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Novel somatic UBA1 variant in a patient with VEXAS syndrome

Blanka Stiburkova, PhD[1,2], **Katerina Pavelcova, PhD**[1], **Monika Belickova, PhD**[3], **Samuel J. Magaziner, MPhil**[4], **Jason C. Collins, PhD**[5], **Achim Werner, PhD**[5], **David B. Beck, MD PhD**[4,7], **Veronika Balajkova, MD**[1], **Cyril Salek, MD PhD**[3,6], **Martin Vostry, MSc**[3], **Herman Mann, MD PhD**[1], **Jiri Vencovsky Prof, MD PhD**[1]

[1]Institute of Rheumatology and Department of Rheumatology, First Faculty of Medicine, Charles University, Prague, Czech Republic

[2]Department of Pediatrics and Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic

[3]Institute of Hematology and Blood Transfusion, Prague, Czech Republic

[4]Center for Human Genetics and Genomics, New York University School of Medicine, New York, New York, United States

[5]National Institute for Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD, United States

[6]Institute of Clinical and Experimental Haematology, First Faculty of Medicine, Charles University, Prague, Czech Republic

[7]Division of Rheumatology, Department of Medicine, New York University School of Medicine, New York, New York, United States

Abstract

Objective—Somatic mutations in UBA1 have recently been causally linked to a severe adult-onset inflammatory condition referred to as VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome. UBA1 is of fundamental importance to the modulation of ubiquitin homeostasis and to the majority of downstream ubiquitylation-dependent cellular processes. Direct sequencing analysis of exon 3 containing prevalent variants p.Met41Leu, p.Met41Val, and/or p.Met41Thr is usually used to confirm the disease associated mutations.

Corresponding Author: Blanka Stiburkova, Institute of Rheumatology, Na Slupi 4, 120 00 Prague 2, Czech Republic, stiburkova@revma.cz.

Authors' contributions

VB, HM, JV and CS contributed to the clinical observation of the study. BS, KP, MB and MV contributed to the acquisition of data of the study. DB, SJM, AW, and JCC performed the functional study. BS, KP, and MB contributed to the analysis and interpretation of sequencing data of the study. All authors were involved in drafting the manuscript or revising the content. All authors approved the final version for publication.

Conflicts of interest

The authors declare no conflicts of interest.

Ethics approval and consent to participate

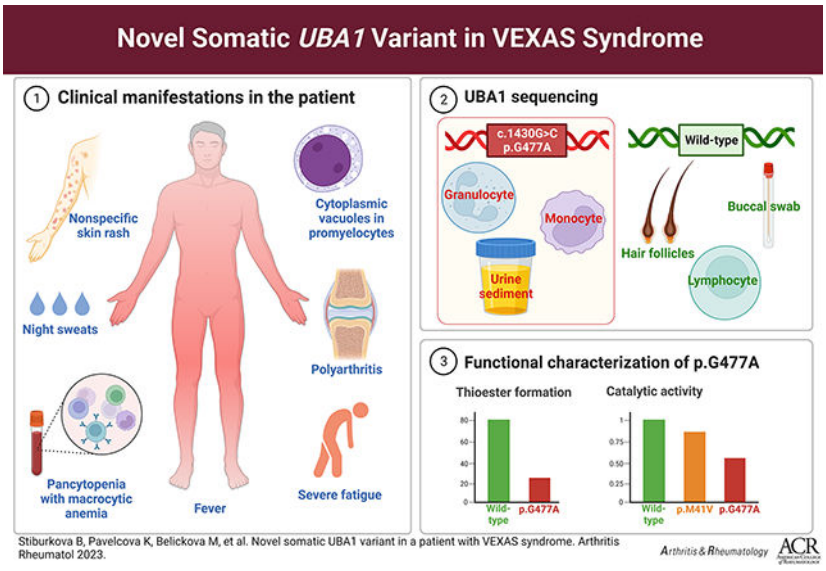
This study was approved by the Ethics Committee of the Institute of Rheumatology (reference number 11058/2021). Patient was fully informed of the aim of the study, and written informed consent was obtained.

Methods—We studied clinical, biochemical and molecular genetic characteristics of a fifty-nine-year-old male with two-year history of arthritis, fever, night sweats, nonspecific skin rash, lymphadenopathy, and myelodysplastic syndrome with multilineage dysplasia.

Results—The mutational analysis revealed a hitherto undescribed sequence variant c.1430G>C in exon 14 (p.Gly477Ala) in *UBA1* gene. *In vitro* enzymatic analyses showed that p.Gly477Ala led to both decreased E1 ubiquitin thioester formation and E2 enzyme charging.

Conclusion—We herein report a case of a patient of European ancestry with clinical manifestations of VEXAS syndrome associated with a newly identified dysfunctional variant UBA1 enzyme. Due to insufficient response to various immunosuppressive treatments, allogeneic hematopoietic stem cell transplantation was performed, which resulted in significant improvement of clinical and laboratory manifestations of the disease.

Graphical Abstract



Introduction

Somatic post-zygotic variants in *UBA1* (ubiquitin like modifier activating enzyme 1, ID HGNC:12469; OMIM * 314370) have been causally linked to a severe adult-onset inflammatory condition referred to as VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome (1–3). VEXAS syndrome (OMIM # 301054) affects mostly adult males older than 50 years. Multisystem inflammation most commonly affects the skin, eyes, joints, blood vessels, cartilage, and lungs (4). Patients with VEXAS syndrome may meet diagnostic criteria for several rheumatic diseases (5) and/or hematological disorders (6). VEXAS syndrome is associated with a high risk of thromboembolic events (7). Common laboratory changes include CRP elevation and macrocytic anemia. Presence of cytoplasmic vacuoles in myeloid and erythroid precursor cells in bone marrow seems to be a universal feature in VEXAS patients (8). VEXAS syndrome is associated with high morbidity and mortality. Optimal management strategy is not yet known (9). Limited evidence suggests that hematopoietic stem cell transplantation (HSCT) may be curative (10).

UBA1 gene is located on the Xp11 chromosome and encodes the most prevalent ubiquitin-activating E1 enzyme crucial in the process of ubiquitylation. The UBA1 protein is large and requires a coordinated effort to activate and transfer ubiquitin to an E2 conjugating enzymes. UBA1 enzyme has two isoforms: UBA1a - the long isoform starting from codon 1 (Met1) of UBA1 localized in the nucleus, and UBA1b - the short isoform starting from codon 41 (Met41) localized in the cytoplasm without a nuclear localization signal. Ubiquitylation is a multi-step post-translational modification that triggers proteasomal degradation, and is essential for multiple cellular processes such as cell cycle progression, DNA damage response, and immune signaling pathways (11). Dysregulation of the ubiquitin-proteasome system results in many disease states, such as lymphoproliferative disorders, malignancy, and autoinflammatory diseases (12).

Somatic mutations found in VEXAS syndrome, mostly missense mutations at the start codon for UBA1b (Met41), lead to a reduction in cytoplasmic UBA1 function and the resultant decreased ubiquitylation activates the unfolded protein response and type I interferon production (11). Patients with VEXAS syndrome showed highly activated inflammatory signatures in multiple pathways, including tumor necrosis factor, interleukin-6, interferon-γ, and interleukin-8 (2).

The diagnosis of VEXAS syndrome in patients with clinically suspicious phenotypes depends solely on the detection of *UBA1* variants confirmed by Sanger sequencing, the next-generation sequencing, including whole-genome sequencing and whole-exome sequencing. Direct sequencing analysis of exon 3 containing variants P.Met41Leu, p.Met41Val, and/or p.Met41Thr is usually used to confirm the disease associated mutations.

In this study we aimed to identify a novel variant in protein-coding regions of *UBA1* outside of exon 3 which could be associated with clinical features of the VEXAS syndrome observed in our patient and perform a functional characterization study to provide a causal link between a hitherto undescribed variant c.1430G>C (p.Gly477Ala, exon 14) in the *UBA1* gene and a VEXAS phenotype.

Patient and Methods

A fifty-nine-year-old male was evaluated in May 2019 for a two-year history of intermittent arthralgias, fever, stiffness, severe fatigue, night sweats, nonspecific skin rash, and lymphadenopathy. Laboratory tests showed mild normocytic anemia, leukopenia due to neutropenia, thrombocytosis, and elevated acute phase reactants. Histological examination of the lymph node revealed reactive changes only. Initial bone marrow (BM) aspirate contained 5% of blasts with normal karyotype, no cytoplasmic vacuoles were present in myeloid precursor cells. Skin biopsy revealed only non-specific perivascular infiltrate without signs of vasculitis. Patient responded well to initial treatment with high-dose oral glucocorticoids; however, the symptoms recurred after tapering to ≤ 15mg prednisolone daily. His condition further deteriorated over the next two years, during which he continued to have various forms of skin rash, developed persistent polyarthritis, pancytopenia with macrocytic anemiaand became red blood cell transfusion-dependent (Figure 1A–C).

Treatment with methotrexate, infliximab, and tofacitinib was ineffective. Tocilizumab only partially alleviated his symptoms.

Targeted gene sequencing of 54 genes involved in hematological malignancies by the TruSight Myeloid Sequencing Panel Kit (Illumina, San Diego, CA, USA) revealed the variant c.394C>T (rs121913499; p.Arg132Cys, NM_005896.3) in *IDH1* gene with variant allele frequency (VAF) of 31% in the patient's bone marrow DNA. A diagnosis of clonal cytopenia of undetermined significance was made. Repeated bone marrow biopsy showed dysplastic changes corresponding to the diagnosis of myelodysplastic syndrome with multilineage dysplasia (Figure 1D), cytoplasmic vacuoles in the promyelocytes, and VAF of IDH1 mutation increased to 47%.

Based on the clinical, laboratory, and genetic findings, a diagnosis of the newly described VEXAS syndrome was considered. We performed a sequencing analysis focusing on the *UBA1* gene. Genomic DNA was extracted from EDTA whole blood using a QIAmp DNA Mini Kit (Qiagen, GmbH., Hilden, Germany). Mononuclear cells and granulocytes were separated by Ficoll-Paque (Sigma, St Louis, MO, USA) density gradient centrifugation. The MACS kits (Myltenyi Biotech, Bergisch Gladbach, Germany) were used for isolation of CD3+ (T-lymphocytes), CD14+ (monocytes), and CD19+ (B- lymphocytes) cells according to manufacturer's protocol. All coding regions of the *UBA1* gene, including intron-exon boundaries, were amplified using PCR and purified using a PCR DNA Fragments Extraction Kit (Geneaid, New Taipei City, Taiwan). DNA sequencing was performed with a DNA sequencer (Applied Biosystems 3130 Genetic Analyzer; Thermo Fisher Scientific, Waltham, MA, USA). Primer sequences and PCR conditions are available upon request. The reference sequence was defined as version ENST00000335972. Prediction of the possible impact of finding non-synonymous allelic variants on protein function was determined using PolyPhen, Provean, Mutation Taster, SIFT, Human Splicing Finder and MutPred predictive software. Next, the detected mutation in the *UBA1* gene was verified by the NGS method using SureSelectXT HS Reagent Kits (Agilent, Technologies, Santa Clara, CA, USA).

Written informed consent was obtained from the proband prior to enrollment in the study. All the procedures were performed in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of the Institute of Rheumatology (reference number 11058/2021).

Immunoblotting from Chinese Hamster Ovary cells

Parental Chinese hamster ovary (CHO) cell line (e36) and temperature-sensitive UBA1 knockdown CHO cell line (ts20) were cultured in complete CHO ts20 medium (MEM α [Gibco, 12571063] supplemented with 1.8 g/mL glucose, 10% FBS, and Penicillin-Streptomycin 100 U/mL) and maintained at 30.5°C with 5% carbon dioxide (13). CHO ts20 cell lines were generated by lentiviral transduction in complete CHO ts20 medium supplemented with 1.6 ug/mL polybrene using lentiviral particles (described in 2) containing pHAGE HA-FLAG HA tagged WT UBA1, M41V or G477A. Stable expressing clones were selected in 5 ug/mL puromycin and confirmed via whole cell lysate immunoblot against the HA tag (Biolegend, 901501).

Cell lines for UBA1, E2, and poly-ubiquitin characterization were collected by trypsinization, counted, resuspended in complete CHO ts20 medium with normalization of one million cells per mL of media. 1 mL of resuspended cells were then transferred to 1.5 mL microcentrifuge tubes and moved to 39.5°C for 6 hours at 500 rpm restrictive temperature studies. Following incubation, heat treated samples extracts were generated in urea-SDS lysis buffer. Whole cell lysates were separated by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis and analyzed by immunoblotting. Primary antibodies for UBA1 (Cell Signaling, 4891S), UBED3 (Protein Tech, 116771–1-AP), and β-actin (Cell Signaling, 4970) were used at a concentration of 1:1000 and visualized using HRP-conjugated secondary antibodies (anti-rabbit [Cell Signaling, 7074S] or anti-mouse [Cell Signaling, 7076S]).

In vitro enzymatic activity performed as in Beck DB et al. (2).

Results

The genetic testing of exon 3 of the *UBA1* gene in genomic DNA did not identify any difference compared to the reference by direct Sanger sequencing. Extended investigation of gDNA isolated from granulocytes did not differ from the reference sequence as well (Figure 1E). An analysis of all coding regions of the *UBA1* gene revealed a hitherto undescribed sequence variant c.1430G>C in exon 14 (p.Gly477Ala) in heterozygous (cells of urine sediment) and homozygous states (monocytes, Figure 1E) depending on the tissue source of genomic DNA (Figure 1F). Variant c.1430G>C was not detected in gDNA from cells of lymphoid lineages (T and B lymphocytes), cells from buccal swab, and hair follicles. No other exonic variants were identified. Novel variant p.Gly477Ala has not yet been identified in the *UBA1* gene; however, the nature of this substitution strongly suggested pathogenicity: eight software for the prediction of the effects on protein function (Provean, PolyPhen2, MutPred2, PredictSNP, MAPP, SIFT, SNAP, and PhD-SNP) identified p.Gly477Ala as deleterious with high confidence. The variant was verified by NGS with variant allele frequency of 92%.

To examine the effect of p.Gly477Ala variant on the expression and function of the UBA1 protein, functional characterization of this novel variant was performed using both recombinant purified enzyme, and *in vivo* transfection experiments. First, using purified UBA1 enzyme we observed a significant thioester formation defect with p.Gly477Ala as compared to wild-type (WT) UBA1, similar to the reported defect with canonical mutations (Figure 2A–B) (2). We also performed complementation or rescue experiments to assess whether these p.Gly477Ala altered UBA1 activity using a Chinese hamster ovary (CHO) cell line, where endogenous UBA1 can be destabilized at restrictive temperatures, and ubiquitylation and E2 charging are impaired (13). Wild-type, *UBA1* variants rescued E2 charging (E2-Ub/E2) but p.Met41Val and p.Gly477Ala did not, demonstrating that p.Gly477Ala led to a catalytic defect *in vivo* (Figure 2C–D). These results together with the clinical phenotype support that p.Gly477Ala is a VEXAS-causing variant.

The identification of a novel somatic variant of UBA1 and the failure of response to various immunosuppressive treatments led to the recommendation of HSCT as a treatment

option for our patient. Allogeneic peripheral blood progenitor cell (PBPC) transplantation from an HLA and ABO matched unrelated donor was performed after a myeloablative preparative regimen of fludarabine and treosulfan (GvHD prophylaxis: ATG, cyclosporine, mycophenolate). Eight months after HSCT, patient's clinical condition has significantly improved, he is free of previously present severe disease manifestations, he is no longer transfusion dependent and his chronically elevated C-reactive protein levels have normalized. He is currently receiving cyclosporine and valacyclovir as a standard regimen after HSCT along with a low dose of hydrocortisone. Sequencing analysis of the *UBA1* gene, exon 14, from gDNA isolated 21 days after HSCT revealed the reference sequence only; the p.Gly477Ala variant was not identified in white blood cells. Vacuolization of hematopoietic precursor cells was no longer evident in control BM biopsy (Supplemental Figure).

Discussion

VEXAS syndrome is a recently described acquired monogenic disease in adults associated with somatic mutation of UBA1 in hematopoietic progenitor cells that leads to systemic inflammation with multiple organ involvement. The ubiquitin-activating enzyme E1, UBA1, functions at the top of the enzymatic ubiquitination cascade and catalyzes ubiquitin activation. Mutations at Met41 variants of UBA1 identified in VEXAS patients promote the formation of a functionally defective cytoplasmic isoform of UBA1, UBA1c. Mutant cells derived from VEXAS patients exhibit loss of ubiquitination, resulting in upregulation of stress and unfolded protein responses, as well as dysregulation of autophagy.

Other mutations in *UBA1* with functional consequences have been recently described. p.Ser56Phe (NM_153280:c.167C>T) variant was identified predominantly in the myeloid lineage populations, whereas B- and T-cell lymphoid lineages were predominantly wild-type (14). Variant p.Ser56Phe did not affect UBA1 cellular localization or resulted in isoform expression abnormalities in HEK293 cells transfected with the mutant transcript, however, the functional study showed the temperature-dependent impairment of UBA1 catalytic activity. Another novel variant c.118–1G>C was identified at the splice acceptor site of exon 3, and analysis of patient-derived RNA revealed a reduction in properly spliced transcript and the creation of multiple incorrectly spliced products leading to production of UBA1c (14).

Here we describe another novel variant p.Gly477Ala that leads to significantly decreased catalytic activity, similar to other reported pathogenic variants for VEXAS syndrome.

A growing number of reports suggest that VEXAS syndrome is not rare. In patients with clinical suspicion, typically *UBA1* sequencing analysis of hematopoietic cells is performed in hot spot sites of p.Met41 in exon 3 only. We propose that an extended analysis of all coding regions of the *UBA1* gene may uncover other mutations with putative functional consequences. Confirming the diagnosis of VEXAS syndrome by genetic analysis may have important therapeutic consequences. Detection of a pathogenic *UBA1* variant facilitates the decision to refer patients for a potentially curative HSCT, as was the case with our patient. The goal of HSCT in VEXAS is to eradicate the pathogenic *UBA1* clone, thus eliminating

the cause of the disease. Given the associated risks, the procedure is currently reserved for patients after failure of immunomodulatory therapy (15). Many unanswered questions regarding the indication and optimal timing of HSCT remain. An ongoing clinical trial should help to position HSCT in the treatment algorithm of VEXAS syndrome. In our patient, HSCT resulted in significant clinical improvement and led to full donor chimaerism 8 months after the procedure with only minimal complications.

In conclusion, we identified a novel variant c.1430G>C in the *UBA1* gene in a patient with VEXAS syndrome. A series of functional assays revealed that this UBA1 p.Gly477Ala is functionally deficient. Together with several other described mutations in the UBA1 gene with functional consequences on the UBA1 protein, our finding strongly supports a pathogenic impact on disease development. Detailed genetic investigation of *UBA1* gene in suspicion of VEXAS is highly recommended. Response to therapy in VEXAS syndrome is usually poor and HSCT may have a curative effect.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Availability of data and materials

Original data on sequencing analysis are available upon request.

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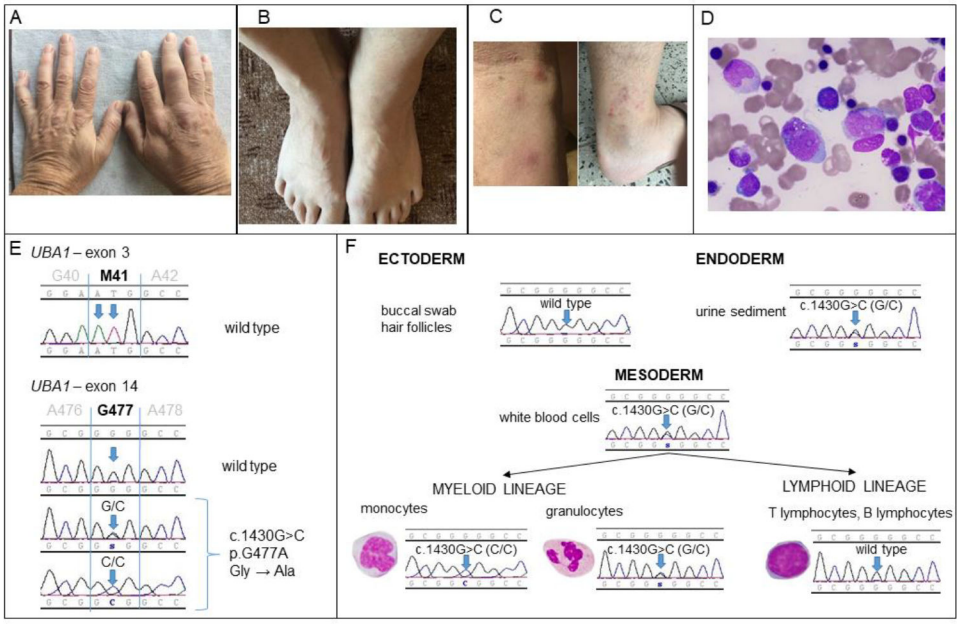

Figure 1:

Clinical manifestations and genetic background of the VEXAS syndrome in our patient.

- A) and B) Severe asymmetrical polyarthritis.
- C) Skin lesions included signs of neutrophilic dermatosis and livedo racemosa.
- D) The representative bone marrow aspirate smears showing dysplasia and characteristic cytoplasmic vacuolation in a promyelocyte (May-Grunwald Giemsa stain).
- E) Electropherograms of sequence of exon 3 showing reference sequence of UBA1: p.Met41 and of exon 14 showing UBA1: p.Gly477 in reference state and new variant p.Gly477Ala (c.1430G>C) in heterozygous and homozygous state, transcript ENST00000335972.
- F) Electropherograms of a partial sequence of exon 14 showed mosaicism of variant p.Gly477Ala (c.1430G>C).

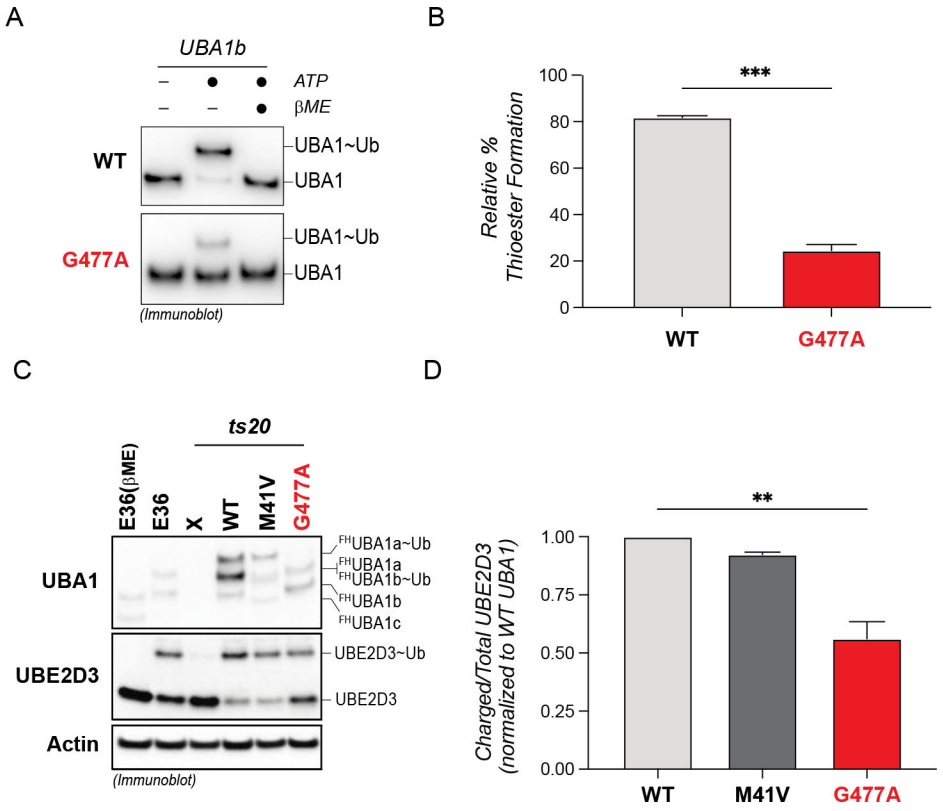

Figure 2:

UBA1 G477A has impaired catalytic activity *in vitro* and *in vivo*

- A) Recombinant UBA1b G477A has impaired thioester formation as compared to wildtype (WT). bME used as a reducing agent to eliminate charged species.
- B) Quantification of 3 biologic replicates for A) with *** p<.05 (.003) with Welsch's t-test.
- C) Chinese hamster ovary cells (CHO) either parental (E36) or temperature sensitive (ts20), were grown at 39.5° C for 6 hr where ts20 demonstrates loss of UBA1 and loss E2 charging (UBE2D3 upper band). ts20 were either untreated (X) or transduced with FLAG-HA tagged (FH) UBA1 WT, M41V, or G477A. bME used as a reducing agent to eliminate charged species. Actin used as an immunoblotting control.
- D) Quantification of 3 biologic replicates for A) with ** p<.05 (.0058) with Welsch's t-test.