Supplemental Online Content

Beck DB, Bodian DL, Shah V, et al. Estimated Prevalence and Clinical Manifestations of *UBA1* Variants Associated With VEXAS Syndrome in a Clinical Population. *JAMA*. Published online January 24, 2023. doi:10.1001/jama.2022.24836

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

eMethods:

Additional sequencing information:

NimbleGen probes (SeqCap VCRome) and xGEN probes from Integrated DNA Technologies (IDT) and were used for target sequence capture. Sequencing was performed by paired end 75bp reads on an Illumina NovaSeq or HiSeq at coverage >20x at >80% of the targeted bases. Alignments and variant calling were based on GRCh38 human genome reference sequence. Variants were called with the WeCall variant caller version 1.1.2. (https://github.com/Genomicsplc/wecall).

Variant detection/interpretation:

All genotype calls of pathogenic and likely pathogenic variants supported by ≥3 alt reads with genotype quality ≥30 were selected for analysis. Two of the patients had low variant allele fraction (VAF) (<0.10), which can be associated with lower confidence genotype calls. However, both were sequenced twice, on two different exome platforms, and variants were detected in both sequencing results, and both patients had symptoms consistent with VEXAS syndrome. Low VAF variant was identified in P9 using mutect2, and manual analysis, although this variant caller was not used for the entire cohort.

Clinical definitions:

Disease onset date refers to first symptoms related to VEXAS syndrome mentioned in the chart. Given that patients were examined at different times, by different physicians, it is possible that clinical diagnoses were present but not assessed or accurately reported leading to missing values and participants lost to follow-up. This is apparent when evaluating cause of death, which was only identified in a small subset of patients (5/9).

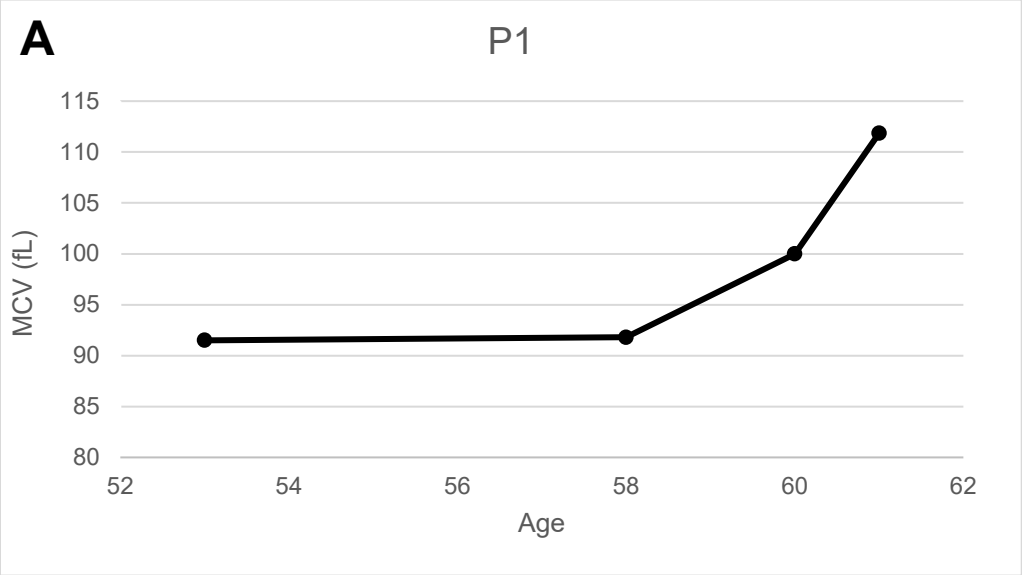
Immunoblotting from Chinese Hamster Ovary cells:

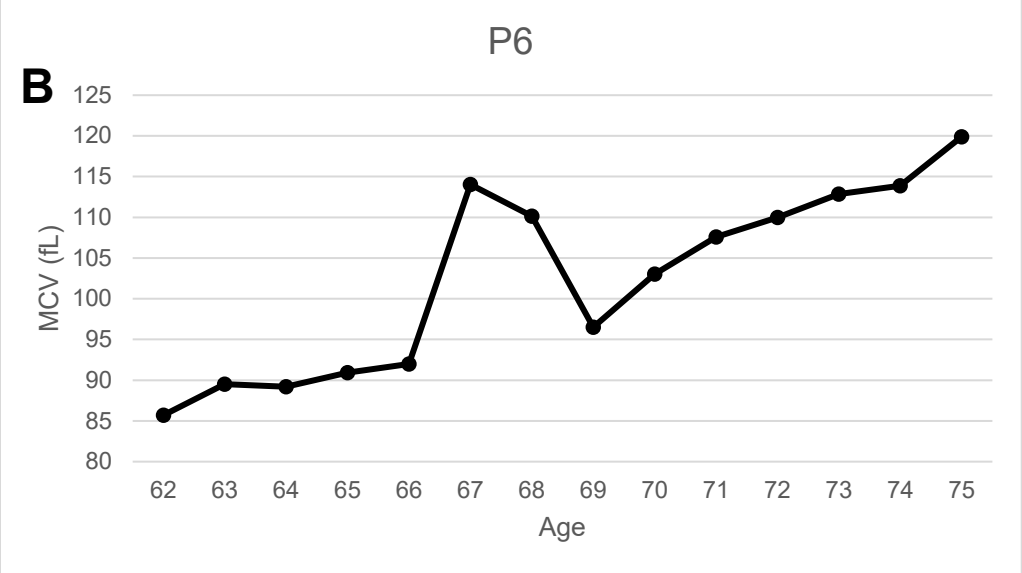
Parental Chinese hamster ovary (CHO) cell line (e36) and temperature-sensitive UBA1 knockdown CHO cell line (ts20) were cultured in complete CHO ts20 medium (MEM α [Gibco, 12571063] supplemented with 1.8 g/mL glucose, 10% FBS, and Penicillin-Streptomycin 100 U/mL) and maintained at 30.5°C with 5% carbon dioxide. [17]

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 elsewhere[5]) containing pHAGE FLAG-HA tagged WT UBA1, Ser621Cys (S621C), Gly213Val (G213V), or Val75Ile (V75I). Stable expressing clones were selected in 5 ug/mL puromycin and confirmed via whole cell lysate immunoblot against the HA tag (Biolegend, 901501).

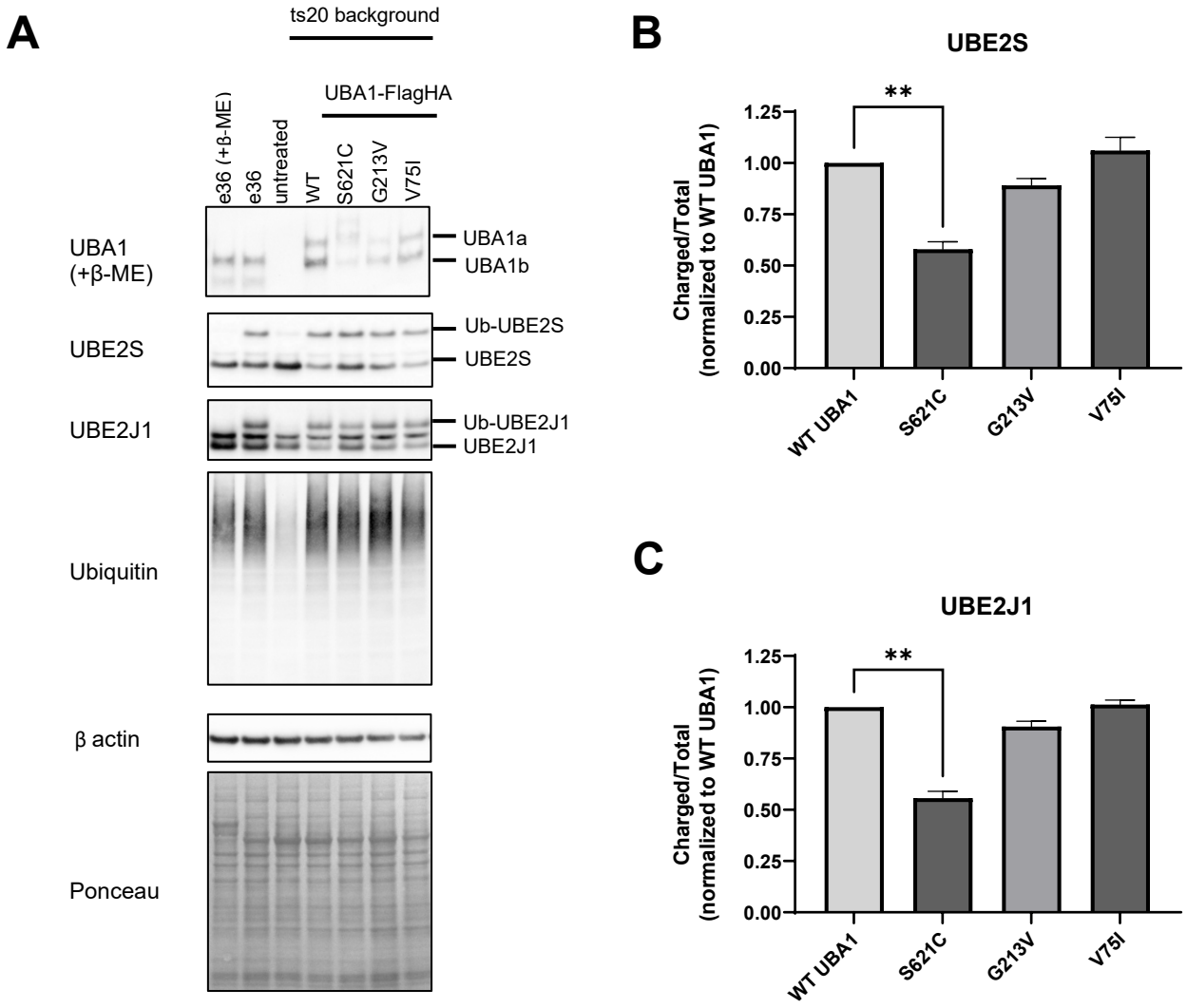
Cell lines for UBA1, E2, and poly-ubiquitin characterization were collected by trypsinization, counted, resuspended in complete CHO ts20 medium with normalization of one million cells per mL of media. 1 mL of resuspended cells were then transferred to 1.5 mL microcentrifuge tubes and moved to 39.5 ° for 6hrs at 500rpm. Following incubation, heat treated samples proteins were extracted 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), UBE2S (Protein Tech, 14115-1-AP), UBE2J1 (Invitrogen, MA5-26025), Poly-ubiquitin (Cell Signaling, 3936S), 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]).

eFigures:





eFigure 1. Macrocytosis in individuals with *UBA1* pathogenic variants. Progressive macrocytic anemia in two representative individuals with VEXAS syndrome are shown for (A) patient P1 and (B) patient P6.



eFigure 2. UBA1 mutant S621C shows decreased Ubiquitin-conjugating enzyme (E2) charging *in vitro*. A) Parental Chinese hamster ovary (CHO) cell line (e36), temperature-sensitive UBA1 knockdown CHO cell line (ts20), and CHO ts20 cell lines containing lentiviral transduced human *UBA1* variants (WT *UBA1*, S621C, G213V, and V75I) were incubated for 6 hours at the restrictive temperature of 39.5°C. Whole cell lysates were immunoblotted for UBA1, UBE2S, UBE2J1, poly-ubiquitin and b-actin were visualized. Note the loss of poly-ubiquitin and ubiquitin-charged UBE2S and UBE2J1 enzymes in the absence of UBA1 the lane containing only ts20. Also note the increased intensity of the uncharged UBE2S and UBE2J1 bands in the lane containing ts20

expressing S621C. β-actin and a ponceau stain are provided as loading controls. No samples contain a reducing agent (β-ME) except as noted in order to visualize the ubiquitin-charged (Ub) isoforms. B/C) Graphical representations of 3 biologic replicate Western blots of the E2 enzymes UBE2S and UBE2J1 in the presence of WT UBA1, S621C, G213V, or V75I. Values represent band intensity of ubiquitylated products over total enzyme (ubiquitylated + non-ubiquitylated) and are normalized to WT UBA1. Both UBE2S and UBE2J1 demonstrate decreased ratios of ubiquitylated species with UBA1 S621C compared to WT UBA1. G213V and V75I show no appreciable change in charged/total E2 ratio. ** p values (UBE2S, p=0.0024; UBE2J1, p=0.0019) are based on paired parametric t-tests using Welch's correction.

eTable 1.

Hematologic	Autoimmune	Dermatologic	Pulmonary
Other anemias, Other aplastic anemias and other bone marrow failure syndromes, Myelodysplastic syndromes, Myeloid leukemia, Iron deficiency anemia, Vitamin B12 deficiency anemia, Other aplastic anemias and other bone marrow failure syndromes, Other and unspecified diseases of blood and blood-forming organs, Purpura and other hemorrhagic conditions, Other disorders of white blood cells, Other peripheral vascular diseases, Arterial embolism and thrombosis, Other venous embolism and thrombosis, Other abnormal immunological findings in serum, Neutropenia, Enlarged lymph nodes	Other joint disorder, not elsewhere classified, Elevated erythrocyte sedimentation rate and abnormality of plasma viscosity, Other and unspecified arthropathy, Synovitis and tenosynovitis, Other necrotizing vasculopathies, Fever of other and unknown origin, Pyogenic arthritis, Other systemic involvement of connective tissue, Other inflammatory liver disease, Other nectrozing vasculopathies	Other disorders of skin and subcutaneous tissue, not elsewhere classified, Cellulitis and acute lymphangitis, Dermatopolymyositis, Other and unspecified dermatitis, Erythema nodosum	Other interstitial pulmonary diseases, Other symptoms and signs involving the circulatory and respiratory system, Respiratory failure, not elsewhere classified, Emphysema, Other chronic obstructive pulmonary disease, Sarcoidosis, Pleural effusion, not elsewhere classified, Bronchitis, not specified as acute or chronic, Acute bronchitis, Abscess of lung and mediastinum

eTable 1. Structured ICD10 code EHR queries for individuals with *UBA1* pathogenic/likely pathogenic variants. Summary level data for ICD10 codes, identified within *UBA1* pathogenic variant carriers, divided by clinical subspecialty. These ICD10 codes form the basis for the clinical characteristics in Table 3.

eTable 2.

	Hematologic			Autoimmune				Dermatologic	Pulmonary		Other		
Patient ID	**VEXAS Flare**	**DVT/PE**	**Stroke**	**Eye inflammation**	**Arthritis**	**Periorbital edema**	**Skin involvement**	**Pulmonary infiltrates**	**Pleural effusion**	**Pericardial effusion**	**Hearing loss**	**Prior transfusion**	**Cause of Death**
1	Yes	No	No	No	Yes	No	Yes	Yes	Yes	Yes	No	No	Sepsis
2	Yes	No	No	No	No	No	No	Yes	No	No	No	Yes	Alive
3	No	No	No	No	No	No	No	Yes	No	No	Yes	Yes	Respiratory Failure (PNA)
4	No	No	Yes	Yes	No	No	Yes	Yes	No	No	Yes	No	Sepsis
5	Yes	No	No	No	Yes	No	Yes	No	No	No	No	No	Alive
6	No	No	No	No	Yes	No	No	No	No	No	No	Yes	Unknown (Found Unresponsive)
7	No	No	No	No	Yes	No	Yes	No	Yes	No	Yes	No	Unknown
8	Yes	Yes	Yes	No	Yes	Yes	Yes- Erythema Nodosum	No	Yes	Yes	No	Yes	Unknown
9	No	No	No	No	Yes	No	No	Yes	No	Yes	No	Yes	Respiratory Failure (ILD)
10	No	No	Yes	No	Yes	No	No	Yes	No	No	Yes	No	Unknown
11	No	No	Yes	No	No	No	No	Yes	Yes	No	Yes	No	Hospice (aspiration PNA)
Total (#)	4	1	4	1	7	1	5	7	4	3	5	5	
Total (%)	36	9	36	9	64	9	45	64	36	27	45	45	

eTable 2. **Manual review of EHR for individuals with *UBA1* pathogenic/likely pathogenic variants.** Detailed data about clinical symptoms and characteristics based on both manual and structured EHR review. No patients were identified with nose, ear or costochondritis, fever of unknown origin, periodic fever syndrome, aseptic meningitis, or pericarditis on physician review. DVT= deep vein thrombosis, PE= pulmonary embolism, ILD= interstitial ling disease and PNA= pneumonia.

eTable 3.

Patient ID	# of bone marrow biopsy (BM Bx) performed	Bone marrow biopsy timing (years from symptoms onset)	Cytoplasmic vacuoles identified during re-review	Medical indications for bone marrow biopsy	Megaloblastic changes
2	4	1,1,2, 4	Yes on aspirates of 2nd and 4th biopsies but not on 1st and 3rd (aspirate aparticulate and pauci-cellular)	Pancytopenia including anemia, leukopenia, and thrombocytopenia. Macrocytic anemia was identified at a later stage.	Yes
3	1	4	Yes	Pancytopenia including macrocytic anemia, leukopenia, and thrombocytopenia	Yes
5	2	2, 4	Yes on both aspirates/biopsies	Pancytopenia including macrocytic anemia, leukopenia, and thrombocytopenia.	Yes
6	2	1, 4	No	Leukocytosis and chronic myeloid leukemia (CML) diagnosed with 1st BMBx; macrocytic anemia at second biopsy	No
8	1	3	Yes	Macrocytic anemia	No
9	1	3	Yes	Anemia (hypochromic and normocytic)	Yes

eTable 3. Bone marrow biopsy features in individuals with *UBA1* pathogenic/likely pathogenic variants. Bone marrow biopsy information for patients including number of biopsies, timing from symptom onset, presence of vacuoles and medical indication for bone marrow biopsy.

eTable 4.

#	Sex	Identified Variant	VAF	Age at Last Encounter	Status	Sample Age	ICD codes	Medications/Procedures	Rheum, Heme, Pulmonary Visits	Clinician Review
12	Male	c.1861A>T; p.Ser621Cys	0.2	80	Alive	73	Other inflammatory liver diseases, Other diseases of liver, Seborrheic dermatitis, Other and unspecified osteoarthritis, Other necrotizing vasculopathies, Other and unspecified, soft tissue disorders, not elsewhere classified, Purpura and other hemorrhagic conditions	Mycophenolate Mofetil (MMF)	R, H	VEXAS flare
13	Male	c.638G>T; p.Gly213Val	0.217	56	Alive	54	Other and unspecified soft tissue disorders, not elsewhere classified	methylprednisone, prednisone,	R	No
14	Male	c.223G>A; p.Val75Ile	0.641	60	Alive	54	Other and unspecified malignant neoplasm of skin Other and unspecified diseases of blood and blood-forming organs, other joint disorder	no medications	H, P	No

eTable 4. **Variants of uncertain significance in *UBA1* and associated phenotypes based on structured and manual review of EHR.** Demographics for Geisinger participants with variants of uncertain significance in *UBA1*. VAF= Variant allele fraction. Variants were annotated using transcript (NM_003334.4) and protein (NP_003325.2) were used for UBA1. H- hematologist, P-pulmonologist, R- rheumatologist.

Geisinger-Regeneron DiscovEHR collaboration contributors:

Goncalo Abecasis; Josh Backman; Xiaodong Bai; Suganthi Balasubramanian; Nilanjana Banerjee; Aris Baras; Mathew Barber; Christian Benner; Andrew Blumenfeld; Michael Cantor; Shan Chen; Giovanni Coppola; Amy Damask; Andrew Deubler; Lee Dobyn; Aris Economides; Gisu Eom; Manuel AR Ferreira; Arkopravo Ghosh; Lauren Gurski; Lukas Habegger; Alicia Hawes; Marcus B. Jones; Eric Jorgenson; Bindu Kalesan; Hyun Min Kang; Katia Karalis; Shareef Khalid; Jack A. Kosmicki; Dadong Li; Alexander Li; Nan Lin; Daren Liu; Adam E. Locke; Luca A. Lotta; Johathan Marchini; Anthony Marcketta; Evan K.

Maxwell; Joelle Mbatchou; Lyndon J Mitnaul; Arden Moscati; Colm O'Dushlaine; John D. Overton; Charles Paulding; Jeffrey G. Reid; Jonathan Ross; William Salerno; Deepika Sharma; Alan Shuldiner; Eli Stahl; Jeffrey C. Staples.