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Molecular and clinical presentation of *UBA1*-mutated myelodysplastic syndromes

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De-identified patient data including clinical annotations and UBA1 mutations has been provided in the supplementary tables. These will also be deposited to CBioPortal. Myeloid driver gene mutations have been previously published in Bernard E. et al NEJM Evidence 2022 (DOI: 10.1056/EVIDoa2200008) and deposited to CBioPortal.

Key Points

- *UBA1* mutations were identified in 1% of MDS patients, enriched in patients with few myeloid mutations or established disease classification.
- Inflammatory clinical presentation and vacuoles were observed in 83% and 71%, respectively, of patients with pathogenic *UBA1* mutations.

Abstract

Mutations in *UBA1*, which are disease-defining for VEXAS syndrome, have been reported in patients diagnosed with myelodysplastic syndromes (MDS). Here, we define the prevalence and clinical associations of *UBA1* mutations in a representative cohort of patients with MDS. Digital droplet PCR profiling of a selected cohort of 375 male patients lacking MDS disease-defining mutations or established WHO disease classification identified 28 patients (7%) with *UBA1* p.M41T/V/L mutations. Using targeted sequencing of *UBA1* in a representative MDS cohort (n=2,027), we identified an additional 27 variants in 26 patients (1%), which we classified as likely/pathogenic (n=12) and unknown significance (n=15). Among the total 40 patients with likely/pathogenic variants (2%), all were male and 63% were classified by WHO^{2016} as MDS-MLD/SLD. Patients had a median of one additional myeloid gene mutation, often in *TET2* (n=12), *DNMT3A* (n=10), *ASXL1* (n=3), or *SF3B1* (n=3). Retrospective clinical review where possible showed that 83% (28/34) *UBA1*-mutant cases had VEXAS-associated diagnoses or inflammatory clinical presentation. The prevalence of *UBA1*-mutations in MDS patients argues for systematic screening for *UBA1* in the management of MDS.

Introduction

Patients with myelodysplastic syndromes (MDS) have heterogeneous clinical presentation, response to therapy and outcomes. The recent integration of genetic alterations alongside established clinical and morphologic features in disease classification and risk stratification have informed clinical management[1–3]. However, 5-10% of patients have no established disease defining mutations[3,4]. Furthermore, inflammatory symptoms of poorly-understood etiology complicate diagnosis and clinical management in 10-30% of MDS patients, and are associated with high-risk disease[5–10].

One such overlap between MDS and inflammatory presentation is VEXAS (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) syndrome, a severe systemic autoinflammatory disease[11]. VEXAS is linked to mutations in the *UBA1* gene, which encodes the major E1-activating enzyme required for ubiquitylation. While mutations at the p.M41 hotspot were the first to be associated with VEXAS, additional variants in *UBA1* have since been characterized as pathogenic[12–17]. *UBA1* mutations typically co-occur with common myeloid driver gene mutations including *DNMT3A*, *TET2*, and *SF3B1* in VEXAS[11], clonal hematopoiesis[18], and MDS[19,20]. The prevalence of MDS in VEXAS patients is also high (25-55%)[11,12,20], however, *UBA1* mutations have not been systematically screened in a representative diagnostic MDS population. In this study, we define the prevalence, co-mutation patterns, and clinical profiles of MDS patients with *UBA1* mutations.

Study Design

Molecular data from a systematic sequencing screen of 152 myeloid driver genes used for the development of the IPSS-M, were evaluated across a representative diagnostic and treatment-naive MDS cohort (n=3,323)[3]. Detailed molecular, cytogenetic, and clinical data were available. We hypothesized that *UBA1* mutations would be prevalent in male patients without myeloid driver mutations or established disease classification. First, 375 cases (Cohort A) with inclusion based on: 1) male gender; and 2) no identified driver mutations or mutations only in DTA (*DNMT3A*, *TET2*, *ASXL1*) genes; or 3) MDS unclassifiable (eg. MDS-U) according to the WHO revised 4th edition classification (WHO[2016])[21,22] or 4) subsets of MDS/MPN overlap syndromes (MDS/MPN-U, MDS/MPN-RS-T, aCML) per WHO 2016 Classification **(Figure 1A)** were profiled using *UBA1* p.M41 digital droplet PCR (ddPCR). To determine the prevalence of *UBA1* mutations in MDS, the cohort was then expanded to profile 2,027 representative patients, both male and female across WHO[2016] subtypes (Cohort B) using targeted sequencing of the *UBA1* locus. *UBA1* variants were called as previously described using the Isabl platform[3,23,24]. 204 cases were profiled with both assays. Retrospective review of inflammation-associated clinical history and morphology was performed for *UBA1*-mutated cases where possible.

This study is approved under Memorial Sloan Kettering Institutional Review Board IRB #15-017.

Results and Discussion

Profiling of *UBA1* by ddPCR for Cohort A identified 30 *UBA1* p.M41T/V/L mutations in 7% (28 of 375) of the patients (mutant droplet fraction median: 0.376, range: 0.00018-0.877). This included 15 patients with p.M41T (c.122T>C), 7 with p.M41V (c.121A>G), 4 with p.M41L (c.121A>C), and 2 patients with more than one mutation (1 with p.M41V/T, and 1 with p.M41L/V) **(Figure 1B)**. The median age at diagnosis in cases with *UBA1* p.M41 mutation was 72 years (range 44-89) and 71 years (range 21-93) for cases without **(Supplemental Table 1)**. There was no significant difference in available clinical parameters (age, blast percentages, blood counts) between *UBA1*-mutant and -wildtype MDS. These data demonstrate that mutations in *UBA1* are frequent (7%) in male MDS patients without established disease classification or typical disease-associated mutation patterns.

In order to 1) determine whether *UBA1* mutations were indeed enriched in patients without clear MDS disease classification, 2) detect recently described mutations beyond the p.M41 locus[12–17], and 3) enable discovery of novel variants in *UBA1*, we sequenced the entire *UBA1* gene across an expanded cohort of 2,027 MDS patients (Cohort B) **(Figure 1A** and **Supplemental Table 2)**.

By NGS in Cohort B, we identified 35 *UBA1* mutations in 34 (1.7%) patients, of which 20 (0.97%, all male) had likely pathogenic variants and 14 had variants of unknown significance (VUS) (3/14 female) **(Figure 1B)**. The 20 likely pathogenic variants comprised 13 p.M41T/V/L hotspot mutations with a broad range of variant allele fractions (VAF) (0.013-0.94), and 7 previously described non-p.M41 mutations to include 2 p.S56F (VAF 0.87 and 0.93), 2 p.A478S (VAF 0.72 and 0.80), 2 p.S621C (VAF 0.80 and 0.82) and 1 p.Y55H (VAF 0.025) **(Figure 1C, Supplemental Table 3)**. The VUS are provided in **Supplemental Table 3** to support future

studies. Within 204 cases profiled by both assays, all 8 p.M41T/V/L mutations were identified with concordant VAF estimations (R^2=0.999 p<0.0001) **(Figure 1D)**. This data demonstrates that 1% (20/2027) of MDS patients had established pathogenic mutations in *UBA1*, and that *UBA1* mutations are strongly enriched (7%), but not limited to, male subjects diagnosed with MDS but without disease defining genetic alterations.

Integration of Cohort A and B (n=2,198) yielded 40 patients (all male) with pathogenic *UBA1* variants **(Table 1)**. The WHO[2016] classification (available for 39) was MDS-SLD/MLD (24), MDS-EB1 (4), MDS-U (3), CMML (2), aCML (2), MDS-RS-MLD (1), MDS/MPN-RS-T (1), MDS/MPN-U (1) **(Figure 1E)**. Two patients were re-classified as AML after clinical review due to a history of AML diagnosis. Most patients had IPSS-M Very-Low/Low Risk (73% 27/37) **(Figure 1F)**. These patients typically had no myeloid driver gene co-mutations or only one, often in the epigenetic regulators *TET2* (n=12) or *DNMT3A* (n=10) **(Figure 1G, 2A)**. They had fewer *ASXL1* (n=3, p=0.022) and *SF3B1* (n=3) co-mutations **(Figure 2B)**. Loss of the Y chromosome was frequent (13%, n=5). In 8 patients with pathogenic *UBA1* mutations with VAF>2% and co-occurring *DNMT3A* mutations, *DNMT3A* and *UBA1* VAF were correlated (r^2=0.87, p=0.0005), suggesting that co-mutation leads to clonal expansion **(Figure 2C)**. Conversely, *TET2* co-mutations were either subclonal (n=4) or clonally dominant (n=8) to *UBA1*. The distinct clonal architectures in *DNMT3A*- or *TET2*-co-mutated cases suggest different mechanisms of cooperation and selection.

VEXAS syndrome has been associated with a wide range of clinical characteristics and is not yet defined by specific diagnostic criteria. We conducted a detailed clinical review of *UBA1* positive cases focusing on inflammatory presentation and treatment. Sufficient clinical history was available for 85% (34/40) of cases with pathogenic *UBA1* variants **(Figure 2A, Supplemental Tables 4-5)**. Of these, 4 patients had rheumatologic diagnoses (all had p.M41T/V/L) and 12 additional patients had inflammatory symptoms associated with VEXAS-like presentation. Of these 16 cases, 12 had multi-organ system involvement. Eleven patients were treated with steroids (n=11) and disease-modifying anti-rheumatic drugs or biologics (n=4). An additional nine patients had elevated acute phase reactants (APRs) suggesting subclinical levels of inflammation **(Figure 2A)**. Inflammatory manifestations were usually reported preceding or around the time of MDS diagnosis (median time 227 days prior; range 4.4 years prior to 4.8 years after) **(Supplemental Figure 1)**. Five patients had monoclonal gammopathy of unknown significance. Vacuoles, a cardinal feature in VEXAS, were identified in all 13 patients with *UBA1* p.M41 VAF >2% and in 71% (15/21) of patients with available slides. Taken together, 83% (28/34) of patients with pathogenic variants had inflammatory clinical presentations **(Figure 2D)**.

Patients with *UBA1* VUS (n=14; 3 female) had distinct co-mutation patterns and risk compared to those with pathogenic variants. They had more myeloid driver gene mutations, with 50% (7/14) having 3 or more co-mutations, than the cases with pathogenic *UBA1* mutations (18%; 7/40) and were more likely to harbor alterations associated with adverse-prognosis[3] **(Figure 1G, 2A)**. Consequently, patients with VUS had a higher proportion of moderate or high IPSS-M risk (64%; 7/11) compared to those with pathogenic variants (27%) **(Figure 1F)**. Three patients out

of 13 cases with *UBA1* VUS and available clinical history had inflammatory presentations associated with VEXAS and an additional 7 had elevated APRs. Three had vacuoles. Functional validation is needed to determine whether these VUS are linked to the inflammatory clinical presentation.

Acute myeloid leukemia (AML) transformation has been reported in only a few *UBA1*-mutated MDS patients[25,26]. Among *UBA1*-mutated patients in Cohorts A and B, 3 transformed to AML. One patient had a low VAF *UBA1* p.M41V (0.0002 by ddPCR) subclonal to *TET2*, *SF3B1*, *FLT3*, and *ASXL2* co-mutations. The second had *UBA1* p.S56F (VAF 0.868) and 7q deletion. The third had VUS *UBA1* p.R869L (VAF 0.222) and *IRF1*, *NFE2* and *RRAS* co-mutations. Among the other *UBA1*-mutant patients that died or were censored after 1 year (n=37), none transformed to AML.

We next assessed whether *UBA1* mutations carried prognostic value in Cohort A, which lacked prognostic biomarkers. We did not identify a significant difference in overall survival between *UBA1*-mutant (n=27) and wildtype (n=312) patients (log-rank test p=0.4) **(Figure 2E)**. Further studies are required to understand whether *UBA1* mutations with or without co-occurring myeloid driver mutations and/or inflammatory presentation are prognostic in low-risk MDS.

In summary, within a large representative diagnostic cohort of MDS patients, 1% of patients harbored likely pathogenic *UBA1* mutations. Notably, *UBA1* mutations were frequent (7%) in the subset of male patients with few or no mutations in myeloid driver genes. *UBA1*-mutated patients were predominantly IPSS-M low risk (73%) with a median of 1 additional mutation, usually in *DNMT3A* or *TET2*. *UBA1* mutations were seen in both dominant clones or as subclones. Of all patients with *UBA1* pathogenic variants, inflammatory presentation was seen in 83% and vacuoles were present in 71%. Importantly, vacuoles were found in all patients with p.M41 mutations at >2% VAF. Our data confirms that for the majority of *UBA1*-mutated MDS cases, there was overlapping inflammatory clinical presentation. Limitations of our study include ascertainment of the MDS cohort prior to the association of *UBA1* mutations with VEXAS resulting in lack of standardized inflammation assessment across the entire cohort.

Our study highlights the need to consider *UBA1* mutations in the diagnostic workup for MDS and MDS/VEXAS overlap. The enrichment of *UBA1* mutations in patients lacking MDS disease-defining alterations may represent a VEXAS population diagnosed as MDS, while others have cardinal genomic and clinical presentations of MDS along with *UBA1* mutations and inflammatory phenotype, suggesting a potential VEXAS/MDS overlap syndrome. The systematic incorporation of *UBA1* mutations in the diagnostic workup of MDS and inflammatory conditions will provide the necessary data to inform diagnostic criteria that may differentiate MDS to VEXAS, and/or MDS/VEXAS overlap. Future prospective studies are needed to define the clinical implications of *UBA1* mutations, associated inflammatory presentation, and co-occurring myeloid driver mutations as prognostic factors.

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Authorship

M.S., E.B. and E.P. designed the study, analyzed the data, and wrote the paper. M.Creignou designed the retrospective clinical review form. A.F. and K.H. designed and performed the *UBA1* ddPCR assays and targeted DNA sequencing for UBA1. M.S., E.B. and D.B.B. curated NGS variants and clinical annotations. D.D. and J.E.A.O. executed bioinformatics pipelines. L.M., F.S., M. Creignou, U.G., A.A.L., M.J., M.T., O.K., M.Y.F., B.S., E.O., K.Z., L.N.E., W.R.S., R.T., F.T., R.F.P., V.S., I.K., J.B., F.P.S.S., V.M.K., M.R.S., M.B., C.G., L.P., L.A., M.G.D.P., P.F., A.P., U.P., M.H., P.V., C.F., M.T.V., L.-Y.S., M.F., J.H.J., J.C., N.G., M. Cazzola, E.H.-L. and S.O. provided clinical data and DNA specimens. E.B. and E.P. coordinated sample acquisition. All authors provided feedback on the paper.

The authors declare the following competing interests. U.G. has received honoraria from Celgene, Novartis, Amgen, Janssen, Roche and Jazz and research funding from Celgene and Novartis. C.A.C. has received research funding from Celgene. A.A.L. is in advisory boards of Celgene, Amgen, Roche, Novartis and Alexion and has received research funding from Celgene. F.T. is on the advisory boards of Jazz, Pfizzer and Abbvie and has received research funding from Celgene. I.K. is on the advisory board of Genesis Pharma and has received research funding from Celgene and Janssen Hellas. F.P.S.S. has received honoraria from

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Figure Legends

Figure 1: Prevalence of *UBA1* mutations in MDS

(A) Schematic of study design showing Cohort A (top) profiled by ddPCR and Cohort B (bottom) profiled by NGS for UBA1 mutations.
(B) Lollipop plot showing likely-pathogenic mutations (top) and VUS (bottom) in *UBA1* detected by ddPCR and/or NGS. Variants are colored by VAF and point shape indicates whether the variant was detected by ddPCR, NGS, or both. VUS in the non-canonical transcripts are reported in the box below the axis.
(C) VAF of *UBA1* mutations detected by NGS in Cohort B.
(D) Correlation of *UBA1* NGS VAF and ddPCR mutant droplet fraction for patients (n=8) with *UBA1* p.M41T/V/L variants detected by both assays.
(E) Stacked barplot of WHO 2016 class (available for n=53/54).
(F) Stacked barplot of IPSS-M Risk Category (available for n=48/54).
(G) Stacked barplot of number of co-occurring mutations in 54 *UBA1*-mutated patients (left) and Cohort B (right).

Figure 2: Co-mutation patterns and clinical presentation of *UBA1*-mutant MDS

(A) Oncoprint for the total cohort of *UBA1*-mutant patients (n=54) including results of retrospective review of clinical history for inflammatory features (bottom). Patients are ordered by decreasing *UBA1* VAF per group (p.M41, non-p.M41 pathogenic, VUS).
(B) Frequency of co-mutations in *UBA1*-mutated and -wildtype patients.
(C) VAF of pathogenic *UBA1* and co-occurring *DNMT3A* (top) and *TET2* (bottom) mutations. Grey dashed line represents the identity line. Black solid line represents a linear model fit to the data.
(D) Stacked barplot of clinical inflammatory manifestations for n=54 patients.
(E) Kaplan-Meier curve for overall survival in Cohort A (ddPCR) of MDS patients with or without mutations in *UBA1.* Number of patients per group and p-values are indicated on the plot.

References

1. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia*. 2022;36(7):1703–1719.
2. Arber DA, Orazi A, Hasserjian RP, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200–1228.
3. Bernard E, Tuechler H, Greenberg PL, et al. Molecular international prognostic scoring system for myelodysplastic syndromes. *NEJM Evid.* 2022;1(7.):
4. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*. 2014;28(2):241–247.
5. Braun T, Fenaux P. Myelodysplastic Syndromes (MDS) and autoimmune disorders (AD): cause or consequence? *Best Pract. Res. Clin. Haematol.* 2013;26(4):327–336.
6. Zhao L-P, Boy M, Azoulay C, et al. Genomic landscape of MDS/CMML associated with systemic inflammatory and autoimmune disease. *Leukemia*. 2021;35(9):2720–2724.
7. de Hollanda A, Beucher A, Henrion D, et al. Systemic and immune manifestations in myelodysplasia: a multicenter retrospective study. *Arthritis Care Res.* . 2011;63(8):1188–1194.
8. Mekinian A, Grignano E, Braun T, et al. Systemic inflammatory and autoimmune manifestations associated with myelodysplastic syndromes and chronic myelomonocytic leukaemia: a French multicentre retrospective study. *Rheumatology* . 2016;55(2):291–300.
9. Montoro J, Gallur L, Merchán B, et al. Autoimmune disorders are common in myelodysplastic syndrome patients and confer an adverse impact on outcomes. *Ann. Hematol.* 2018;97(8):1349–1356.
10. Oganesyan A, Jachiet V, Chasset F, et al. VEXAS syndrome: still expanding the clinical phenotype. *Rheumatology* . 2021;60(9):e321–e323.
11. Beck DB, Ferrada MA, Sikora KA, et al. Somatic Mutations in UBA1 and Severe Adult-Onset Autoinflammatory Disease. *N. Engl. J. Med.* 2020;383(27):2628–2638.
12. Bourbon E, Heiblig M, Gerfaud Valentin M, et al. Therapeutic options in VEXAS syndrome: insights from a retrospective series. *Blood*. 2021;137(26):3682–3684.
13. Poulter JA, Collins JC, Cargo C, et al. Novel somatic mutations in UBA1 as a cause of VEXAS syndrome. *Blood*. 2021;137(26):3676–3681.
14. Collins JC, Balanda N, Magaziner SJ, et al. Novel Disease-Causing Mutations in UBA1 Reveal Disease Mechanisms in Bone Marrow Failure and Inflammation. *Blood*. 2022;140(Supplement 1):2914–2915.
15. Sakuma M, Blombery P, Meggendorfer M, et al. Novel causative variants of VEXAS in UBA1 detected through whole genome transcriptome sequencing in a large cohort of hematological malignancies. *Leukemia*. 2023;
16. 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*. 2023;329(4):318–324.
17. Stiburkova B, Pavelcova K, Belickova M, et al. Novel somatic UBA1 variant in a patient with VEXAS syndrome. *Arthritis Rheumatol*. 2023;
18. Gutierrez-Rodrigues F, Kusne Y, Fernandez J, et al. Spectrum of clonal hematopoiesis in VEXAS syndrome. *Blood*. 2023;142(3):244–259.
19. Zhao L-P, Schell B, Sébert M, et al. Prevalence of UBA1 mutations in MDS/CMML patients with systemic inflammatory and auto-immune disease. *Leukemia*. 2021;35(9):2731–2733.
20. Georgin-Lavialle S, Terrier B, Guedon AF, et al. Further characterization of clinical and laboratory features in VEXAS syndrome: large-scale analysis of a multicentre case series of 116 French patients. *Br. J. Dermatol.* 2022;186(3):564–574.

21. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391–2405.
22. Campo E, Harris NL. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. International Agency for Research on Cancer; 2017.
23. Bernard E, Nannya Y, Hasserjian RP, et al. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. *Nat. Med.* 2020;26(10):1549–1556.
24. Medina-Martínez JS, Arango-Ossa JE, Levine MF, et al. Isabl Platform, a digital biobank for processing multimodal patient data. *BMC Bioinformatics*. 2020;21(1):549.
25. Gurnari C, Pascale MR, Vitale A, et al. Diagnostic capabilities, clinical features, and longitudinal UBA1 clonal dynamics of a nationwide VEXAS cohort. *Am. J. Hematol.* 2023;
26. Battipaglia G, Vincenzi A, Falconi G, Fiore A. New scenarios in Vacuoles, E1 enzyme, X linked, Autoinflammatory, Somatic (VEXAS) syndrome: evolution from myelodysplastic syndrome to acute …. *Curr. Res. Agric. Sci.* .

Tables

Table 1: Characteristics of n=54 *UBA1*-mutated cases. Table is split by *UBA1* mutation: p.M41, non-p.M41 pathogenic, and VUS.

Table 1: Characteristics of *UBA1*-mutated patients (n=54)

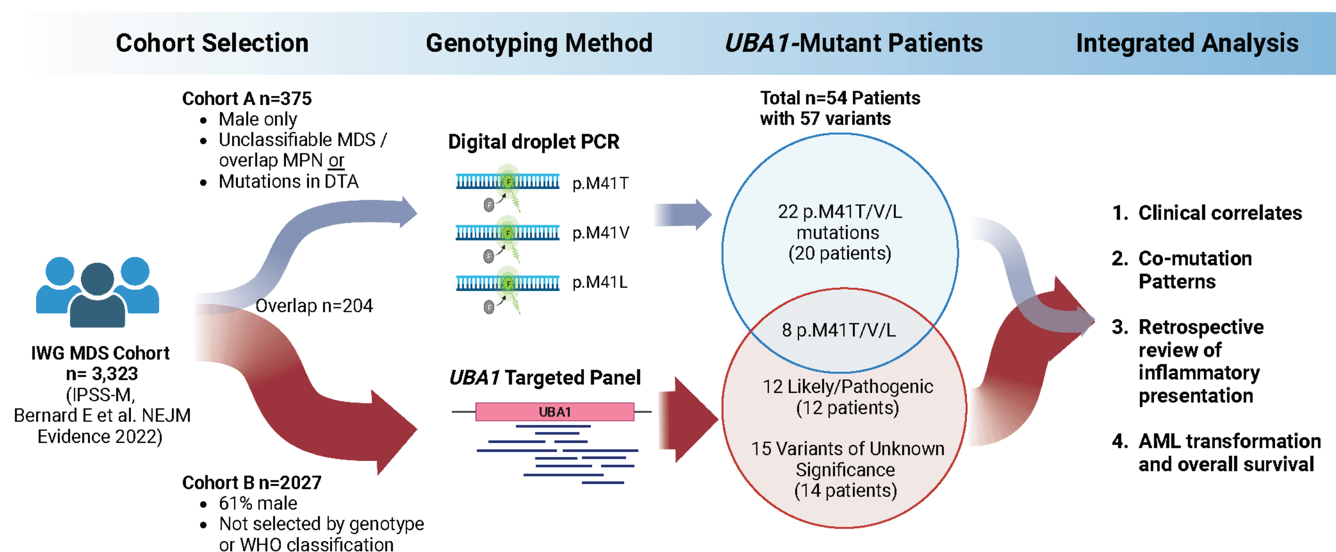
Characteristic	**p.M41T/V/L**, N = 33[1]	**Other Pathogenic**, N = 7[1]	**VUS**, N = 14[1]
Age at sample time	73 (65, 79)	69 (64, 73)	72 (55, 77)
WHO 2016 Classification			
MDS-SLD/MLD	20 (62%)	4 (57%)	6 (43%)
MDS-RS-SLD/MLD	0 (0%)	1 (14%)	1 (7.1%)
MDS/MPN-RS-T	1 (3.1%)	0 (0%)	0 (0%)
MDS-U	2 (6.2%)	1 (14%)	0 (0%)
MDS/MPN-U	1 (3.1%)	0 (0%)	0 (0%)
MDS-EB1/2	4 (12%)	0 (0%)	5 (36%)
CMML	2 (6.2%)	0 (0%)	1 (7.1%)
aCML	2 (6.2%)	0 (0%)	0 (0%)
AML	0 (0%)	1 (14%)	1 (7.1%)
Unknown	1	0	0
IPSS-M			
Very-Low	7 (23%)	2 (33%)	1 (9.1%)
Low	16 (52%)	2 (33%)	3 (27%)
Moderate-Low	4 (13%)	0 (0%)	1 (9.1%)
Moderate-High	2 (6.5%)	2 (33%)	2 (18%)
High	2 (6.5%)	0 (0%)	2 (18%)
Very-High	0 (0%)	0 (0%)	2 (18%)
Unknown	2	1	3
SEX			
F	0 (0%)	0 (0%)	3 (21%)
M	33 (100%)	7 (100%)	11 (79%)
Bone Marrow Blast (%)	2.00 (1.00, 4.00)	1.00 (1.00, 1.00)	4.00 (3.00, 8.00)
Unknown	0	0	1
White Blood Count (1e9/L)	4.30 (3.42, 7.61)	2.45 (2.04, 4.95)	3.80 (2.59, 5.23)
Unknown	2	1	2
Absolute Neutrophil Count (1e9/L)	3.00 (1.80, 4.66)	1.10 (0.94, 3.23)	1.58 (1.00, 2.84)

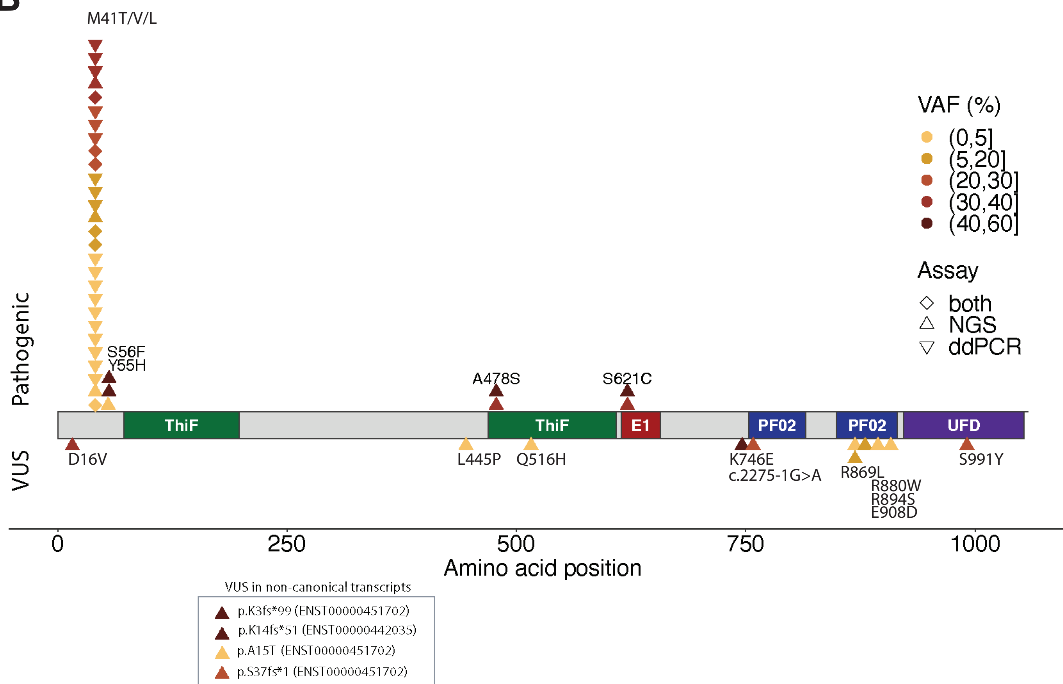
Unknown	1	0	2
Monocytes (1e9/L)	0.31 (0.10, 0.52)	0.36 (0.20, 0.65)	0.10 (0.10, 0.44)
Unknown	5	2	3
Hemoglobin (g/dL)	10.45 (8.75, 11.15)	9.90 (8.48, 11.60)	10.35 (8.17, 11.07)
Unknown	1	0	0
Platelets (1e9/L)	118 (86, 174)	57 (25, 65)	50 (31, 70)
Unknown	1	0	1

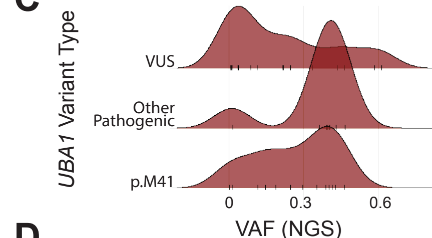
[1] Median (IQR); n (%)

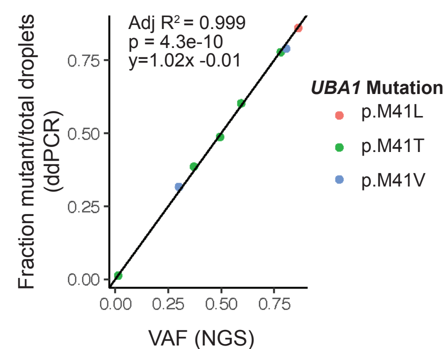
Figure 1

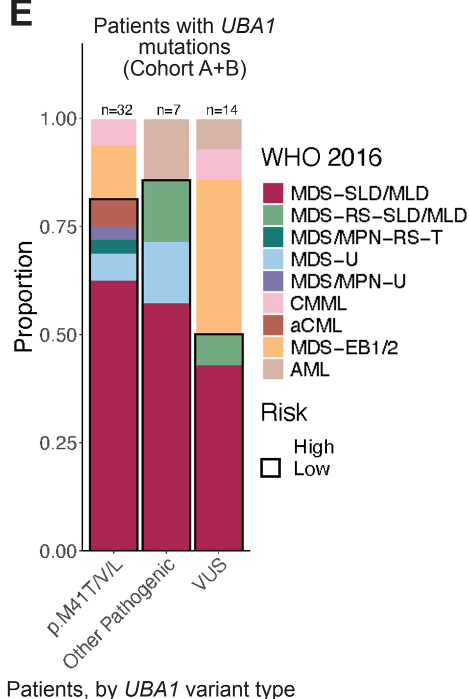
Figure 1

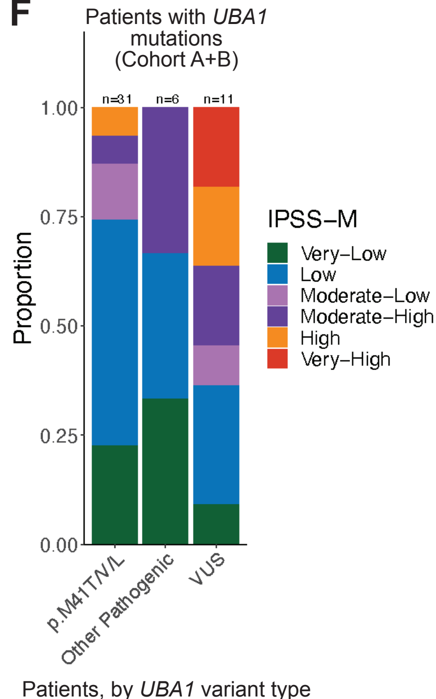












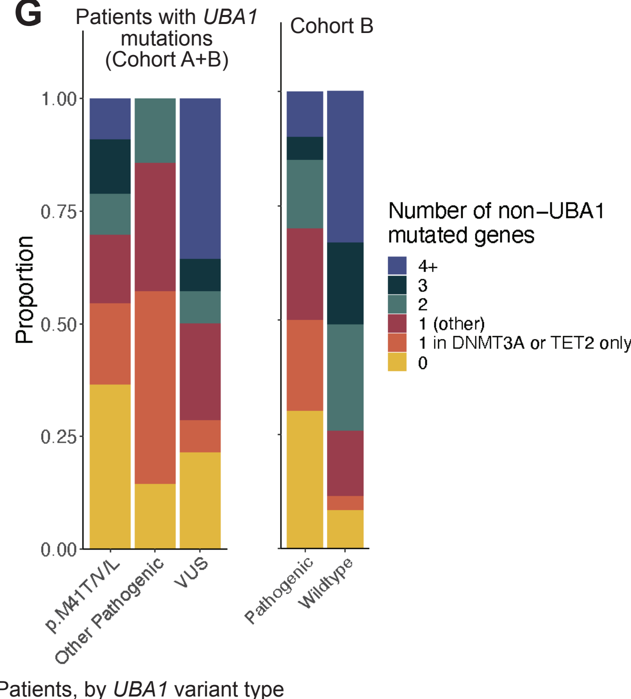


Figure 2

Figure 2

A

p.M41T/V/L p.Y55H p.S56F p.A478S p.S621C VUS

Protein Change
Cohort
WHO 2016
IPSS-M Risk
Sex
Age
UBA1 VAF 0 20 40
TET2
DNMT3A
ASXL1
SF3B1
Loss of Y
RUNX1
TP53
EZH2
BCOR
SRSF2
del(7)
del(7q)
del5q
+8
del(20q)
inv(16)
Total non-UBA1 events
Clinical History Available
Rheumatic Diagnoses
Skin/cartilage
Arthritis/joint pain
Pulmonary
Ocular
Constitutional
Thrombotic
Other hematologic
Treatment with steroids
Treatment with DMARDs/biologics
Vacuoles
C-reactive protein
LDH or ESR or Ferritin
AML Transformation
Alive last follow up

Clinical history review
Morphology
Laboratory Values
Outcomes

Alterations
- MT
- WT

Protein Change
- p.M41T
- p.M41V
- p.M41L
- p.M41T_p.M41V
- p.Y55H
- p.S56F
- p.A478S
- p.S621C
- VUS

Cohort
- both
- ddPCR
- NGS

WHO 2016
- MDS-SLD/MLD
- MDS-RS-SLD/MLD
- MDS/MPN-RS-T
- MDS-U
- MDS/MPN-U
- CMML
- aCML
- MDS-EB1/2
- AML

IPSS-M Risk
- Very-Low
- Low
- Moderate-Low
- Moderate-High
- High
- Very-High

Sex
- F
- M

Age
- <50
- 51-60
- 61-70
- 71-80
- 81-90

UBA1 VAF
1
0.5
0

Total non-UBA1 events
- 0
- 1
- 2
- 3
- 4+

Clinical History Available
- Reviewed
- Not available

Rheumatic Diagnoses
- Seronegative rheumatoid arthritis
- Relapsing polychondritis
- Polymyalgia rheumatica
- Psoriatic arthritis
- Systemic lupus erythematosus and Rheumatoid arthritis
- No
- NA

Skin/cartilage, Arthritis/joint pain, Pulmonary, Ocular, Constitutional, Thrombotic
- True
- False
- NA

Other hematologic
- Monoclonal gammopathy of undetermined significance
- T-cell large granular lymphocytic leukemia and Immune thrombocytopenic purpura
- Thalessemia minor
- Chronic lymphocytic leukemia
- Hodgkin's lymphoma
- No
- NA

Treatment with steroids or DMARDs/biologics
- Treated
- No
- NA

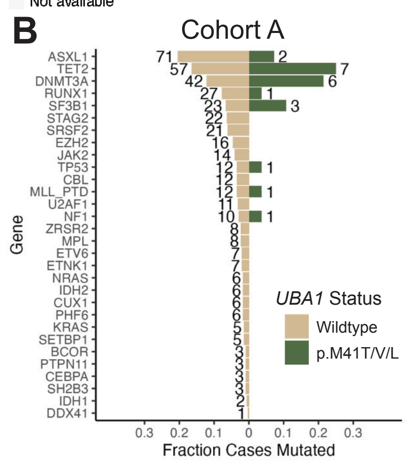
Vacuoles
- Vacuoles
- No Vacuoles
- Slides not available

C-reactive protein LDH or ESR or Ferritin
- Elevated
- Not elevated
- NA

AML Transformation
- True
- No
- NA

Alive last follow up
- Alive last follow up
- Dead last follow up
- NA

B



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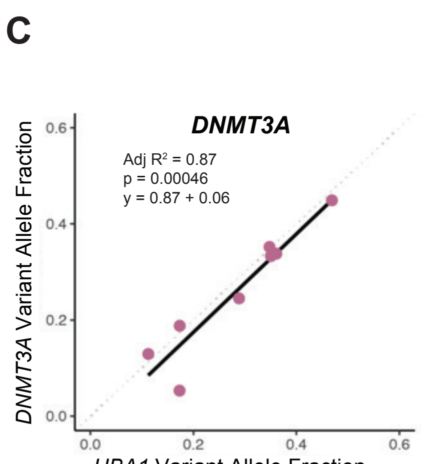

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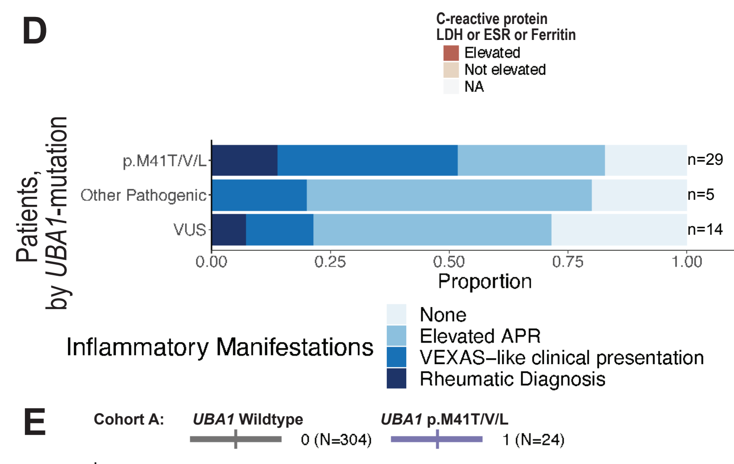
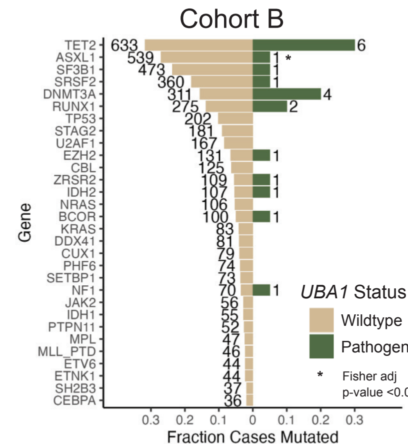
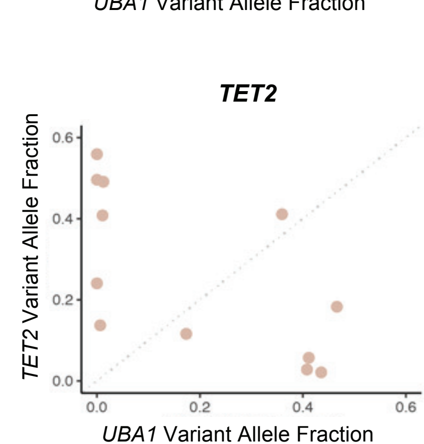
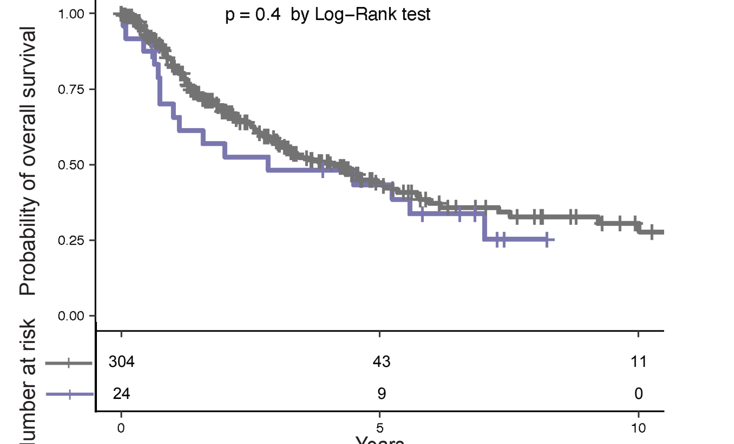






E



Molecular and Clinical Presentation of *UBA1*-Mutated Myelodysplastic Syndromes (MDS)

Context of Research

VEXAS syndrome is a monogenic disease caused by somatic mutations in *UBA1* in hematopoietic cells

A portion of VEXAS patients meet established diagnostic criteria for MDS

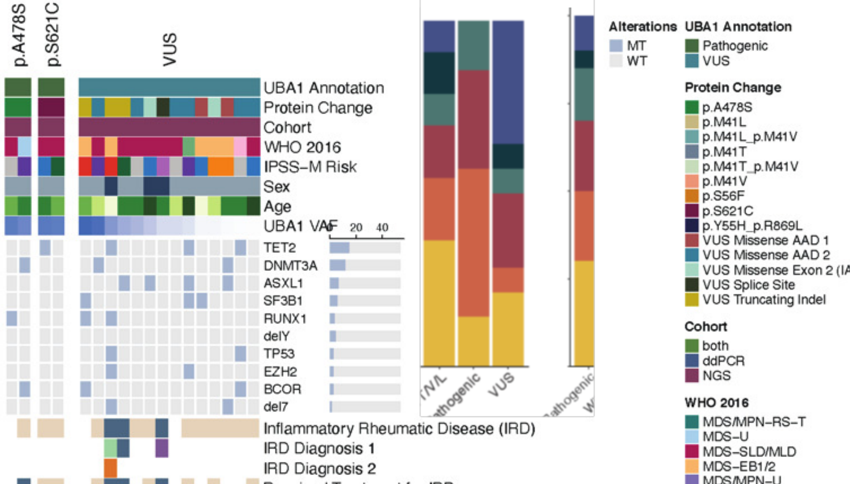
Patients and Methods

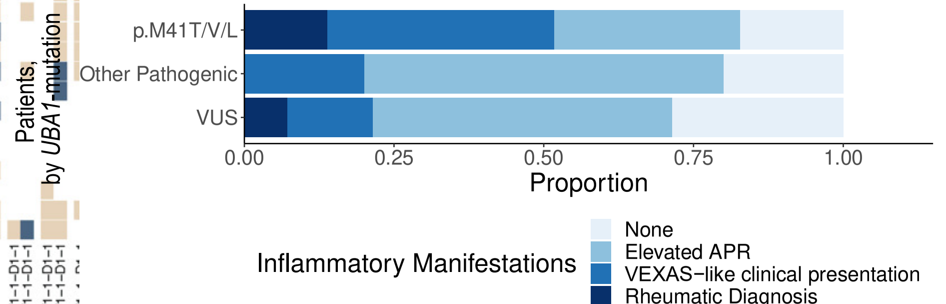
2,198 MDS patients

- *UBA1* ddPCR in 375 patie
- *UBA1* NGS in 2,027 patie
- Correlation with 152 gene myeloid panel and inflamm clinical presentation

Main Findings

UBA1 mutations enriched in low risk MDS wi





Conclusions: **In a large MI**
were frequent (7%) in the subse... o mutations in
(83%) associated with inflamma

*Sirenko et al. DOI: 10.xxxx/**blood***

mutations