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## Supplemental Data

# Truncating Mutations in UBAP1 Cause Hereditary Spastic Paraplegia

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**Supplementary Material**

**Figures and Legends**

**Figure S1**

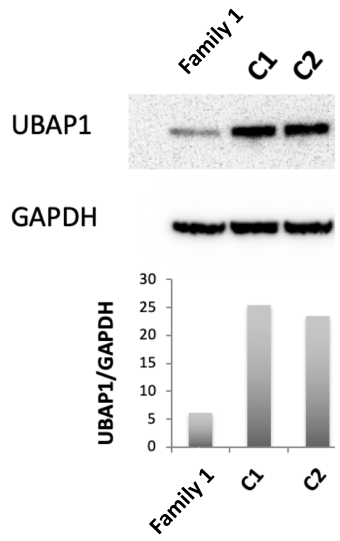


**Figure S1**. RT-PCR of a patient (Family 1) and two controls (C1, C2) from fibroblasts indicate that truncated UBAP1 mRNA escapes nonsense mediated decay.

## Figure S2

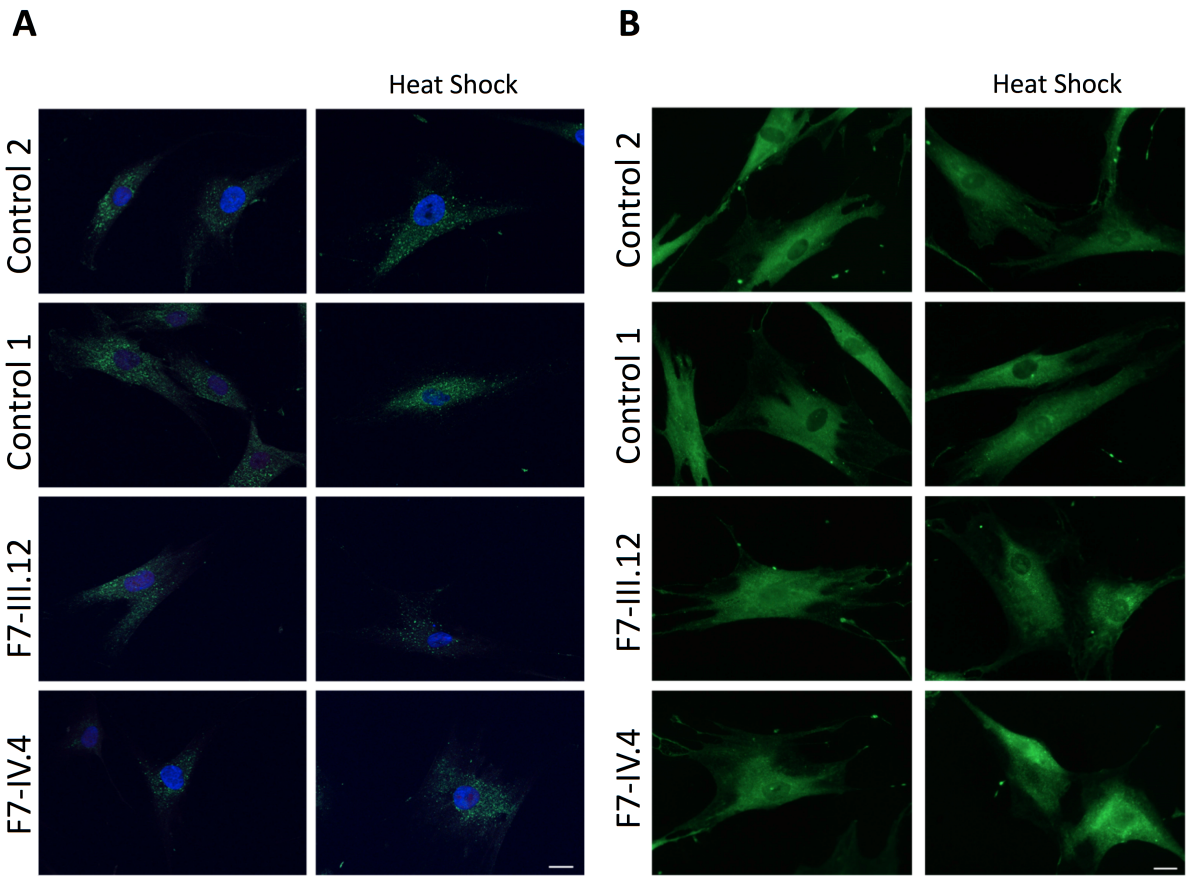


**Figure S2. Patient fibroblast immunohistochemistry studies in family 7.**

(A) Immunofluorescence staining of control and patient fibroblast lines with LAMP1 antibody under normal conditions (left) as well as after heat shock (right). Nuclei were stained with DAPI, scale bar 20µm.

(B) Immunofluorescence staining of control and patient fibroblast lines with Ubiquitin (FK2) antibody under normal conditions (left) as well as after heat shock (right). Scale bar 20µm.

## Material and Methods

*Subjects and Family Members*

All affected cases studied were from non-consanguineous and unrelated families. All families gave written informed consent, and the study protocol was approved by the institutional review board of the participating institutions. Patients were clinically evaluated by Neurologists.

*Whole-Exome Sequencing*

Whole-exome sequencing was performed in the seven index individuals with autosomal-dominant HSP. The SureSelect Human All Exon Kit (Agilent) was used for in-solution enrichment, and the HiSeq 2500 instrument (Illumina) was used to produce 100 bp paired-end sequence reads. The Burrows-Wheeler aligner and Freebayes were used for sequence alignment and variant calling. Exome data were uploaded into the GENESIS software and analyzed with strict filtering approach for heterozygous variants. Sanger sequencing confirmed segregation of the loss-of-function variants detected by whole-exome sequencing in the seven HSP families.

*Western Blot*

Fibroblasts from affected individual from family 1 were cultured and cell lysates were collected for Western blot analysis. The following antibodies were used to detect UBAP1 and GAPDH: Rabbit polyclonal anti-UBAP1 antibody (Abcam) and mouse monoclonal GAPDH antibody (Santa Cruz).

*Reverse Transcription Polymerase Chain Reaction (RT-PCR) and Sanger Sequencing*

Total RNA was isolated from patient's fibroblast using the RNeasy Plus Mini Kit (Qiagen). cDNA was synthesized from purified RNA with the SuperScript™ III First-Strand Synthesis SuperMix (ThermoFisher). Primers were used to amplify mutation site including exons 4 and 5 and flanking

intronic region: 5'-CCACAATGCCACCTCCTAT-3' (forward) and 5'-AGAATAGGCCTGGGGACA-3' (reverse). Polymerase chain reaction (PCR) was performed with Platinum Taq (ThermoFisher) and PCR products were purified with the PCR purification kit (Qiagen). Sanger sequencing was performed by Eurofins Genomics, and trace files were analyzed with the Sequencher software.

*Plasmids transfection and immunofluorescence*

Plasmid encoding open-reading frame of UBAP1 transcript 1 (NM_016525.4) fused with a 3X-HA tag in the N-terminus end of the protein was obtained from Genecopoeia. Plasmid encoding VPS28 transcript (NM_016208.3) fused to a Myc tag at the C-terminus region was also obtained from Genecopoeia. Site-direct-mutagenesis was performed to generate frameshift mutation (HA-FS-UBAP1) corresponding to variant c.361dupC (p.Leu121Profs*18). One base insertion was introduced by PCR using the Q5 Site-Direct Mutagenesis kit (NEB). U2OS cells were transfected with Lipofectamine 3000. Cells were plated on cover slips and immonostained with anti-HA and anti-myc (Cell signaling) and fluorescent antibodies, Alexa fluor 488 and Alexa fluor 555 (Invitrogen). Cells were imaged by confocal microscopy (LSM710).

*Co-immunoprecipitations*

HEK293T cells were transiently transfected with VPS28-Myc and HA-WT-UBAP1 or HA-Fs-UBAP1. After 24hrs cells were harvested in IP lysis/wash buffer (Thermofisher) and incubated on ice for 5 minutes. Cell debris was removed by centrifugation (13,0000g). Co-immunoprecipitation was performed according to manufacturer's protocol. Briefly, Antibodies (HA, Myc and IgG) were incubated with magnetic Dynabeads (ThermoFisher) for 10 minuntes and cell lysates were incubated with the dynabeads-antibody complex for 10 minutes. Dynabeads were washed three times with washing buffer and eluted with elution buffer.

*Zebrafish husbandry*

Experiments were carried out using transgenic stain Tg(olig2::DsRed).[1] Adults were kept on a 14-h light/10-h dark cycle at 28 °C. Embryos were collected from natural crosses after removing a divider at the beginning of the light cycle. Embryos were raised in Petri dishes in the system water at 28 °C under standard conditions. All experiments were conducted in accordance with University of Miami Institutional Animal Care and Use Committee guidelines. For live imaging embryos were anesthetized at 48 hpf with 0.02% tricaine methanesulfonate (Sigma).

*sgRNA design and synthesis*

sgRNAs were chosen among top targets with NGG PAM sites generated by the CHOPCHOP software (http://chopchop.cbu.uib.no/) with zero predicted off-targets with at least three mismatches in the 20-mer. The target exons chosen were exon 4 and exon 7 (two guides per exon). sgRNAs were generated by the oligonucleotide assembly method as described in Varshney *et al*.[2] RNAs were synthesized using the HiScribe™ T7 Quick High Yield RNA Synthesis Kit (New England Biolabs) with an incubation time of 12 h for the *in vitro* transcription reaction. RNAs were purified with the RNA Clean & Concentrator™-5 kit (Zymo Research) and eluted with 15 µl water and diluted to working concentrations ~ 200 pg/µl. F

*hRNA rescue synthesis*

hRNA was synthesized from plasmid encoding wild-type and truncated mutant UBAP1 (family 4, p.Leu121Profs*18) using mMessage mMachine$^{tm}$ T7 Ultra kit (Invitrogen).

*Microinjections*

Microinjections were performed into embryos at one-cell stage. Cas9 protein (PNA Bio) and pooled sgRNA were mixed with 1% Fast Green dye (Sigma) and incubated for 5 min at 37 degrees Celsius. For the rescue injections mixtures were supplemented with either wild-type or mutant synthesized human RNA (hRNA). Approximately 1.5 nL of active sgRNA-Cas9 ribonucleoprotein complex plus hRNA were injected per embryo into the cell. The final amounts injected per embryo approximately were: 350 pg of Cas9 protein; 150 pg of sgRNA pool, 50 pg of hRNA. At least three independent injection experiments were performed with spawns from different founder fish.

*In vivo imaging of motor neurons*

Motoneuron outgrowth was assayed at 48 h.p.f. Live fish were dechorionated by tweezers and anesthetized with tricaine methanesulfonate, embedded in 1% low-melting point agarose and imaged using a Leica confocal microscope with a 20× air lens. 1-µm *z* stacks were imaged between myotome segments 6 and 13, and the motoneuron morphology was evaluated for its normal shape and outgrowth trajectory.[3] Images were processed with Fiji software (ImageJ). LUT:edges was used to generate the figure.

*CRISPR efficiency testing by Fragment analysis*

Embryos were euthanized and DNA was extracted using 50 mM NaOH digestion at 95 degrees Celsius for 20 min. DNA then was used to run Fluorescent PCR as described in Varshney *et al*.[2] The reaction products were run on a Genetic Analyser 3130xl using POP-7 polymer and analyzed for the disruption of the wild-type peaks as described in Carrington *et al*.[4]

*Statistical analysis*

Images from three separate experiments were blindly evaluated for qualitative inclusion into either "normal" group or "affected" group. The normal group was assigned to images with motor axons shaped into normal hooks as in images of uninjected controls. The affected group was assigned to images with any amount of drastically misshaped or truncated axons. The Fisher exact test was performed for these two groups comparing the wild-type rescue injected group with the mutant rescue group. Differences in the number of observations were considered significant at $p \leq 0.05$. To quantify the motoneuron axon length the Simple Neurite Tracer plugin in Fiji was used for tracing.[5] Four axons per sample embryo from the same area closer to the yolk were traced, and the statistical comparison was performed by using one-tailed Student's t test with the p-value considered significant under $p \leq 0.005$.

**References**


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