A homozygous missense variant in **UBE2T** is associated with a mild Fanconi anemia phenotype

Fanconi anemia (FA) is a rare multi-system disorder characterized by bone marrow failure, congenital abnormalities, and cancer predisposition. Pathogenic variants have been described in 22 known FA genes (FANCA-FANCI) that are required for the proper repair of DNA interstrand crosslinks. A key step in the repair of interstrand crosslinks is FA pathway activation via monoubiquitination of FANCD2 and FANCI by FANCL, an E3 ubiquitin-ligase working with UBE2T/FANCT, an E2 ubiquitin-conjugating enzyme. Pathogenic germline variants in **UBE2T** have been described in three individuals with FA, thus, the knowledge of the phenotypic spectrum is limited for the FA-T complementation group. Here we describe a mild presentation of FA resulting from a hypomorphic missense variant in **UBE2T** that partially disrupts the function of the encoded protein. This report highlights the importance of an algorithmic approach to bone marrow failure that combines genetic testing and functional cellular assays.

Three patients have previously been reported with biallelic pathogenic variants in **UBE2T** consistent with an autosomal recessive disease. All three patients presented with the classic features of FA (Online Supplementary Table S1). Hira et al. reported two unrelated patients both harboring a c.4C>G, p.Gln2Glu missense variant in trans with either a 23 kb whole gene deletion (patient 1) or a c.180+5G>A, p.Gln37Argfs*47 frameshift variant (patient 2). Both patients developed hematologic abnormalities and bone marrow failure. Patient 2 developed myelodysplastic syndrome which evolved to acute myeloid leukemia. Both patients required hematopoietic stem cell transplantation. Rickman et al. and Virts et al. reported the findings of a maternally inherited Alu-mediated duplication, c.-64_468dup, producing an unstable transcript and a paternally inherited Alu-mediated deletion, c.-64_468del, leading to loss of the majority of the gene. However, this patient did not develop bone marrow failure as a result of somatic mosaicism identified in his peripheral blood.

The patient reported here is a 22-year-old Hispanic female who was unaffected at birth, had a normal developmental history, and a negative family history with no reported consanguinity. She originally presented to another institution at 8 years of age and was reported to have mild neutropenia and thrombocytopenia; a bone marrow biopsy at that time was non-diagnostic. At 21 years, the patient presented with persistent neutropenia and macrocytosis, intermittent thrombocytopenia, episodic fevers, an urticarial erythematous rash, and metromenorrhagia (Online Supplementary Tables S1 and S2). No developmental anomalies or cutaneous hypo/hyperpigmentation were noted. Chromosomal
breakage assays performed on peripheral blood lymphocytes showed increased breakage (Online Supplementary Table S1). A repeat bone marrow biopsy revealed moderate hypocellularity (40-50%) with no evidence of dysplasia or a lymphoproliferative process and a normal karyotype. A gene panel to investigate periodic fever was negative (Online Supplementary Table S3). Due to the patient’s undiagnosed neutropenia, panel-based next-generation sequencing was performed on whole blood (Online Supplementary Table S4) and revealed a homozygous c.196C>A, p.P66T (NM_014176.3, Chr1(GRCh37):202302667G>T) missense variant of uncertain significance in UBE2T. This variant is absent from the gnomAD database. GeneDx exon level deletion/duplication calling from sequencing data (with manual verification) did not detect any evidence of a multi-exon copy number variant in UBE2T, suggesting the patient is not hemizygous. Parental samples were not available for testing.

The p.P66T variant identified causes a substitution of a hydrophobic to polar uncharged amino acid at a highly conserved position in the UBC fold domain (Online Supplementary Figure S1). Multiple in silico tools predict that this variant is likely to be damaging (Online Supplementary Table S5). The proline 66 resides at the base of one of multiple loops comprising the FANCL binding region (Online Supplementary Figure S2A and B). When modeled, P66T is predicted to change the position of the loop because of changes in the backbone ϕ/ψ angles. The loop is moved out, as compared to the wild-type (WT) structure, and the interacting residues are moved away from the UBE2T and FANCL interface (Online Supplementary Figure S2C). As P66T changes the range of peptide backbone flexibility, making the base of the loop much more flexible, the binding with FANCL is expected to be dysregulated from a stricter cis/trans switch.

In order to confirm the pathogenicity of the c.196C>A (p.P66T) variant in UBE2T, functional in vitro studies were performed. Sanger sequencing of genomic DNA and complementary DNA from patient-derived fibroblasts (PM085) confirmed the presence of this variant and absence of splicing defects (Figure 1A and B). Immunoblotting of whole cell extract from these cells demonstrated decreased, but not absent, UBE2T protein expression (Figure 1C). This is consistent with the p.P66T missense variant causing instability in the UBE2T protein, resulting in the observed decrease in protein level.

To determine whether the c.196C>A (p.P66T) variant affects the E2 function of UBE2T, FANCD2 monoubiquitination was assessed after treatment with the DNA
interstrand crosslinking agent mitomycin C. Normal
FANCD2 monoubiquitination was observed in the WT
control cell line (BJ fibroblasts), was absent in UBE2T
null fibroblasts (UBE2T−/− RA2627) and FANCA−/− fibroblasts and was
reduced in the proband’s fibroblasts (Figure 1E).

Expression of WT UBE2T in the patient’s fibroblasts fully
rescued FANCD2 monoubiquitination (Figure 1D and E),
recruitment of FANCD2 to chromatin after mitomycin C
treatment, and sensitivity of the proband’s fibroblasts to
mitomycin C (Figure 1F-H). These results indicate that
the proband belongs to FA-T complementation group
and suggest that the patient’s missense variant is hypo-
morphic, resulting in reduced function.

To further demonstrate that the missense variant
reduces UBE2T function and is indeed likely pathogenic,
UBE2T−/− fibroblasts were transduced with either WT or P66T
HA-tagged UBE2T (Figure 2A). P66T variant
expressed at a lower level compared to WT UBE2T, con-
sistent with decreased stability of UBE2T carrying that
variant. Expression of P66T UBE2T also only partially
rescued cell survival, FANCD2 ubiquitination, and
FANCD2 foci formation upon treatment with mitomycin C
compared to WT UBE2T expression (Figure 3B-D). This provides further evidence that the missense variant is a likely pathogenic hypomorph.

The cellular and patients’ phenotypes described for the
FA-T complementation group are thus far consistent with
defective FA pathway activation and a defect in interstrand
crosslink repair. However, it was previously reported that
UBE2T-deficient DT40 cells were sensitive to ultraviolet
irradiation and the replication stress-inducing agent,
hydroxyurea.11 To determine whether UBE2T is important
for resistance to other types of DNA damage, RA2627 cells
were tested for sensitivity to a number of other genotoxic
agents. RA2627 cells were not found to be hypersensitive
to ultraviolet irradiation, ionizing radiation, camptothecin,
hydroxyurea, or the PARP inhibitor olaparib (Figure 3A-E).
These data suggest that UBE2T does not have a major role
in responding to DNA lesions or replication stress pro-
duced by these agents and its primary function is in inter-
strand crosslink repair and that the patients’ phenotypes
reflect defects in the repair of interstrand crosslink lesions.

In conclusion, we report a novel presentation of FA-T
complementation group resulting from a likely pathogen-
ic missense variant (c.196C>A) in UBE2T. The patient
presented with atypical, mild FA, characterized by per-
sistent macrocytosis and neutropenia with intermittent
thrombocytopenia but no severe bone marrow failure
(without evidence of somatic reversion in blood) or con-
genital abnormalities common to FA. Clinical chromoso-
mal breakage assays were consistent with a diagnosis of
FA and subsequent functional analysis of patient-derived

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**Figure 2.** P66T UBE2T is a partial loss-of-function variant. (A) Immunoblot with anti-HA antibody of RA2627 (UBE2T−/−) primary fibroblasts expressing empty vec-
tor (EV) or C-HA-FLAG P66T UBE2T or wild-type (WT) UBE2T. (B) Cell survival of RA2627 (UBE2T−/−) fibroblasts expressing EV, P66T UBE2T, or WT UBE2T after
treatment with mitomycin C (MMC). (C) FANCD2 ubiquitination with and without MMC treatment in RA2627 (UBE2T−/−) fibroblasts expressing EV, P66T UBE2T,
or WT UBE2T. (D) Quantification of FANCD2 foci formation after MMC treatment in RA2627 (UBE2T−/−) fibroblasts expressing EV, P66T UBE2T, or WT UBE2T.
Approximately 300 HA-expressing cells were analyzed for the presence of FANCD2 foci in three separate coverslips. The mean percent nuclei with FANCD2 foci
was plotted and tested for significance using one-way analysis of variance with multiple comparisons. ns: not significant, ****P≤0.0001.
fibroblasts and the p.P66T UBE2T variant performed here demonstrate that the hypomorphic variant is the likely cause of disease in this patient and can be classified as likely pathogenic following the recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.12

The c.196C>A (p.Pro66Thr) UBE2T variant is likely damaging to UBE2T function by conferring both reduced E2 activity and reduced stability as immunoblotting demonstrated decreased protein levels. The p.P66T variant affects a residue highly conserved across various E2 and likely affects the interaction with FANCL due to the amino acid residue substitution being at the hydrophobic E2-E3 interface.10

The patients previously reported by Hira et al. who also had a missense variant, p.Q2E, were likewise demonstrated to be hypomorphic in RA2627 cells,13 but heterozygous and in trans to loss of function variants suggesting the possibility of UBE2T dosage sensitivity, as the two patients presented with more severe disease. Disease severity may also be increased in those two patients because of the presence of the ALDH2* variant which is known to interact genetically with the FA pathway.14

We hypothesize that the hypomorphic variant and resulting residual function of the c.196C>A (p.P66T) variant in UBE2T explains our patient’s mild phenotype. This case adds to the limited knowledge regarding this rare FA-T complementation group. It is possible that there are other undiagnosed patients with mild phenotypes, emphasizing the utility of an algorithmic approach utilizing genomic sequencing and functional analysis for patients with non-specific hematologic phenotypes.

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Contributions: FPL, KAR, LSR, AF, KJC, EWK and AS designed the study and interpreted the results. FPL, KAR, TLS and CTS performed the study. MMP, AM and ML were the treating team at the Mayo Clinic where the patient was seen in the institutional inherited bone marrow failure clinic. NJ oversaw the patient’s care at Hennepin.
County Medical Center. NRD, and MTZ performed in silico protein modeling. LSR, KAR, FPL, MMP and AS wrote the manuscript with input from the other authors.

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