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Novel germline missense *DDX41* variant in a patient with an adult-onset myeloid neoplasm with excess blasts without dysplasia

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To the editor,

DEAD/H-box helicase gene, *DDX41* is a tumor suppressor gene located on chromosome 5 (5q35) which plays a role in pre-mRNA splicing and processing[1]. Germline *DDX41* canonical frameshift insertions induce a haploinsufficiency which is leukemogenic, although somatic perturbations in this gene also have similar effects[1]. Often, these mutations portend an unfavorable prognosis and aggressive disease biology in myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML). The majority of del(5q) MDS cases are theorized to include the *DDX41* locus, in particular the longer interstitial deletions, and suggest therapeutic responsiveness to lenalidomide[1,2]. Several families with *DDX41* germline variants have been reported[3]. The inheritance pattern is variable with presentations often later in life, suggesting that germline *DDX41* mutation is more a ‘first hit’ or an ‘ancestral event’, requiring a somatic mutation or ‘second hit’ to develop MDS/AML phenotype[1].

Here we report a case of a 70-year-old man, who was referred to our pre-myeloid and bone marrow failure precision genomics clinic (NCT#02958462, www.clinicaltrials.gov) for adult-onset pancytopenia without definitive evidence of marrow dysplasia and was subsequently found to have a novel *DDX41* pathogenic variant. Prior to this referral, his work-up included a vitamin B12 level of 330 [normal range (NR): >200] pg/mL

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Conflict of interests

No conflict of interest.

(methylmalonic acid level not available) and serum folate level of 11.3 ng/mL (normal range: 2-20). Hence, he was started on vitamin B12 injections. Additional laboratory tests showed a white blood cell count of 2,200/ μ L (NR: 3,500-10,500), hemoglobin of 11.9 g/dL (NR: 14-18), and platelet count of 2,000/ μ L (NR: 150,000-450,000). MCV was elevated at 105.2 fL (NR: 78.2-97.9 fL). Peripheral blood smear did not show dysgranulopoiesis, circulating blasts or absolute monocytosis. For a clinical concern of idiopathic thrombocytopenic purpura (ITP), a dose of intravenous immunoglobulin (IVIG) and a 4-day course of dexamethasone were administered, without response.

Both a bone marrow aspirate (BMA) and core biopsy were obtained. These were normocellular (30%), but BMA differential showed 17% blasts (Figure 1, panel A and B) which was confirmed by CD34 immunohistochemistry on the core biopsy (Figure 1, panel D). No Auer rods were seen. Flow cytometric immunophenotyping showed a myeloid blast population without definitive immunophenotypic aberrancy. Blasts expressed CD34, CD45 (dim), CD13, CD33, CD117, HLA-DR and CD38, and were negative for CD19, CD10, CD3, CD15, CD16, CD2, CD7, CD56, CD36 and CD64. The background trilineage hematopoiesis showed a myeloid to erythroid ratio of 1:1, but with no significant dysplasia in any of the three cell lines (Figure 1, panel C). Iron stain showed no ring sideroblasts. Given these findings, in spite of the increased blasts, a reactive process could not be completely excluded at this time.

Chromosome studies revealed a normal male karyotype: 46,XY[20]. FISH for abnormalities involving KMT2A (MLL), MYH11, and CBFB loci were performed to exclude an early acute myeloid leukemia and were negative. A next generation sequencing gene panel did not reveal any abnormalities. Genes included on this panel were: *ASXL1*, *BCOR*, *BRAF*, *CALR*, *CBL*, *CEBPA*, *CSF3R*, *DNMT3A*, *ETV6*, *EZH2*, *FLT3*, *GATA1*, *GATA2*, *IDH1*, *IDH2*, *JAK2*, *KIT*, *KRAS*, *MPL*, *MYD88*, *NOTCH1*, *NPM1*, *NRAS*, *PHF6*, *PTPN11*, *RUNX1*, *SETBP1*, *SF3B1*, *SRSF2*, *TERT*, *TET2*, *TP53*, *U2AF1*, *WT1*, and *ZRSR2*.

A comprehensive work up for alternative causes of the patient's cytopenias was unrevealing. A repeat bone marrow after 5 months revealed identical findings including normocellularity, persistence of approximately 15% myeloid blasts, and lack of significant dysplasia. Chromosome studies were again normal. Due to persistent thrombocytopenia, eltrombopag was initiated with modest response and subsequently transitioned to romiplostim 5 μ g/kg (due to insurance issues), with a gradual improvement in his platelet counts. Subsequently, a research-based whole exome sequencing from peripheral blood DNA was performed, which showed a variant of uncertain significance in *DDX41* (NM_016222.2(DDX41):c.773C>T; p.Pro258Leu) later confirmed to be germline by targeted testing in fibroblast DNA. The variant is not reported in general population databases such as gnomAD (<http://gnomad.broadinstitute.org>) nor in clinical databases (ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar/>) and Human Gene Mutation Database, HGMD (<http://www.hgmd.cf.ac.uk/>)). *In silico* prediction tools (M-CAP[4] and PredictSNP2[5]) deemed the variant deleterious. It is highly conserved among paralogs and orthologues and lies on the DEAD-box domain which is a region scored intolerant to variations by subRVIS[6]. The patient's family history is negative for myeloid neoplasms, although his sister was recently diagnosed with chronic lymphocytic leukemia.

Population-based studies have highlighted familial aggregation of myeloid neoplasms[8], thereby resulting in the inclusion of ‘myeloid neoplasm with germline predisposition’ as a separate entity in the 2017 revision of the WHO classification of myeloid neoplasms[9]. Germline mutations in *CEBPA*, *RUNX1*, *ANKRD26*, *ETV6* and *DDX41* genes have been linked with familial myeloid neoplasms[10,11]. *DDX41* encodes for a DEAD-box RNA helicase and is part of a family of proteins involved in many aspects of RNA metabolism[12]. *DDX41* is involved in innate immunity and foreign DNA recognition, since it binds DNA and STING via the DEAD domain and triggers activation of signaling pathways resulting in interferon production[12].

After the first report of *DDX41* germline variants in association with myeloid neoplasms[1], over 70 families have been reported with a wide range of malignancies[14]. The most common phenotype of families with germline *DDX41* variants involve the development of MDS/AML, although families with lymphoma, multiple myeloma, and sarcoidosis have also been reported[13]. Although the most common variant in Caucasian families is the *DDX41* p.Asp140Glyfs*2, several frameshift, nonsense and missense mutations have been identified. Pathogenicity of some of these previously described variants were further confirmed using a cell-based assay based on the overexpression of the mutant *DDX41* in 293T cells that proved loss of function of *DDX41*[7]. We would need to perform similar experiments on our variant in order to conclude on its pathogenicity. Segregation studies would also be informative in that sense, but unfortunately no other family members were available for testing.

Interestingly, due to the location of *DDX41* on the long arm of chromosome 5 (5q35), its alterations are associated with responsiveness to lenalidomide[1]. Given the severe thrombocytopenia and the lack of conclusive data supporting this, we have not treated our patient with the same.

In summary, we report a novel germline *DDX41* variant presenting with pancytopenia and excess marrow blasts without dysplasia. Initially we were reticent to consider this patient as having myelodysplastic syndrome, given the striking *lack* of dysplasia. Although technically he meets WHO criteria for MDS with excess blasts-2 (MDS-EB2), in our experience these patients virtually always have some degree of bone marrow cytologic atypia. We considered an oligoblastic AML; however, lack of confirmatory cytogenetic findings and progression of his blast count over several months argue against this. Further study of similar patients with elevated blast counts without dysplasia, as well as patients with *DDX41* mutations is warranted. Whether the best diagnosis for this patient is MDS-EB2, versus an unclassifiable myeloid neoplasm with excess blasts and *DDX41* mutation, versus simply germline *DDX41* mutation and marrow findings of uncertain significance, remain up for debate.

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Figure 1:

Initial bone marrow aspirate smear and core biopsy. Panels A and B show bone marrow aspirate with increased numbers of small blasts with fine chromatin and basophilic cytoplasm. Erythroid precursors and granulocytic precursors show no significant dysplasia. Panel C shows the core biopsy with normocellular marrow for age and cytologically normal megakaryocytes and immature myeloid cells. CD34 stain (Panel D) shows 10-20% blasts (400 $\times$). Wright Giemsa, 1000 $\times$ (A,B), Hematoxylin and Eosin, 400 $\times$ (C).