica, MA). Cells were seeded at a density of 2 x 10^4 cells/well 24 hours before the assay. OCR was measured basally and followed with sequential addition of 1 µM oligomycin, 1.5 µM carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone (FCCP) and 1 µM antimycin A with 1 µM rotenone. Values for OCR were normalized to total protein. Protein was quantified with Pierce BCA Protein Assay (Thermo Fisher Scientific, Waltham, MA).