# A Case Series of 17 Patients with VEXAS Syndrome due to *UBA1 p.*Met41 Variants

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## Introduction

VEXAS syndrome (vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic) is an adult-onset inflammatory condition primarily affecting males and was first described by Beck et al. in December 2020[a]. Affected individuals develop rheumatologic and hematologic symptoms, including recurrent fevers, vasculitis, arthralgias, chondritis of the ear and nose, and pulmonary and dermatologic manifestations. Clinical suspicion of VEXAS is molecularly confirmed when a disease-associated somatic variant in the *UBA1* gene on the X chromosome is identified in a DNA specimen containing myeloid tissue.

The most common genomic variants associated with VEXAS affect the *UBA1* Met41 residue, including NM_003334.4 c.122T>C (p.Met41Thr), c.121A>G (p.Met41Val), and c.121A>C (p.Met41Leu)[a]. Other variants in *UBA1* associated with VEXAS continue to be identified[b]. Males showing somatic mosaicism for a disease-associated *UBA1* variant are affected, whereas females are typically unaffected unless monosomy X has also been identified. Specimen type selection for analysis by next-generation sequencing (NGS) is important, as *UBA1* variants in affected individuals are commonly identified in DNA extracted from blood or bone marrow but absent from non-heme-derived samples[c,d].The median variant allele fraction (VAF) of Met41 variants reported in peripheral blood or bone marrow specimens of affected, untreated males is between 44% to 80%[a,c,e] but can be as high as 96%[d]. Bone marrow transplant and treatment with hypomethylating agents can be expected to lower VAFs of characteristic *UBA1* variants[e]. As this syndrome is newly described, no published reports to date have shared the experience of a referral laboratory regarding molecular testing for VEXAS. In this study, we summarize the results from individuals with *UBA1* Met41 variants who received NGS-based genetic testing and describe VAFs with respect to specimen type.

## Methods

We performed a retrospective analysis of an internal sequencing database consisting of deidentified data from patients who underwent clinical genetic testing at Blueprint Genetics to identify those with reported variants at the NM_003334.4 *UBA1* c.122 and c.121 genomic positions affecting the Met41 residue. Validation of the NGS-based assay used for genetic testing showed 100% of the *UBA1* target region (all coding exons +/- 20 base pairs into the intron-exon boundary) was covered at >20x with 99.89% sensitivity for detection of sequence variants (Blueprint Genetics unpublished data). Assay validation also showed that mosaic variants with an allelic ratio of as low as 14.6% can be detected with greater than 90% probability. Specimen types accepted for genetic testing included peripheral blood, saliva, and DNA extracted from other tissue sources. The genetic test, variant results, and variant allele fractions were extracted from our internal sequencing database, and patient sex, age at testing, and specimen type were collected from test requisition forms. A two-tailed t-test was used to analyze the difference in mean VAF

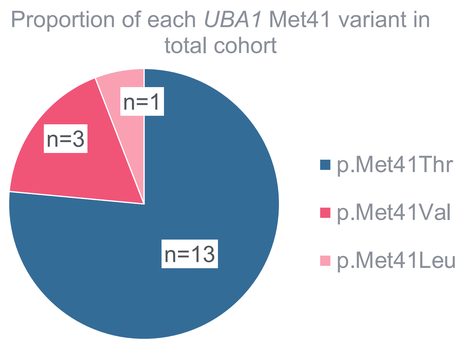
## Results

Seventeen patients were identified with one of the common Met41 variants: p.Met41Thr (n=13; 76%); p.Met41Val (n=3; 18%); p.Met41Leu (n=1; 6%) (Figure 1a). All 3 variants were classified as pathogenic with respect to VEXAS syndrome. All patients were male, and age at testing ranged from 62 to 91 years (median=68) (Table). Patients received either *UBA1* single gene testing (n=14) or a panel test including up to 336 genes (n=3) (Figure 1b). Most specimens submitted were peripheral blood (82%, 14/17), along with 2 saliva specimens and 1 specimen of DNA extracted from bone marrow aspirate. VAFs in blood or bone marrow specimens ranged from 41% to 91% (median=62%). The 2 cases where saliva specimens were submitted for analysis show the lowest VAFs, at 4% and 28% (Table). A significant difference was observed in mean VAF from specimens where DNA was extracted from blood or bone marrow (M= 64.73, SD=17.50) compared to those from specimens extracted from saliva (M=16.00, SD=16.97), t(15)=3.71, p=.002.

Analysis of next-generation sequencing data from a validated assay can identify *UBA1* p.Met41 somatic variants associated with VEXAS syndrome.

| Study ID | Sample type submitted to lab | Patient age at testing in years | *UBA1* variant identified | Variant allele fraction (VAF) |
|---|---|---|---|---|
| 001 | Peripheral Blood | 68 | p.Met41Thr | 91% |
| 002 | Peripheral Blood | 68 | p.Met41Thr | 85% |
| 003 | Peripheral Blood | 72 | p.Met41Leu | 84% |
| 004 | Peripheral Blood | 70 | p.Met41Thr | 82% |
| 005 | Peripheral Blood | 75 | p.Met41Thr | 80% |
| 006 | Peripheral Blood | 63 | p.Met41Thr | 79% |
| 007 | Peripheral Blood | 62 | p.Met41Val | 67% |
| 008 | Peripheral Blood | 64 | p.Met41Val | 62% |
| 009 | Peripheral Blood | 73 | p.Met41Thr | 61% |
| 010 | Peripheral Blood | 64 | p.Met41Thr | 51% |
| 011 | DNA from peripheral blood | 91 | p.Met41Thr | 51% |
| 012 | DNA from peripheral blood | 71 | p.Met41Thr | 49% |
| 013 | DNA from bone marrow aspirate | 85 | p.Met41Thr | 46% |
| 014 | DNA from peripheral blood | 74 | p.Met41Val | 42% |
| 015 | DNA from peripheral blood | 67 | p.Met41Thr | 41% |
| 016 | Saliva | 67 | p.Met41Thr | 28% |
| 017 | Saliva | 66 | p.Met41Thr | 4% |

Table. Characteristics of patient cases with *UBA1* Met41 variants identified via single gene testing or panel testing



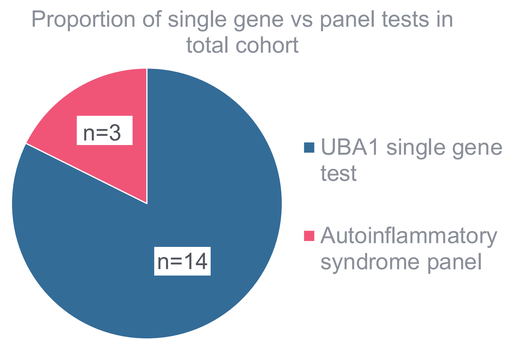




**Figure 1. Variant identified and test ordered characteristics in total cohort (n=17)**
**a) Proportion of each *UBA1* Met41 variant in total cohort**
**b) Proportion of single gene vs panel tests in total cohort**

## Conclusions

- Analysis of NGS data from a validated assay can identify Met41 somatic variants in the *UBA1* gene associated with VEXAS.
- Median variant allele fraction of variants detected in DNA extracted from blood and bone marrow (62%) is consistent with findings reported to date and much lower variant allele fractions were seen in the 2 saliva specimens in this study.
- Caution may be warranted in sending saliva specimens for molecular confirmation of VEXAS, as the variant allele fractions reported here are under the level of mosaic variant detection for many NGS assays. Further studies are needed to confirm if variant allele fractions of *UBA1* Met41 variants are consistently lower in DNA samples extracted from saliva than those from blood or bone marrow aspirate when patients have not undergone bone marrow transplant or treated with hypomyelinating agents.

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Conflict of interest statement: All authors are employed by Blueprint Genetics.

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