

**Spanish Cohort of VEXAS syndrome: Clinical Manifestations, Outcome of Treatments
and Novel Evidences About *UBA1* Mosaicism**

SUPPLEMENTARY TABLES

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Supplementary Table S1. List of genes included in the targeted gene panel for autoinflammatory diseases. Abbreviations: ADA2, adenosin deaminase 2; ARPC1B, actin related protein C1B; NOCARH, Neonatal-onset cytopenia, autoinflammation, rash and hemophagocytic lymphohistiocytosis; IL-10, interleukin-10; DIRA, deficiency of interleukin-1 receptor antagonist; DITRA, deficiency of interleukin-36 receptor antagonist; FMF, familial Mediterranean Fever; PAAND, Pyrin-associated autoinflammation with neutrophilic dermatosis; MK, mevalonate kinase; AIFEC, Autoinflammation with infantile enterocolitis; NAIAD, NLRP1-associated autoinflammation with arthritis and dyskeratosis; FCAS2, familial cold-induced autoinflammatory syndrome type 2; CAPS, cryopyrin-associated periodic syndromes; PLAID, PLCG2-associated antibody deficiency, and immune dysregulation; APLAID, Autoinflammation and PLCG2-associated antibody deficiency, and immune dysregulation; CANDLE, Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature; PRAAS, Proteasome-associated autoinflammatory syndrome; PAPA, Pyogenic arthritis, pyoderma gangrenosum and acne; Hz/Hc, Hyperzincemia and hypercalprotectinemia syndrome; HOIL-1, Heme-oxidized IRP2 ubiquitin ligase 1; CRIA, cleavage-resistant RIPK1-induced autoinflammatory syndrome; HOIP, HOIL-1-interacting protein; SAVI, STING-associated vasculopathy with onset in infancy; TRAPS11, TNFRSF11A-associated periodic syndrome; TRAPS, TNF Receptor I-associated periodic syndrome; SIFD, Sideroblastic anemia, B-cell immunodeficiency, periodic fevers, and developmental delay; PFIT, periodic fever, immunodeficiency, and thrombocytopenia syndrome.

Gene	Disease	Ref Seq		Gene	Disease	Ref Seq
ADA2	ADA2 Deficiency	NM_001282225.1		PLCG2	PLAID-APLAID	NM_002661.3
ADAR	Aicardi-Goutières syndrome Type 6	NM_001111.5		POMP	CANDLE/PRAAS	NM_015932.5
AP1S3	Pustular Psoriasis	NM_001039569.1		PSMA3	CANDLE/PRAAS	NM_002788.3

<i>ARPC1B</i>	ARPC1B Deficiency	NM_005720.4		<i>PSMB4</i>	CANDLE/PRAAS	NM_002796.2
<i>CARD14</i>	Pustular Psoriasis	NM_024110.4		<i>PSMB8</i>	CANDLE/PRAAS	NM_148919.3
<i>CDC42</i>	NOCARH	NM_001791.4		<i>PSMB9</i>	CANDLE/PRAAS	NM_002800.4
<i>IFIH1</i>	Aicardi-Goutières syndrome Type 7	NM_022168.3		<i>PSMG2</i>	CANDLE/PRAAS	NM_020232.4
<i>IL10</i>	IL-10 Deficiency	NM_000572.2		<i>PSTPIP1</i>	PAPA-Hz/Hc	NM_003978.3
<i>IL10RA</i>	IL-10R1 Deficiency	NM_001558.3		<i>RBCK1</i>	HOIL-1 Deficiency	NM_031229.3
<i>IL10RB</i>	IL-10R2 Deficiency	NM_000628.4		<i>RELA</i>	Rel-A Haploinsufficiency	NM_021975.3
<i>IL1RN</i>	DIRA	NM_173842.2		<i>RIPK1</i>	CRIA	NM_003804.5
<i>IL36RN</i>	DITRA	NM_173170.1		<i>RNASEH2A</i>	Aicardi-Goutières syndrome Type 4	NM_006397.2
<i>LACC1</i>	Laccase Deficiency	NM_153218.3		<i>RNASEH2B</i>	Aicardi-Goutières syndrome Type 2	NM_024570.3
<i>LPIN2</i>	Majeed syndrome	NM_014646.2		<i>RNASEH2C</i>	Aicardi-Goutières syndrome Type 3	NM_032193.3
<i>MEFV</i>	FMF-PAAND	NM_000243.2		<i>RNF31</i>	HOIP Deficiency	NM_017999.4
<i>MVK</i>	MK Deficiencies	NM_000431.3		<i>SAMHD1</i>	Aicardi-Goutières syndrome Type 5	NM_015474.3
<i>NCSTN</i>	Hidradenitis suppurativa	NM_015331.2		<i>TMEM173</i>	SAVI	NM_198282.3
<i>NLRC4</i>	AIFEC	NM_021209.4		<i>TNFAIP3</i>	A20 Haploinsufficiency	NM_006290.3

<i>NLRP1</i>	NAIAD	NM_033004.3		<i>TNFRSF11A</i>	TRAPS11	NM_003839.3
<i>NLRP12</i>	FCAS2	NM_144687.2		<i>TNFRSF1A</i>	TRAPS	NM_001065.3
<i>NLRP3</i>	CAPS	NM_001243133.1		<i>TREX1</i>	Aicardi-Goutières syndrome Type 1	NM_033629.5
<i>NOD2</i>	Blau syndrome	NM_022162.2		<i>TRNT1</i>	SIFD	NM_182916.2
<i>OTULIN</i>	Otulipenia	NM_138348.5		<i>WDR1</i>	PFIT	NM_017491.4

Supplementary Table S2. Primer sequences used for the Sanger method of DNA sequencing.

Gene	Exon	Forward Primer (5' to 3')	Reverse Primer (5' to 3')
<i>UBA1</i>	2 +3	AGTCCTCACCTTTCTGTGCC	CTCATGGCCCAACACATACC
<i>UBA1</i>	4+5	AGACGAGGGCCTTTACTCCC	GCCTGCATACTCTAGTCCCC
<i>UBA1</i>	6+7	TTTGTGAAAGAGGCTGACCC	ATCCAGGCAGGTAACCACAC
<i>UBA1</i>	8	GCCACTCAGTGCTATGGTTTC	ACACCAGGATGTGTCTTCCC
<i>UBA1</i>	9+10	CCTGGAGCTGAGGGAAGAC	GGGCCAGAAAGAACCAAGAG
<i>UBA1</i>	11+12	AATGAGGTGGGTGAGTGGG	GTCTGAGCCAAACACAGCC
<i>UBA1</i>	13	TCTCCCTGAGGACAAAGAGG	GGCTTCCCTTCAAGATAAGGAG
<i>UBA1</i>	14	TGCAACCTGAAGATGTGGTG	AAAGTGAGACGACAAAGCCC
<i>UBA1</i>	15	GCCTGTGTGATGGAGAGAGAC	GAGAGCTAGGAAAGAAACATCAGG
<i>UBA1</i>	16	GGGCAAAGTTGTGTGTGTTTC	ATGCAACATCTGAAGGCAAG
<i>UBA1</i>	17	TTCCCCACTTCCAGAGTAGC	ACCCCAAACAACATGAGGC
<i>UBA1</i>	18	CATGAATGAGTGGCTGTTGG	TCTCAGATCCCCATGCCTC
<i>UBA1</i>	19+20	GTGATTGGCTCCAGTCCG	GGTGTGGGTAAACCTCAGGC
<i>UBA1</i>	21+22	CCAGGATGTCTCTCCTTCC	TTGCTCTGAGAGACAGAAGGG
<i>UBA1</i>	23	GCACAAGGTGAGGGGAATC	TTTTCGCACGTAICTCAAATAATG

<i>UBA1</i>	24+25	TGACTAAACACGTCTGCATGG	GTAGGGGCAGTGA ACTGGAG
<i>UBA1</i>	26	CTAGGGGAGGCCCTGTATG	GGGATGATGAGGGAACACC

Supplementary Table S3. Classification of Post-zygotic *UBA1* Variants. ¹Genome Build: GRCh37/hg19. ²RefSeq: NM_003334.4. ³Classification of pathogenicity of gene variants performed on the basis of standards and guidelines proposed in the consensus recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Abbreviations: Chr, chromosome; 1000 GP, 1000 Genomes Project Phase 3; NHLBI-ESP, National Heart, Lung and Blood Institute-Exome Sequencing Project; gnomAD, Genome Aggregation Database; CSVS, Collaborative Spanish Variant Server; SIFT, Sorting Intolerant from Tolerant; CADD, Combined Annotation Dependent Deletion; ACMG / AMP, American College of Medical Genetics and Genomics / Association for Molecular Pathology; n.a., not applicable.

Structural Features of Variants				
Chromosome position ¹	Chr X: 47058446	Chr X: 47058450	Chr X: 47058450	Chr X: 47058451
Reference allele	G	A	A	T
Variant allele	C	C	G	C
Gene ²	<i>UBA1</i>	<i>UBA1</i>	<i>UBA1</i>	<i>UBA1</i>
Exon/Intron	Intron 2	Exon 3	Exon 3	Exon 3
cDNA alteration	c.118-1G>C	c.121A>C	c.121A>G	c.122T>C
Predicted amino acid alteration	Splice acceptor site variant	p.(Met41Leu)	p.(Met41Val)	p.(Met41Thr)
Population Genetics (MAF) (%)				
1000 Genomes Project Phase 3 (2015 release)	0	0	0	0
NHLBI-ESP (ESP6500SI-V2 version)	0	0	0	0
Kaviar database (160204 version)	0	0	0	0
gnomAD (v2.1.1)	0	0	0	0
CSVS (3.0.1. version; February 2021 release)	0	0	0	0

Bioinformatics				
Polyphen-2 (Hum Var)	n.a.	Benign (0.021)	Benign (0.002)	Benign (0.008)
SIFT (Score)	n.a.	Deleterious (0.05)	Tolerated (0.11)	Tolerated (0.1)
Mutation Taster	Disease causing	Disease causing	Disease causing	Disease causing
CADD PHRED	35	23.2	21.6	22.3
Phenotype-Genotype Databases				
ClinVar database	Pathogenic	Pathogenic	Pathogenic	Pathogenic
INFEVERS database	Pathogenic	Pathogenic	Pathogenic	Pathogenic
ACMG / AMP Variant Classification ³	Pathogenic	Pathogenic	Pathogenic	Pathogenic

Supplementary Table S4. Results of *UBA1* Genotyping in Patient's Healthy Daughters. ¹RefSeq: NM_003334.4. ²Mean of data collected from three independent experiments. Abbreviations: ADS, amplicon-based deep sequencing; n.p., not performed.

Patient			Patient's Healthy Daughter		ADS	
Patient's Code	Nucleotide <i>UBA1</i> Variant ¹	Amino Acid Exchange ¹	Number	Sample	Mutant Allele Fraction (%) ²	Coverage ²
Pt 6	c.122T>C	p.Met41Thr	# 1	Peripheral Blood	0.0	1935x
Pt 8	c.121A>C	p.Met41Leu	# 1	Peripheral Blood	0.0	1900x
Pt 26	c.122T>C	p.Met41Thr	# 1	Peripheral Blood	0.0	1675x
			# 2	Peripheral Blood	0.0	2019x
			# 3	Peripheral Blood	0.0	1305x
Pt 29	c.122T>C	p.Met41Thr	# 1	Peripheral Blood	0.0	1884x
Pt 32	c.121A>G	p.Met41Val	# 1	Peripheral Blood	0.0	1862x
Pt 38	c.121A>C	p.Met41Leu	# 1	Peripheral Blood	0.0	1440x

Supplementary Table S5. Demographic data of genetically-confirmed VEXAS patients. Abbreviations: allo-HSCT, allogeneic haematopoietic stem cell transplantation.

	Sex	Status	Current Age or age at death (years)	Age at disease onset (years)	Years of disease (alive patients)	Years of disease until death or allo-HSCT	Cause of death
Pt 1	Male	Deceased	73	72	-	1	Respiratory insufficiency
Pt 3	Male	Deceased	73	67	-	6	Unknown
Pt 4	Male	Deceased	77	73	-	4	Pneumonia and respiratory insufficiency
Pt 6	Male	Alive	56	52	4	-	
Pt 8	Male	Alive	84	78	6	-	
Pt 9	Male	Alive	66	62	4	-	
Pt 10	Male	Deceased	62	52	-	3 (allo-HSCT)	Multiple complications after bilateral leg amputation to treat diabetes mellitus-related arterial disease
Pt 12	Male	Alive	68	61	7	-	
Pt 14	Male	Deceased	79	75	-	4	Unknown
Pt 15	Male	Deceased	67	65	-	4	Septic shock
Pt 16	Male	Alive	79	77	2	-	
Pt 17	Male	Deceased	77	70	-	7	Septic shock
Pt 20	Male	Alive	63	60	3	-	
Pt 21	Male	Deceased	74	66	-	7	Pneumonia and respiratory

							insufficiency
Pt 22	Male	Alive	72	67	5	-	
Pt 24	Male	Alive	75	72	3	-	
Pt 25	Male	Alive	81	74	7	-	
Pt 26	Male	Deceased	74	63	-	11	Pneumonia and respiratory insufficiency
Pt 29	Male	Alive	71	65	6	-	
Pt 30	Male	Alive	58	55	3	-	
Pt 32	Male	Alive	72	69	3	-	
Pt 33	Male	Alive	74	66	8	-	
Pt 34	Male	Deceased	63	60	-	3	Unknown
Pt 35	Male	Alive	78	74	4	-	
Pt 36	Male	Deceased	68	59	-	8	Pneumonia and respiratory insufficiency
Pt 38	Male	Alive	86	85	1	-	
Pt 39	Male	Deceased	72	64	-	8	Septic shock
Pt 40	Male	Alive	88	86	2	-	
Pt 41	Male	Alive	70	70	0.5	-	
Pt 42	Male	Alive	71	67	4	-	

Supplementary Table S6. Detailed clinical data of genetically-confirmed VEXAS patients. Abbreviations: Rec, recurrent; MAS, macrophage activation syndrome; +, present; -, absent; Mon, monoarthritis; Olig, oligoarthritis; Pol, polyarthritis.

	Skin lesions						Rec. fever	Pulmonary manifestations		Ocular manifestations					Recurrent chondritis				Musculoskeletal manifestations			Others		
	Erythematous Papules/Plaques	Purpuric Plaques	Vesicular lesions	Urticaria-like lesions	Livedo racemosa	Panniculitis		Lung infiltrates	Respiratory Insufficiency	Conjunctivitis	Palpebral Edema	Episcleritis	Uveitis	Papilledema	Ear	Nose	Respiratory tract	Costal cartilages	Arthralgia	Arthritis	Myalgia / Myositis	Headache	Venous thromboembolism	Arterial thrombosis
Pt 1	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	Olig	+	+	+	-	-
Pt 3	+	-	+	+	+	+	+	-	-	-	-	+	-	+	-	-	-	+	Pol	-	-	-	-	-
Pt 4	+	-	-	-	-	+	+	+	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-
Pt 6	+	+	-	-	+	+	+	+	-	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-
Pt 8	+	+	-	-	-	-	+	+	-	-	+	-	-	+	-	-	-	+	Pol	-	-	+	-	-
Pt 9	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	Pol	-	-	+	-	-
Pt 10	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pt 12	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pt 14	+	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Pt 15	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	Olig	+	-	-	-	-
Pt 16	-	+	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	Olig	+	+	-	-	+
Pt 17	+	-	-	-	-	+	+	-	-	-	-	+	-	+	-	-	-	+	Pol	-	-	-	-	-	
Pt 20	-	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Pt 21	+	-	-	-	+	-	-	-	-	-	+	-	-	-	+	-	-	-	+	Pol	-	-	-	-	-
Pt 22	+	+	-	+	+	-	+	+	+	+	-	-	-	-	+	+	-	-	+	Mon	-	-	+	+	-
Pt 24	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Pt 25	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-	+	Pol	-	-	-	-	-
Pt 26	+	-	-	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	Pol	-	-	-	-	-
Pt 29	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	Pol	-	-	+	-	-
Pt 30	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+	-	+	-	-
Pt 32	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	+	Pol	+	+	-	-	-
Pt 33	-	-	-	+	-	+	+	+	-	-	-	-	-	-	+	-	+	-	+	Pol	-	-	+	+	-
Pt 34	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
Pt 35	-	+	-	-	-	-	+	+	+	-	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-
Pt 36	+	-	+	-	-	-	+	-	+	+	+	-	-	+	+	+	-	-	+	Mon	-	+	+	-	-
Pt 38	-	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pt 39	+	+	-	-	-	-	+	-	-	+	+	+	-	+	+	+	+	-	+	-	-	-	+	-	-
Pt 40	+	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	+	Pol	-	-	-	-	-
Pt 41	+	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pt 42	-	-	-	+	-	-	-	-	+	+	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-

Supplementary Table S7. Detailed results of biochemistry tests performed in genetically-confirmed VEXAS patients. Abbreviations: RR, reference range; ESR, erythrocyte sedimentation rate; n.p., not performed; n.a., not available.

	Ferritin (ng/mL) RR: 20-400 ng/mL		Increased Ferritin (>400 ng/mL)		High Increased Ferritin (>1000 ng/mL)		Increased ESR (>10 mm/h)		High Increased ESR (>50 mm/h)		Bone Marrow Vacuoles
	Mean	Range	%	n/total	%	n/total	%	n/total	%	n/total	
Pt 1	1889	421-5032	100	13/13	61.5	8/13	100	23/23	87	20/23	Yes
Pt 3	3712	1000-7747	100	15/15	100	15/15	100	13/13	100	13/13	Yes
Pt 4	384	295-472	50	1/2	0	0/2	100	13/13	100	13/13	Yes
Pt 6	1374	224-5157	88.6	39/44	52.3	23/44	100	24/24	87.5	21/24	Yes
Pt 8	167	92-327	0	0/11	0	0/11	86.5	32/37	35.1	13/37	Yes
Pt 9	150	54-329	0	0/11	0	0/11	87.5	35/40	75	30/40	Yes
Pt 10	1554	380-2377	85.7	6/7	57.1	4/7	100	4/4	100	4/4	Yes
Pt 12	2058	1617-2602	100	4/4	100	4/4	100	6/6	100	6/6	n.p.
Pt 14	2453	1758-4223	100	4/4	100	4/4	100	12/12	100	12/12	n.p.
Pt 15	763	204-2167	60	3/5	20	1/5	100	9/9	88.9	8/9	n.p.
Pt 16	3615	292-11931	87.5	14/16	56.2	9/16	100	12/12	91.7	11/12	Yes
Pt 17	1252	545-3256	100	13/13	53.8	7/13	100	12/12	100	12/12	Yes
Pt 20	973	654-1971	100	5/5	20	1/5	100	5/5	60	3/5	Yes
Pt 21	396	158-558	50	4/8	0	0/8	91.7	11/12	41.7	5/12	Yes
Pt 22	289	54-698	33.3	3/9	0	0/9	100	19/19	73.7	14/19	Yes
Pt 24	362	362-362	0	0/1	0	0/1	100	2/2	0	0/2	Yes
Pt 25	1276	633-2844	100	19/19	63.1	12/19	95.5	21/22	68.2	15/22	Yes
Pt 26	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	100	7/7	57.1	4/7	Yes
Pt 29	711	307-1209	75	6/8	25	2/8	100	13/13	92.3	12/13	Yes
Pt 30	517	408-622	100	4/4	0	0/4	100	1/1	100	1/1	Yes

Pt 32	1216	281-5414	77.8	7/9	22.2	2/9	100	10/10	50	5/10	n.p.
Pt 33	702	250-1210	80	12/15	20	3/15	94.4	17/18	50	9/18	Yes
Pt 34	1438	1073-1992	100	7/7	100	7/7	37.5	3/8	0	0/8	Yes
Pt 35	807	645-1024	100	3/3	33.3	1/3	100	10/10	100	10/10	n.p.
Pt 36	856	203-1726	71.4	5/7	57.1	4/7	100	29/29	86.2	25/29	Yes
Pt 38	208	179-266	0	0/5	0	0/5	85.7	6/7	57.1	4/7	Yes
Pt 39	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	100	6/6	50	3/6	Yes
Pt 40	1282	1282-1282	100	1/1	100	1/1	100	4/4	50	2/4	n.p.
Pt 41	1471	1264-1624	100	5/5	100	5/5	100	6/6	100	6/6	Yes
Pt 42	693	641-744	100	2/2	0	0/2	n.a.	n.a.	n.a.	n.a.	n.p.

Supplementary Table S8. Detailed results of haematological tests performed in genetically-confirmed VEXAS patients. Abbreviations: MCV, mean corpuscular volume; RR, reference range; WBC, white blood cells; n.a., not available.

	Haemoglobin (g/L) RR: 130-170 g/L		Haemoglobin <120 g/L		MCV (fL) RR: 80-100 fL		Macrocytosis MCV >100 fL		Hematocrit <36%		Platelets (10 ⁹ /L) RR: 140-400·10 ⁹ /L		Platelets <140·10 ⁹ /L		WBC (10 ⁹ /L) RR: 4-11·10 ⁹ /L	
	Mean	Range	%	n/total	Mean	Range	%	n/total	%	n/total	Mean	Range	%	n/total	Mean	Range
Pt 1	88.1	67-131	93.5	58/62	102	95-117	51.6	32/62	93.5	58/62	202	46-383	24.2	15/62	3	0.6-8.4
Pt 3	79.7	64-111	100	20/20	103	92-119	65	13/20	100	20/20	68	15-125	100	20/20	9.9	6.2-16.2
Pt 4	105	88-125	93.8	30/32	96	88-103	21.9	7/32	87.5	28/32	267	94-430	9.4	3/32	6.1	0.2-15.9
Pt 6	85.6	65-138	96.7	117/121	109	99-128	99.2	120/121	95	115/121	133	15-740	66.9	81/121	7.9	1.5-21
Pt 8	119.2	79-143	59.6	31/52	110	95-125	84.6	44/52	40.4	21/52	143	73-250	48.1	25/52	5.7	3.7-8.7
Pt 9	101.8	70-145	88.2	45/51	107	98-118	94.1	48/51	80.4	41/51	241	70-753	15.7	8/51	3.6	1.6-6.4
Pt 10	91.9	66-119	100	21/21	115	102-128	100	21/21	100	21/21	126	61-335	76.2	16/21	3.6	2-5.8
Pt 12	104.5	68-161	72.2	13/18	109	91-126	83.3	15/18	72.2	13/18	134	34-317	72.2	13/18	11	3.9-33.3
Pt 14	87.2	66-106	100	17/17	107	91-128	76.5	13/17	100	17/17	136	61-304	52.9	9/17	6.7	2.5-15.7
Pt 15	105.9	93-126	93.3	14/15	100	85-113	53.3	8/15	93.3	14/15	143	76-237	60	9/15	4	2.5-5.6
Pt 16	100.6	67-131	91.7	22/24	95	88-101	12.5	3/24	95.8	23/24	222	145-308	0	0/24	4.7	2.2-8.3
Pt 17	103.8	70-122	93.3	14/15	108	100-124	100	15/15	86.7	13/15	159	77-237	26.7	4/15	5.1	1.6-9.9
Pt 20	97.3	79-115	100	15/15	107	98-117	86.7	13/15	93.3	14/15	120	66-187	73.3	11/15	7.3	3.3-11.6
Pt 21	91.4	59-113	100	29/29	105	89-120	65.5	19/29	100	29/29	93	9-280	86.2	25/29	5.5	2.1-12.1
Pt 22	100.1	73-127	92.9	39/42	103	97-116	85.7	36/42	90.5	38/42	142	54-255	47.6	20/42	3.4	1.3-7.8
Pt 24	97.1	84-109	100	7/7	106	103-109	100	7/7	100	7/7	140	77-236	57.1	4/7	4.5	3.5-5.5
Pt 25	102.3	68-143	70.5	31/44	109	96-123	88.6	39/44	72.7	32/44	160	63-413	59.1	26/44	5	2.4-15.1
Pt 26	117	88-137	62.5	10/16	102	93-107	75	12/16	68.8	11/16	149	105-215	43.8	7/16	5.7	3.5-7.4
Pt 29	105.6	64-130	89.5	17/19	106	97-117	84.2	16/19	89.5	17/19	143	27-225	42.1	8/19	5.8	2.2-13.9

Pt 30	114.8	88-131	62.5	5/8	107	105-110	100	8/8	62.5	5/8	140	126-169	62.5	5/8	7.3	4.9-10.6
Pt 32	118.4	92-138	53.8	7/13	101	97-105	53.8	7/13	53.8	7/13	260	113-539	7.7	1/13	5.7	3.3-9.9
Pt 33	107	72-142	75	27/36	93	86-101	2.8	1/36	72.2	26/36	222	121-329	5.6	2/36	6.3	3.2-11
Pt 34	84.1	61-105	100	20/20	117	100-133	90	18/20	100	20/20	20	4-48	100	20/20	11.6	6.4-25.5
Pt 35	100.3	74-119	100	15/15	109	99-121	93.3	14/15	93.3	14/15	144	61-230	46.7	7/15	7.2	2.8-15.8
Pt 36	102.6	64-146	80.6	54/67	102	88-112	58.2	39/67	70.1	47/67	152	80-305	40.3	27/67	4.4	1.1-8.5
Pt 38	106.8	98-117	100	8/8	105	102-109	100	8/8	87.5	7/8	183	130-234	12.5	1/8	7.5	4.9-11
Pt 39	113.9	74-158	57.9	11/19	103	92-114	78.9	15/19	68.4	13/19	163	101-273	42.1	8/19	5.4	2.5-11.3
Pt 40	83.3	61-127	90	9/10	100	91-108	40	4/10	90	9/10	79	53-101	100	10/10	2.9	1.5-3.9
Pt 41	79.3	64-91	100	8/8	97	95-99	0	0/8	100	8/8	156	113-248	50	4/8	3.9	3.1-5.1
Pt 42	82.1	71-92	100	16/16	104	99-112	81.3	13/16	100	16/16	77	22-113	100	16/16	6.6	4.6-10.6

Supplementary Table S9. Main characteristics of *UBA1*-negative patients. Abbreviations: MCV, mean corpuscular volume; +, present; -, absent.

	Sex	Status	Age at Onset (years)	Neutrophilic dermatosis	Recurrent Fever	Pulmonary Manifestations	Relapsing polycondritis	Recurrent arthritis	Venous thromboembolism	Hematology			
										Hemoglobin <120 g/L g/L	MCV >100 fL	Leukocytes <4·10 ⁹ /L	Platelets <140·10 ⁹ /L
Pt 2	Male	Deceased	60	+	-	+	-	-	-	+	+	-	+
Pt 5	Male	Deceased	67	+	+	-	+	-	-	+	-	-	+
Pt 7	Male	Alive	69	+	+	+	-	-	-	+	+	-	-
Pt 11	Male	Alive	38	+	+	-	-	-	-	+	-	-	-
Pt 13	Female	Alive	63	+	+	-	-	-	-	+	-	+	+
Pt 18	Male	Alive	55	-	-	-	+	+	+	+	+	-	-
Pt 19	Male	Alive	39	-	+	+	+	-	-	-	+	-	+
Pt 23	Male	Deceased	71	-	-	+	-	-	+	+	+	+	+
Pt 27	Male	Alive	51	-	+	+	+	-	-	+	-	-	-
Pt 28	Male	Alive	52	+	-	+	+	-	-	+	+	-	-
Pt 31	Male	Alive	45	+	+	+	-	+	-	+	-	+	-
Pt 37	Male	Alive	50	-	-	-	+	+	-	+	+	-	-

Supplementary Table S10. ADS-Leukocyte Subpopulations. Amplicon-based Deep *UBA1* Sequencing Analyses of Isolated Leukocyte**Subpopulations.** ¹RefSeq: NM_003334.4. ²Mean of data collected from three independent experiments. Abbreviations: SD, standard deviation.

Patient	Leukocyte Subpopulation	<i>UBA1</i> Variant ¹	Mutant Allele Frequency - Mean (SD) ²	Coverage – Mean (SD) ²
Pt 3	Peripheral blood	p.Met41Thr	76.3% (1.1)	7486x (893)
	Neutrophils	p.Met41Thr	97.9% (0.5)	8842x (415)
	Monocytes	p.Met41Thr	98.0% (0.2)	24941x (2271)
	T cells	p.Met41Thr	0.5% (0.1)	22253x (4110)
	B cells	p.Met41Thr	0.3% (0.1)	21167x (2580)
	CD34 ⁺ cells	p.Met41Thr	97.1% (0.2)	5752x (2536)
Pt 9	Peripheral blood	p.Met41Thr	60.7% (1.0)	4621x (525)
	Neutrophils	p.Met41Thr	60.5% (10.9)	9255x (875)
	Monocytes	p.Met41Thr	87.7% (0.3)	10629x (519)
	T cells	p.Met41Thr	0.7% (0.2)	10983x (2582)
	B cells	p.Met41Thr	0.9% (0.4)	8670x (826)

Supplementary Table S11. Evaluation of post-zygotic *UBA1* variants in non-hematopoietic tissues. ¹RefSeq: NM_003334.4. ²Mean of data collected from three independent experiments. Abbreviations: SD, standard deviation.

Patient	Nucleotide <i>UBA1</i> Variant ¹	Amino Acid Exchange ¹	Sample	Mutant Allele Frequency - Mean (SD) ²	Coverage – Mean (SD) ²
Pt 1	c.121A>G	p.Met41Val	Nails	5.4% (0.4)	634x (255)
Pt 6	c.122T>C	p.Met41Thr	Nails	73.7% (8.4)	906x (188)
Pt 9	c.122T>C	p.Met41Thr	Nails	8.5% (1.6)	1556x (157)
Pt 16	c.121A>G	p.Met41Val	Nails	2.7% (2.7)	429x (112)
Pt 21	c.121A>C	p.Met41Leu	Nails	62.9% (3.8)	4217x (274)
Pt 22	c.121A>G	p.Met41Val	Nails	16.3% (0.3)	2289x (142)
Pt 25	c.122T>C	p.Met41Thr	Nails	9.9% (1.3)	1609x (188)
Pt 30	c.122T>C	p.Met41Thr	Nails	16.5% (5.7)	866x (498)
Pt 36	c.122T>C	p.Met41Thr	Nails	18.4% (2.1)	4763x (513)

Supplementary Table S12. Outcome of administered treatments in genetically-confirmed VEXAS patients. Abbreviations: NSAID, non-steroidal anti-inflammatory drugs; GCs, Glucocorticoids; MTX, Methotrexate; MMF, Mycophenolate mofetil; AZA, Azathioprine; CYF, Cyclophosphamide; IVIGs, intravenous immunoglobulins; Inh, inhibitor; ATB, antibiotics; CyA, Cyclosporine A; allo-HSCT, allogeneic haematopoietic stem cell transplantation; HCQ, Hydroxychloroquine; Neg, negative; PR, partial response; CR, complete response; n.a., not administered.

	Colchicine	NSAIDs	GCs	MTX	MMF	AZA	CYF	IVIGs	TNF-inh	IL-6-inh	IL-1-inh	Anti-CD20	Jak-inh	Others
Pt 1	Neg	Neg	CR	Neg	-	Neg	n.a.	n.a.	n.a.	Neg	n.a.	n.a.	Neg	ATB
Pt 3	PR	n.a.	CR	PR	n.a.	n.a.	n.a.	n.a.	n.a.	Neg	PR	PR	n.a.	CyA
Pt 4	Neg	PR	CR	Neg	n.a.	Neg	n.a.	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 6	Neg	Neg	CR	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	Neg	PR	Neg	Neg	Azacytidine
Pt 8	n.a.	Neg	CR	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 9	n.a.	n.a.	PR	PR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Decitabine
Pt 10	n.a.	n.a.	CR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	Allo-HSCT
Pt 12	n.a.	PR	CR	n.a.	n.a.	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 14	n.a.	PR	CR	n.a.	n.a.	PR	PR	PR	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 15	Neg	PR	CR	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	HCQ
Pt 16	n.a.	Neg	CR	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	ATB
Pt 17	n.a.	Neg	CR	PR	PR	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 20	n.a.	PR	CR	Neg	Neg	n.a.	n.a.	Neg	n.a.	PR	Neg	PR	PR	CyA
Pt 21	n.a.	Neg	PR	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 22	n.a.	PR	CR	PR	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	PR	HCQ
Pt 24	Neg	n.a.	PR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	CR	
Pt 25	Neg	n.a.	CR	PR	n.a.	n.a.	n.a.	n.a.	Neg	PR	n.a.	PR	n.a.	

Pt 26	n.a.	PR	PR	PR	n.a.	PR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 29	Neg	Neg	PR	n.a.	n.a.	Neg	n.a.	Neg	n.a.	Neg	PR	n.a.	n.a.	ATB
Pt 30	n.a.	n.a.	CR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 32	n.a.	n.a.	CR	Neg	Neg	Neg	n.a.	n.a.	n.a.	CR	Neg	n.a.	n.a.	
Pt 33	n.a.	n.a.	CR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 34	n.a.	n.a.	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 35	Neg	PR	PR	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	Neg	n.a.	n.a.	n.a.	
Pt 36	n.a.	PR	CR	Neg	Neg	n.a.	n.a.	n.a.	Neg	n.a.	n.a.	n.a.	n.a.	ATB
Pt 38	Neg	n.a.	CR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 39	n.a.	Neg	PR	n.a.	n.a.	n.a.	Neg	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 40	n.a.	n.a.	CR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	
Pt 41	n.a.	n.a.	CR	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	ATB
Pt 42	PR	n.a.	PR	n.a.	Neg	Neg	n.a.	n.a.	Neg	n.a.	n.a.	n.a.	n.a.	