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Overall cancer risk in people with deleterious germline *DDX41* variants

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Abstract

Germline loss of function (LoF) *DDX41* variants predispose to late-onset hematopoietic malignancies (HMs), predominantly of myeloid lineage. Among 43 families with germline *DDX41* LoF variants, bone marrow (BM) biopsies in those without (n=8) or with malignancies (n=21) revealed mild dysplasia in peripheral blood (57%) and BM (88%), long before the average age of *DDX41*-related HM onset. Therefore, we recommend baseline bone marrow biopsies in people with germline $DDX41^{LoF}$ alleles to avoid over-diagnosis of myelodysplastic syndromes. A variety of solid tumors were also observed in our cohort, with 24% penetrance by age 75. Although acquired *DDX41* mutations are common in HMs, we failed to identify such alleles in solid tumors arising in those with germline $DDX41^{LoF}$ variants (n=15), suggesting an alternative mechanism driving solid tumor development. Furthermore, 33% of pedigrees in which $\geq$15% of first-degree relatives including the proband were diagnosed with a solid tumor had second germline deleterious variants in other cancer-predisposition genes, likely serving as primary cancer drivers. Finally, both lymphoblastoid cell lines and primary peripheral blood from individuals with germline $DDX41^{LoF}$ variants exhibited differential levels of inflammation-associated proteins. These data provide evidence of inflammatory dysfunction mediated by germline $DDX41^{LoF}$ alleles that may contribute to solid tumor growth in the context of additional germline cancer-associated variants. For those with HMs and personal/family histories of solid tumors, we recommend broad germline testing. DDX41 may be an indirect modifier of solid tumor pathogenesis compared to its tumor suppressor function within hematopoietic tissues, a hypothesis that can be addressed in future work.

Introduction

Germline deleterious variants in many genes are known to predispose to hematopoietic malignancies (HMs), and classification schemes for leukemias now include these entities. (1-3) *DDX41*, encoding DEAD-box RNA helicase 41, is the most common germline-mutated gene in adult myeloid neoplasms (MNs), driving approximately 3% of MNs.[4] Germline *DDX41* loss-of-function (LoF) alleles predispose to late-onset MNs. (4-16) Fifty-four percent of *DDX41*-mutated neoplasms acquire a somatic mutation on the wild-type (WT) allele, usually the "hotspot" variant *DDX41* c.1574G>A (Arg525His), suggesting that DDX41 acts as a tumor suppressor for MNs. (4-9, 11, 12, 14, 15, 17) Multiple *in vivo* studies show an association between germline *DDX41*^{LOF} variants and hematopoietic stem cell expansion, (18-21) with R-loop accumulation (18) and a genomic instability-associated inflammatory response.(20, 21) However, the exact mechanism by which germline *DDX41*^{LOF} alleles contribute to malignancies is unclear.

Those with germline *DDX41*^{LoF} variants develop MNs at a median age of 68, generally with favorable overall survival. (4, 8, 13-15) However, these individuals are at risk for severe acute graft-versus-host disease (GVHD) when they undergo allogeneic hematopoietic cell transplantation even with WT donors, unless they receive post-transplant cyclophosphamide, (22) suggesting an activated inflammatory milieu.(4) Furthermore, solid tumors have been reported in families with germline *DDX41*^{LoF} alleles variants, but it is unknown if that allele contributes directly to their development.(10, 13) DDX41 is an RNA helicase required for activation of the cGAS-stimulator of IFN genes (STING)-type I interferon pathway in response to DNA virus invasion, which suppresses R-loop accumulation. (18, 20, 23) Furthermore, DDX41 can activate cGAS-STING in response to R-loop accumulation. (18) Therefore, DDX41 plays an important role in immune regulation even in the absence of viral DNA invasion. Additionally, the *DDX41* "hotspot" variant Arg525His increases STING activation, while knockout (KO) of *DDX41* decreases STING activation, suggesting that different *DDX41* alleles differentially affect DDX41-mediated immune processes and cancer predisposition. (18, 23) However, the impact of different germline *DDX41* variants on immunity and inflammation has yet to be investigated in patients or human-derived cell lines.

Methods

Additional details are provided in the Supplementary Methods.

Patients

All individuals signed written informed consent to participate in research approved by Institutional Review Boards at the University of Chicago and Northwestern University, conducted in accordance with the Declaration of Helsinki, and protected by National Institutes of Health Certificates of Confidentiality.

Germline Sequencing

Individuals with personal and/or family histories consistent with a deleterious germline *DDX41* variant or those with such alleles identified via tumor profiling (24) underwent clinical germline genetic testing (Supplementary Table 1). DNA was sequenced using an augmented whole exome sequencing platform (25) in the University of Chicago Genetic Services Laboratory (https://genes.uchicago.edu/clinical-genetics). DNA variants in 139 cancer-predisposing genes (Supplementary Table 2) were analyzed. A custom bioinformatic pipeline capable of detecting single nucleotide variants and copy number variants was used (https://github.com/LucyGodley/Pipeline/blob/main/Variant_Calling/WES/hg/Automated/WES_Pipeline.sh). (16) Variants were curated according to the American College of Medical Genetics and Genomics/Association of Molecular Pathology. (26) Deleterious variants in cancer-causing genes were confirmed by Sanger sequencing.

Somatic Solid Tumor Sequencing

DNA derived from formalin-fixed, paraffin-embedded solid tumor tissue derived from eight patients with germline *DDX41*^{LOF} variants was sequenced via the OncoPlus next-generation panel, which includes *DDX41*. (27) Additional tumor-derived sequencing data from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/) were acquired for eleven additional patients with truncating *DDX41* alleles that are likely to be germline based on the frequency with which such alleles are inherited. (17)

LCL Preparation

Lymphoblastoid cell lines (LCLs) were derived from peripheral blood B-cells from individuals with deleterious germline *DDX41*^{LOF} variants (*DDX41*$^{var/+}$), which were transformed using Epstein-Barr Virus cultured in standard LCL growth media. *DDX41*^{WT} LCLs were purchased from the Coriell Institute for Medical

Research (https://www.coriell.org/), which were derived using a virtually identical transformation protocol from three individuals: a 44yo man; a 25yo man; and a 42yo woman.

Protein Isolation and Western Blotting

Whole-cell protein lysates were prepared from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs two days after passaging. Nuclear and cytoplasmic fractions were prepared from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs two days after passaging using the Pierce "NE-PER Nuclear and Cytoplasmic Extraction Reagents" kit (Thermo Fisher Scientific). A standard SDS-PAGE Western blotting protocol was performed to quantify total DDX41 in whole cell lysates and NF-κB in nuclear and cytoplasmic fractions.

RNA Sequencing

RNA-sequencing was performed at the University of Chicago Functional Genomics Laboratory, and data was analyzed using the Cufflinks pipeline (https://cole-trapnell-lab.github.io/cufflinks/manual/; Supplementary Figure 1). Genes of interest were validated using real-time qualitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Measurement of cytokine levels

Quantification of 105 unique cytokines from conditioned LCL growth medium was performed using the "Proteome Profiler Human XL Cytokine Array Kit" (R&D Systems). Quantification of 65 unique cytokines (43 of which were also assessed in the cytokine arrays; Supplementary Figure 2) from conditioned LCL growth medium was performed using the "Human Magnetic Luminex Multiplex Cytokine/Chemokine Array Kit-65 Plex" (Creative Biolabs). Quantification of transforming growth factor-β (TGF-β) was performed using the "Human/Mouse/Rat/Porcine/Canine TGF-beta 1 Quantikine ELISA" (R&D Systems). Levels of ANG, CXCL13, CXCL8, and IL-9 were confirmed using a custom "ProcartaPlex" Luminex panel (Thermo Fisher Scientific) and normalized to a GDF-15 internal control. Conditioned LCL growth media from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs was 8X concentrated for all assays.

UK Biobank Proteomics Analysis

We compared proteomics data from blood plasma in a cohort of 49 individuals with deleterious likely germline *DDX41* variants (cases) to 98 age and sex-matched controls available in the UK Biobank (28) (https://biobank.ndph.ox.ac.uk/ukb/field.cgi?id=30900, Project ID 83200). At the time the peripheral blood was collected, none of these individuals had been diagnosed with cancer. Protein interaction analysis was performed using STRING (https://string-db.org/) with the minimum required interaction score set to "high confidence" (0.700). Pathway enrichment analysis was performed using the STRING database, the Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.genome.jp/kegg/pathway.html), and the DISEASES database (https://diseases.jensenlab.org/Search).

Results

Mild dysplasia in patients with germline DDX41^{LoF} *variants at baseline*

Germline *DDX41* variants were identified in 102 individuals from 52 families (Supplementary Table 1; Supplementary Figures 3-4). Germline pathogenic (P) and likely pathogenic (LP) *DDX41* variants were identified in 93 individuals (91%) from 43 families (83%; Table 1). Germline *DDX41* variants of uncertain significance (VUSs) were identified in 11 individuals (11%) from 11 families (21%). Two families (9 and 26; Supplementary Table 1) had both a germline P/LP (deleterious) *DDX41* variant and a VUS. Among the 28 distinct deleterious variants identified, two (7%) were novel. Of the nine distinct *DDX41* VUSs identified, 5 (56%) were novel (Figure 1A).

Bone marrow biopsies were reviewed from 29 individuals (8 without malignancies and 21 with malignancies) with germline deleterious *DDX41* variants. Both peripheral blood and bone marrow demonstrated dysplasia commonly at baseline regardless of age compared to *DDX41*^{WT} individuals (Table 2; Figure 1). The most frequent morphologies observed at baseline were small, hypolobated megakaryocytes (75%) and macrocytic erythrocytes (50%; Supplementary Table 3), even in individuals as young as 17 years old (yo; Figure 1B-E). A 46yo woman with a germline *DDX41* deletion of exons 12-17 displayed similar, but more severe, dysplastic characteristics at baseline (Figure 1F-I). In contrast, bone marrow from this patient's 73yo father with myelodysplastic syndrome (MDS) showed dysplasia in all three lineages and increased blasts

(Figure 1J-M). Similar observations of multilineage dysplasia and 18% blasts were made in an unrelated 66yo man with MDS and a familial *DDX41* allele encoding p.P258L (Figure 1N-Q).

DDX41 *is not a tumor suppressor in solid tumor development*

Our cohort of 43 pedigrees with deleterious germline *DDX41* variants allowed us to characterize the tumor spectrum and age of diagnosis in those with $DDX41^{LoF}$ alleles. Penetrance of HMs in individuals carrying germline P/LP *DDX41* variants was 54% (n=50/93) by 90yo, similar to what has been observed in other cohorts. (6, 8, 17) HMs included MDS (n=22), acute myeloid leukemia (AML, n=28), chronic myeloid leukemia (CML, n=1), Hodgkin Lymphoma (HL, n=1), and non-Hodgkin lymphoma (NHL, n=1; Supplementary Table 1). The average age of onset for HMs in this cohort was 64yo, consistent with the well-known late onset of *DDX41*-related neoplasms. (4, 16) Additionally, there was higher HM penetrance in individuals $\geq$ 50yo compared to those <50yo (p=0.0005), consistent with the well-known late-onset of HMs associated with *DDX41*.

As in other patient cohorts, (10, 13) we observed solid tumors in people with germline $DDX41^{LOF}$ variants: breast (n=3), melanoma (n=3), prostate (n=3), colon (n=3), basal cell carcinoma (n=3), ovarian (n=2), gastric (n=1), endometrial (n=1), tonsillar (n=1), mesothelioma (n=1), renal (n=1), head and neck (n=1), lung (n=1), and vulvar (n=1; Figure 2A). Penetrance of solid tumors in individuals with germline P/LP *DDX41* variants was 24% (n=22/93) by 75yo, with an average age of onset of 62yo. Fifty-five percent (n=12/22) of these individuals also developed HMs, and in those cases, the solid tumor preceded the HM (mean latency = 8 years; range = 2-13 years) in all but one individual (n=11/12, 92%; Figure 2B). Methods used to treat these solid tumors included radiation and chemotherapy, suggesting that these HMs may have been treatment-related (Figure 2B). Bone marrow biopsies were performed on seven of the 12 patients with HMs and solid tumors and pathologic findings [such as *TP53* mutations and complex karyotypes including t(11;16)(q23;p13)] from five (71%), supported considering these HMs as treatment-related (Supplementary Table 3).

Acquisition of a somatic *DDX41* mutation, typically p.R525H, occurs in 54% of MNs associated with deleterious germline *DDX41* alleles, (17) suggesting that *DDX41* often acts as a tumor suppressor gene in MNs. To determine if *DDX41* has a similar role in solid tumor development, DNA derived from solid tumors was sequenced from individuals with germline P/LP *DDX41* variants (n=5) or VUSs (n=3). No somatic *DDX41* LoF

variants were identified (Table 3), suggesting that somatic mutations like p.R525H are less common in solid tumors or that DDX41 has an alternative role in the development of these tumors. Because some *DDX41* variants have only been observed as germline alleles and others, like truncating variants are virtually always germline,(17) we searched the TCGA database for solid tumors with those *DDX41* alleles (n=10) and failed to identify any additional somatic *DDX41* mutations (Table 3), again suggesting that these solid tumors may have been driven by an alternative mechanism.

Second cancer-risk alleles in those with solid tumors

To test if second germline P/LP variants could drive the formation of solid tumors within the 43 families comprising our cohort, we analyzed DNA variants from 139 cancer-predisposition genes using augmented whole exome sequencing from germline tissue. We divided these pedigrees into those that had solid tumors and HMs, defined as those with a $\geq$15% prevalence of solid tumors in first-degree relatives of the proband, including the proband (n=21/43), versus those with only HMs (n=22/43, Table 1). Among the 21 pedigrees with solid tumors and HMs, seven (33%) had second deleterious germline variants in other cancer risk genes: *APC*, *ATM, ATRX, BRCA1, BRCA2, CDKN2A,* and *PALB2* (Table 1, Supplementary Tables 4-5). The solid tumors in these pedigrees were consistent with the expected tumor spectra of each disorder (Table 1, Supplementary Table 1). In five of these pedigrees (n=5/7, 71%), the additional cancer-predisposing allele was identified in an individual with both the familial *DDX41*^{LoF} allele and a solid tumor(s). In contrast, we identified only one family with a second deleterious germline variant among the 22 pedigrees with only HMs (5%, Table 1, Supplementary Tables 4-5). These findings demonstrate that germline *DDX41*-mutated families with solid tumors are more likely to have germline pathogenic variants in other cancer-predisposition genes than families with only HMs (p=0.02, Supplementary Figure 3), providing support for the recommendation that families with germline *DDX41*^{LOF} alleles with a $\geq$15% prevalence of solid tumors among primary relatives including the proband should have comprehensive testing for cancer risk alleles.

DDX41$^{var/+}$ *patient-derived LCLs exhibit inflammatory dysregulation*

The prevalence of solid tumors in our family cohort as well as prior *in vivo* studies and clinical observations of severe GVHD disease those with germline *DDX41*^{LOF} alleles suggest an important role for

DDX41 in regulating inflammation. (4, 18-20, 23) First, we quantified total DDX41 protein levels by Western blotting using *DDX41*^{WT} LCLs derived from three sex-matched individuals as negative controls and five germline *DDX41*$^{var/+}$ LCLs derived from: a 65yo man with a *DDX41* allele encoding a start-loss mutation, p.M1? (Family #6 individual III-6); a 66yo man with a *DDX41* allele encoding p.P258L (Family #32 individual III-2); a 73yo man with a truncated *DDX41* allele, p.A492Gfs*17 (Family #41 individual III-3); a 65yo woman with a similar truncated *DDX41* allele, p.A500Cfs*9 (Family #21 individual III-1); and a 74yo man with a *DDX41* allele encoding a deletion of exons 12-17 (del ex. 12-17, Family #43 individual III-4; Supplementary Table 1). We found lower DDX41 levels in the context of germline variants associated with nonsense-mediated mRNA decay (p.A492Gfs*17, p.A500Cfs*9, and del ex. 12-17), but relatively unchanged DDX41 levels in the absence of such variants (p=0.04, Supplementary Figure 5). To determine how different patient-associated germline *DDX41* variants affect gene expression, we performed RNA-sequencing revealing differential gene expression between *DDX41*^{WT} *and DDX41*$^{var/+}$ LCLs, and among individual patient-derived *DDX41*$^{var/+}$ cell lines, suggesting that each germline *DDX41* mutation may differentially disrupt DDX41-mediated functions. Furthermore, principal component analysis revealed clustering of *DDX41*$^{P258L/+}$ with *DDX41*$^{A500Cfs*9/+}$, and *DDX41*$^{A492Gfs*17/+}$ with *DDX41*$^{del\ ex.12\text{-}17/+}$, suggesting similar effects of these variants on DDX41 protein function (Figure 3A). Eight genes known to be associated with inflammation (*e.g., CDC14B, CD244, CD9, IL1R1, IL23R, IL32, LTBR,* and *PTPN14*) were upregulated across all five *DDX41*$^{var/+}$ LCLs (Figure 3B). Additionally, upregulation of 22 hallmark pathways was observed in *DDX41*$^{var/+}$ LCLs, including TNF-α signaling via NF-κB ($p=1.3\times10^{-9}$), hypoxia response ($p=1.9\times10^{-8}$), epithelial-mesenchymal transition ($p=7.6\times10^{-7}$), early estrogen response ($p=5.2\times10^{-6}$), IL-2/STAT5 signaling ($p=5.1\times10^{-6}$), angiogenesis ($p=3.4\times10^{-3}$), ultraviolet (UV)-response down ($p=1.2\times10^{-4}$), and inflammatory response signaling ($p=5.0\times10^{-5}$, Figure 3C). Two pathways were downregulated: E2F ($p=2.1\times10^{-4}$) and MYC signaling ($p=1.5\times10^{-3}$, Figure 3C). These findings were validated by qRT-PCR (Supplementary Table 6, Supplementary Figure 6). Taken together, RNA-sequencing and qRT-PCR data suggested that patient-derived *DDX41*$^{var/+}$ LCLs exhibit differential expression of immune-related genes and processes.

Next, we used cytokine arrays and Luminex assays to assess the levels of 127 unique cytokines in *DDX41*$^{var/+}$ and *DDX41*^{WT} LCL-conditioned growth media to investigate inflammatory signaling at the protein level (Supplementary Figure 2). ANG, CXCL13, CXCL8, and IL9 levels were higher in *DDX41*$^{var/+}$ LCL-

conditioned media than in *DDX41*^{WT} LCL-conditioned media (Figure 3D-E), validated by a Luminex panel (Figure 3F-I). Interestingly, RNA expression of these inflammatory cytokines showed no significant overall increases or decreases in *DDX41*$^{var/+}$ LCLs from WT, suggesting that translation and/or protein level regulation may be important in DDX41-mediated inflammatory changes (Supplementary Table 6). Although RNA-sequencing revealed upregulation of several TGF-β-associated pathways, direct measurement of TGF-β levels by ELISA did not show elevation in *DDX41*$^{var/+}$ LCL-conditioned media (Supplementary Figure 7). Overall, assessment of inflammatory cytokines in patient-derived LCL-conditioned media suggests there is inflammatory dysregulation in the context of deleterious germline *DDX41* variants.

A mechanism for inflammatory dysregulation in germline DDX41$^{var/+}$ *LCLs*

Well-studied signaling pathways involving DDX41 and the key inflammatory cytokines, ANG, CXCL13, CXCL8, and IL9, intersect at the transcription factor complex NF-κB (Figure 4). The p65 and p60 subunits of NF-κB translocate to the nucleus upon activation of the NF-κB complex.(29) Therefore, to determine if NF-κB activity increases in the context of germline *DDX41*^{LOF} alleles, we measured NF-κB p65 subunit levels by Western blotting in nuclear and cytoplasmic cellular fractions of patient-derived *DDX41*$^{var/+}$ (n=5) versus *DDX41*^{WT} (n=3) LCLs. Overall, increased NF-κB levels were observed in the nuclear fractions of *DDX41*$^{var/+}$ LCLs compared to WT (p=0.008) while cytoplasmic levels were unchanged (Figure 4), confirming activation of NF-κB in *DDX41*$^{var/+}$ LCLs and suggesting that NF-κB activity may be involved in DDX41-mediated inflammatory dysregulation (Figure 4D).

Inflammatory dysregulation in individuals with likely germline DDX41^{LoF} *variants*

To determine if there is inflammatory dysregulation in individuals with germline *DDX41*^{LoF} alleles, we analyzed UK Biobank proteomic data available for 2,922 proteins measured from participants' primary peripheral blood.(28) We compared protein levels in individuals with likely germline *DDX41*^{LoF} variants without cancer (n=49) to twice the number of age and sex-matched controls (n=98, Supplementary Tables 7-8). Levels of 30 proteins increased in the context of germline *DDX41*^{LoF} alleles, including stress antigens MICA and MICB (p=0.04, Figure 5A). Levels of 114 proteins decreased in the context of germline *DDX41*^{LoF} alleles, including immune-signaling proteins CD79B (p=0.0004), HLA-E (p=0.0004), CD4 (p=0.003), CD28 (p=0.005), and CD80

(p=0.02, Figure 5A). Protein interaction analysis on proteins found to decrease in germline *DDX41*^{LoF} cases compared to WT controls revealed 30 proteins with high confidence (CI=0.700) interactions in which CD4, CD28, and CD80 appear to be central (Figure 5B). STRING pathway enrichment analysis of proteins found to decrease in the context of germline *DDX41*^{LoF} alleles showed that nine of the top ten diminished pathways (90%) involve inflammation (Figure 5C). KEGG pathway enrichment analysis revealed dysregulated NF-κB signaling (FDR=1.0*10^{-2}) and disease-gene associations showed "immune system disease" (FDR=4.0*10^{-8}), "autoimmune disease" (FDR=5.0*10^{-7}), and "primary immunodeficiency disease" (FDR=0.7*10^{-6}) as most likely to be present among our *DDX41*^{LoF} cases (Supplementary Figure 8). Overall, these data suggest that individuals with deleterious likely germline *DDX41*^{LoF} variants have dysregulation of inflammatory proteins years before cancer develops, which could contribute to tumor pathogenesis.

Discussion

Our cohort of 52 families with germline variants in *DDX41* is the largest published to date. Importantly, these families reflect what is known of germline *DDX41*^{LoF} allele carriers in other cohorts such as a 54% HM penetrance by 90 yo and an average age of HM onset of 64 yo. (4, 6, 8, 17) The penetrance of malignancies in an unselected population is lower and has been investigated previously.(30) However, extensive study of the 43 families with deleterious germline *DDX41* variants and molecular studies on patient-derived tissues allowed us to reveal that the phenotypes and cancer risks within such families may be more complex than previously appreciated.

Baseline biopsies in individuals with germline deleterious *DDX41* variants revealed distinct dysplasia in the peripheral blood and bone marrow, particularly in megakaryocytic and erythroid lineages. Most notably, these changes were observed in a 17yo individual indicating that mild dysplasia may be characteristic of individuals with *DDX41*^{LoF} variants many decades before the expected age of onset of *DDX41*-related HMs. Importantly, the two baseline cases discussed are representative of many clinical cases we have observed over the past decade. We caution against overinterpretation of bone marrow dysplasia and misdiagnosis of MDS in individuals with germline *DDX41*^{LoF} alleles, since baseline dysplasia in people with *DDX41*^{LoF} alleles must be distinguished carefully from malignancy-associated changes. We suggest performing a baseline bone

marrow biopsy when an individual is diagnosed with a germline *DDX41*^{LoF} variant to provide a comparator for subsequent bone marrow examinations to allow assessment of dysplastic changes over time.

Although *DDX41* has long been associated with HMs, the presence of solid tumors in our cohort of 43 families with deleterious germline *DDX41* variants and has been reported previously (10, 13) warranted deeper investigation. The spectrum of solid tumors observed in our cohort shares similarities with previous studies: Bannon *et al.* reported an 18% prevalence of solid tumors in individuals with germline *DDX41*^{LoF} alleles (10), similar to our cohort with a 24% penetrance by 75yo. The same study reported prostate cancer and melanoma (10), which were observed frequently in our cohort as well (prostate, n=3; melanoma, n=3). Additionally, our observation that over half of germline *DDX41*^{LoF} carriers who developed solid tumors developed HMs an average of 8 years later suggests a potential compounded effect of the germline cancer-risk allele(s) with the therapies used to treat the solid tumors. We recommend increased surveillance of individuals with germline *DDX41*^{LoF} alleles treated for solid tumors. We recognize the challenge this presents, because currently genetic cancer risk testing for solid tumors often lacks coverage of *DDX41*. We recommend inclusion of *DDX41* in cancer risk testing for families with both HMs and solid tumors.

We failed to identify any somatic *DDX41* mutations in patient-derived solid tumor tissue (n=8) or in TCGA data (n=10). Moreover, we observed that among families with HMs and solid tumors (n=21/43), ~30% had second germline deleterious variants in other cancer-associated genes. Low numbers of tumors identified in those with likely germline *DDX41*^{LoF} alleles with or without additional cancer-risk alleles in public tumor databases precluded our ability to assess differences in tumor prevalence. We hope that broader testing and expansion of public databases will allow this analysis in the future. Together, these data suggest that *DDX41* may contribute indirectly to solid tumor development, arguing for broad comprehensive germline cancer risk testing in families with solid tumors in $\geq$15% of primary relatives including the proband and deleterious germline *DDX41* variants.

Since DDX41 regulates innate immunity, we hypothesized that it might contribute to solid tumor development via inflammation. We generated LCLs from five individuals in our cohort with distinct germline deleterious *DDX41* variants to investigate this hypothesis. Recognizing that each *DDX41*$^{var/+}$ LCL line has a different genetic background and that each *DDX41* mutation was shown to effect protein expression differently, we searched for inflammatory phenotypes that were shared and distinguished *DDX41*$^{var/+}$ from *DDX41*^{WT}

controls. We identified upregulation of inflammation-associated genes and several pathways, including TNF-α signaling via NF-κB ($p=1.3x10^{-9}$) and inflammatory response signaling ($p=5.0x10^{-5}$) across all five *DDX41*$^{var/+}$ LCL lines, indicating an inflammatory phenotype at the transcription-level. Analysis of cytokines present in LCL-conditioned growth media demonstrated elevated levels of four cytokines, ANG, CXCL13, CXCL8, and IL9, suggesting dysregulated inflammatory signaling in the presence of a deleterious germline *DDX41* variant. Increased levels of NF-κB in *DDX41*$^{var/+}$ compared to *DDX41*^{WT} LCL nuclear extracts and by our RNA-sequencing data provide further evidence for an inflammatory imbalance driven by these cytokines, which are known to signal through NF-κB. NF-κB signaling is known to promote tumor proliferation, induce epithelial-mesenchymal transition, and stimulate the immune system in favor of tumor growth, consistent with our hypothesis that the *DDX41*^{LoF}-mediated inflammatory signature modifies solid tumor pathogenesis.(29) Moreover, NF-κB is activated in response to the cGAS-STING-TBK1 axis in *Ddx41*-deficient zebrafish.(18, 31, 32)

Our proteomics analysis of individuals with likely germline *DDX41*^{LoF} variants compared to age and sex-matched controls revealed inflammatory dysregulation as well. Interaction analysis of proteins found to decrease in germline *DDX41*^{LoF} cases compared to WT controls revealed many high confidence interactions, particularly involving immune cell receptors CD4, CD28, and CD80. STRING pathway enrichment analyses showed that 90% of the most dysregulated pathways involved inflammation. Enriched KEGG pathways included NF-κB signaling, supporting our hypothesis that germline *DDX41*^{LoF} variants are associated with dysregulated NF-κB signaling. Interestingly, JAK/STAT signaling was also enriched according to the STRING database. Since NF-κB is known to contribute to JAK/STAT signaling in response to inflammatory cytokines,(33) it is possible these pathways are central to *DDX41*-mediated inflammatory dysregulation. The proteomics data currently available within the UK Biobank (28) are obtained from a single time point. We advocate for similar studies to be performed prospectively in a cohort of individuals with germline *DDX41*^{LoF} alleles compared to familial controls to assess changes in the inflammatory milieu over their lifetimes.

The lack of somatic *DDX41* mutations in solid tumors of those with germline P/LP *DDX41* variants, the presence of other cancer-associated germline pathogenic variants, and inflammatory dysregulation in patient-derived cells and proteomic data suggest that *DDX41* may be an indirect modifier of solid tumor pathogenesis

compared to its tumor suppressor function as seen in HMs. (5-9, 11, 12, 14, 15, 17) Based on our data, we advocate for broad cancer risk testing for families with HMs and solid tumors that includes *DDX41.* We also advocate for screening of other cancer-risk alleles in families known to have a $DDX41^{LOF}$ allele with a history that includes solid tumors in $\geq$15% of primary relatives including the proband. We hope our observations are hypothesis-generating and encourage further research on the mechanism by which germline deleterious *DDX41* variants contribute to malignancies.

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Tables and Figure Legends

Table 1. Deleterious germline variants detected in pedigrees with hematopoietic malignancies with and without solid tumors.

Table 2. Dysplasia identified in peripheral blood and bone marrow examinations in those with deleterious germline *DDX41* variants from our cohort.

Table 3. Somatic sequencing from solid tumor tissue from patients with germline deleterious *DDX41* variants.

Table 1. Deleterious germline variants detected in pedigrees with hematopoietic malignancies with and without solid tumors.

			Family #	Germline *DDX41* variant (P/LP) [NM_016222.4]	DDX41-encoded protein variant [NP_057306.2]	# Primary relatives	# Primary relatives with STs	% Primary relatives wth STs	STs in primary relatives	Additional germline-mutated gene*	Additional germline variant (P/LP)	Additional-encoded protein variant
Families with Germline P/LP *DDX41* Variants* (n=43)	HMs + STs (n=21/43, 49%)	Additional Germline Variant (n=7/21, 33%)	9	c.142C>T	p.Gln48*	7	2	29%	Breast x2	*PALB2*	c.2938del (P) [NM_024675.3]	p.Ser980Alafs*10 [NP_078951.2]
			11	c.268C>T	p.Gln90*	9	3	33%	Endometrial, Lung x2	*ATRX*	c.7219C>T (P) [NM_000489.6]	p.Arg2407* [NP_000480.3]
			26	c. 490C>T	p.Arg164Trp	7	2	29%	Basal Cell Carcinoma, Colon x2	*APC*	c.3920T>A (LP) [NM_000038.6]	p.Ile1307Lys [NP_000029.2]
			28	c. 490C>T	p.Arg164Trp	7	2	29%	Colon, Ovarian, Prostate, Spinal	*BRCA1*	c.68_69delAG (P) [NM_007294.4]	p.Glu23Valfs*17 [NP_009225.1]
			30	c.653G>A	p.Gly218Asp	5	2	40%	Lung, Neuroendocrine Carcinoma	*ATM*	c.2921+1G>A (P) [NM_000051.4]	p.?
			31	c.766G>A	p.Glu256Lys	8	4	50%	Basal Cell Carcinoma, Melanoma, Prostate x2, Renal	*CDKN2A*	c.9_32dup (LP) [NM_000077.5]	p.Ala4_Pro11dup [NP_000068.1]
			38	c.1187T>C	p.Ile396Thr	5	1	20%	Breast	*BRCA2*	c.6174delT (P) [NM_000059.4]	p.Phe2058LeufsTer12 [NP_000050.3]
		DDX41 Only (n=14/21, 67%)	6	c.3G>A	p.Met1?	6	2	33%	Breast, Pancreatic			
			8	c.121C>T	p.Gln41*	4	2	50%	Lung			
			12	c.323del	p.Lys108Serfs*3	13	2	15%	Breast, Colon, Liver, Meningioma			
			13	c.415_418dupGATG	p.Asp140Glyfs*2	4	1	25%	Gastric			
			14	c.415_418dupGATG	p.Asp140Glyfs*2	13	5	38%	Colon, Melanoma x2, Uterine			
			15	c.415_418dupGATG	p.Asp140Glyfs*2	3	2	67%	Bladder, Breast, Melanoma, Prostate			
			19	c.1141A>T	p.Lys381*	6	2	33%	Clear Cell Renal, Prostate			
			23	c.386dup	p.Lys130Glufs*5	13	2	15%	Colon, Ovarian			
			27	c. 490C>T	p.Arg164Trp	5	2	40%	Bladder, Breast, Tonsillar			
			29	c.653G>A	p.Gly218Asp	6	1	17%	Tonsillar			
			32	c.773C>T	p.Pro258Leu	6	2	33%	Melanoma, Prostate			
			34	c.1013G>A	p.Cys338Tyr	6	1	17%	Breast			
			39	c.1187T>C	p.Ile396Thr	5	1	20%	Breast, Endometrial/Uterine			
			41	c.1474dup	p.Ala492Glyfs*17	6	1	17%	Breast			
	HMs Only (n=22/43, 51%)	Additional Germline Variant (n=1/22, 5%)	24	c.435-2_435-1delinsCA	p.?	6	0	0%		*CHEK2*	c.470T>C (P) [NM_007194.4]	p.Ile200Thr [NP_009125.1]
		DDX41 Only (n=21/22, 95%)	1	c.3G>A	p.Met1?	10	0	0%				
			2	c.3G>A	p.Met1?	9	0	0%				
			3	c.3G>A	p.Met1?	12	1	8%				
			4	c.3G>A	p.Met1?	9	1	11%				
			5	c.3G>A	p.Met1?	8	1	13%				
			7	c.121C>T	p.Gln41*	8	1	13%				
			10	c.232_233insAA	p.Pro78Glnfs*3	6	0	0%				
			16	c.415_418dupGATG	p.Asp140Glyfs*2	8	1	13%				
			17	c.415_418dupGATG	p.Asp140Glyfs*2	7	0	0%				
			18	c.946_947del	p.Met316Asp*31	3	0	0%				
			20	c.1285C>T	p.Gln429*	7	0	0%				
			21	c.1496dup	p.Ala500Cysfs*9	8	0	0%				
			22	c.108T>A	p.Tyr36*	3	0	0%				
			25	c. 490C>T	p.Arg164Trp	7	1	14%				
			33	c.847delC	p.Leu283Cysfs*21	6	0	0%				
			35	c.1016G>T	p.Arg339Leu	7	1	14%				
			36	c.1105C>G	p.Arg369Gly	11	0	0%				
			37	c.1118T>C	p.Leu373Pro	7	0	0%				
			40	c.1283T>C	p.Leu428Pro	4	0	0%				
			42	c.1721del	p.Leu574Arg*fs143	6	0	0%				
			43	c.1721del	p.Leu574Arg*fs143	5	0	0%				

Abbreviations used: HM, hematopoietic malignancy; LP, likely pathogenic; P, pathogenic; ST, solid tumor.

*Pedigrees in which >15% of first-degree relatives including the proband were diagnosed with a solid tumor were more likely to have second germline deleterious variants in other cancer-predisposition genes (p=0.0212).

Table 2. Dysplasia identified in peripheral blood and bone marrow examinations in those with deleterious germline *DDX41* variants from our cohort.

	Peripheral Blood			Core Biopsy/Aspirate Smear		
	Dysplasia	**No Dysplasia**	**% with Dysplasia**	**Dysplasia**	**No Dysplasia**	**% with Dysplasia**
Baseline	4	3	57%	7	1	88%
Malignant	20	0	100%	21	0	100%
			p=0.0120*			p=0.2759* (ns)

Abbreviations used: BM, bone marrow; ns, not significant

*P-values determined by two-tailed Fisher's exact tests to determine association between malignancy and presence of dysplasia.

Table 3. Somatic sequencing from solid tumor tissue from patients with germline deleterious *DDX41* variants.

Pedigree ID	Sex	Age, y	Germline Variant Classification	*DDX41* Germline Variant [NM_016222.4]	DDX41 Encoded Protein Variant [NP_057306.2]	Solid Tumor	Presence of "hotspot" (p.Arg525His) or other *DDX41* variant
From TCGA	F	57	LP/P	c.C1105T	p.Arg36*	Breast invasive carcinoma dx. 57	No
From TCGA	M		P	c.C142T	p.Q48*	Prostate adenocarcinoma	No
F23-III-1	M	69	LP	c.386dup	p.Lys130Glufs*5	Colon dx. 69	No
F13-III-1	F	72	P	c.415_418dup	p.Asp140Glyfs*2	Gastric dx. 70	No
From TCGA	M	77	LP/P	c.418_419insGATG	p.Asp140_Pro141delinsGly*	Bladder Urothelial Carcinoma dx. 77	No
From TCGA	M	56	LP/P	c.418_419insGATG	p.Asp140_Pro141delinsGly*	Esophageal carcinoma dx. 56	No
From TCGA	M	32	LP/P	c.418_419insGATG	p.Asp140_Pro141delinsGly*	Pheochromocytoma and Paraganglioma dx. 32	No
From TCGA	F	73	LP/P	c.C475T	p.Arg159*	Lung adenocarcinoma dx. 73	No
From TCGA	M	59	LP/P	c.C475T	p.Arg159*	Head and Neck squamous cell carcinoma dx. 59	No
From TCGA	F	57	LP/P	c.C475T	p.Arg159*	Cervical squamous cell carcinoma and endocervical adenocarcinoma dx. 57	No
F27-III-6	F	69	P	c.490C>T	p.Arg164Trp	Breast dx. 54	No
F29-III-4	M	66	LP	c.653G>A	p.Gly218Asp	Tonsillar dx. 64	No
F30-III-1	F	37	LP	c.653G>A	p.Gly218Asp	Neuroendocrine Carcinoma dx. 31	No
From TCGA	F	64	LP/P	c.946_947del	p.Met316fs	Liver hepatocellular carcinoma dx. 64	No
From TCGA	M	46	LP/P	c.A1789T	p.Lys597*	Bladder Urothelial Carcinoma dx. 46	No
F48-III-1	M	73	VUS	c.465G>A	p.Met155Ile	Melanoma dx. 72	No
F51-III-2	M	77	VUS	c.511G>C	p.Val171Leu	Prostate dx. 72	No
F52-III-2	F	67	VUS	c.926C>T	p.Thr309Ile	Ovarian dx. 67	No

Abbreviations used: F, family(Pedigree ID)/female(Sex); ID, identification; LP, likely pathogenic; M, male; P, pathogenic; P#, pedigree number; VUS, variant of uncertain significance; y, years

Figure Legends

Figure 1. Family-associated germline *DDX41* variants and morphologic features of baseline and malignant peripheral blood and bone marrow in individuals with deleterious germline *DDX41* variants at different ages. (A) Deleterious germline *DDX41* variants identified in patients and families with hematopoietic malignancies (HMs) are shown above the protein schematic, and variants of uncertain significance (VUSs; blue circles) are shown below. Pathogenic variants are indicated by red diamonds, and likely pathogenic variants by orange diamonds. Non-protein coding variants are listed in the bottom right. The likely pathogenic copy number variant (CNV) is indicated by an orange line. Novel variants are shown with glow and previously identified variants are shown without glow. DDX41 protein domains are indicated by color: RecA-like domain 1 (light blue), RecA-like domain 2 (lilac), and zinc finger (ZnF, light green). **(B-Q)** Images shown include **(B, F, J, N)** peripheral blood, **(C, G, K, O)** bone marrow aspirate, **(D, H, L, P)** bone marrow trephine, and **(E, I, M, Q)** immunohistochemistry on trephine. **(B-E)** A 17-year-old female with a pathogenic *DDX41* mutation (DDX41 p.M1?), mild dysplastic changes in erythroid lineage (red arrow) and megakaryocytic lineage (yellow arrow), but insufficient for diagnosis of MDS. **(F-I)** A 46-year-old female with a likely pathogenic *DDX41* deletion of exons 12-17, significant (>10%) dysplastic changes in both erythroid and megakaryocytic lineages, but no granulocytic dysplasia. **(J-M)** A 73-year-old male (father of **(F-I)**) with a likely pathogenic *DDX41* deletion of exons 12-17, 4.6% blasts and significant dyserythropoiesis and dysmegakaryopoiesis as well as dysgranulopoiesis manifested mainly by abnormal nuclear morphology including hyposegmentation, dense chromatin and nuclear membrane projections (orange arrow), but not cytoplasmic hypogranulation, diagnosed with MDS with multilineage dysplasia. **(N-Q)** A 61-year-old male with a likely pathogenic *DDX41* mutation (p.P258L), 18% blasts, and multilineage dysplasia (particularly prominent in granulocytes), diagnosed MDS with excess blasts-2 progressing toward AML.

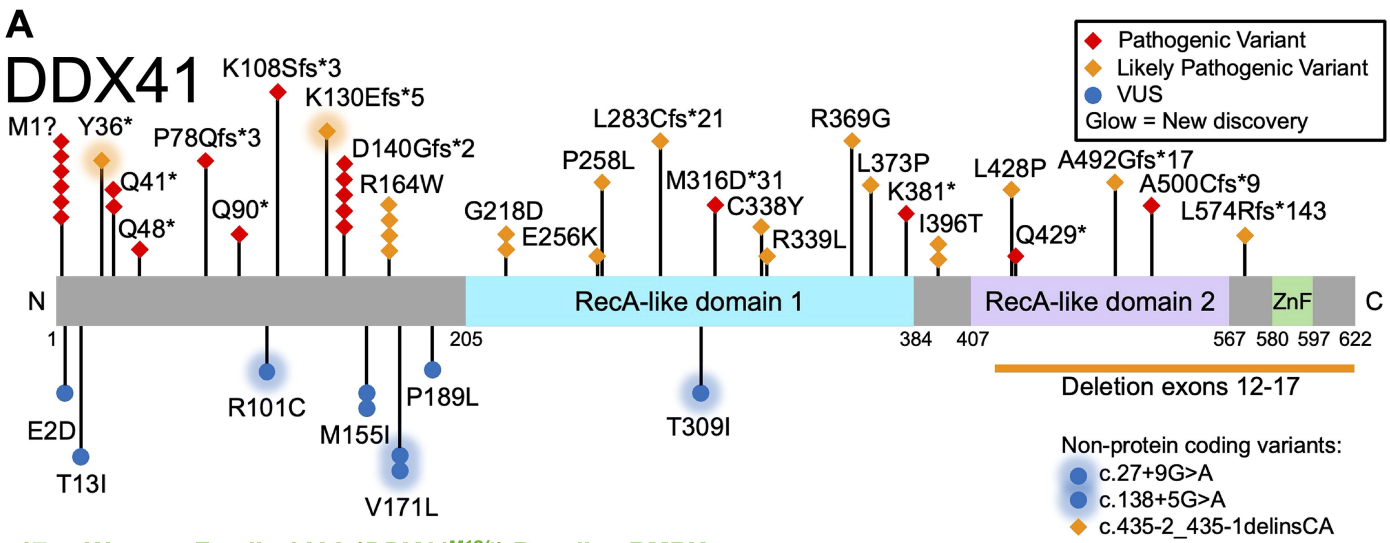
Figure 2. Disease breakdown by *DDX41* variant and timelines of solid tumors and HMs in patients with multiple malignancies. **(A)** DDX41 protein schematic showing all malignancies identified in individuals with *DDX41*^{LoF} alleles plotted by corresponding variant. Diseases represented include hematopoietic malignancies (red), and solid tumors such as breast (pink), prostate (orange), melanoma (yellow), colon (light green), gastric (black), endometrial (light blue), lung (dark blue), tonsillar (magenta), ovarian/vulvar (purple), renal (brown),

neuroendocrine carcinoma (peach), mast cell cytosis (dark green), head and neck (green), small bowel (teal), basal cell carcinoma (gray), fallopian tube (fuchsia), mesothelioma (light pink), and kidney (salmon) cancers. DDX41 protein domains are indicated by color: D-E-A-D (DEAD) Box (light blue), Helicase C (lilac), and zinc finger (ZnF) (light green). **(B)** Age of cancer diagnoses and treatments in individuals with *DDX41*^{LoF} alleles who were diagnosed with more than one cancer. Hematopoietic malignancies are shown in red. Solid tumors represented are breast (pink), prostate (orange), colon (light green), lung (dark blue), renal (brown), basal cell carcinoma (grey), gastric (black), ovarian/vulvar (purple), tonsillar (magenta), and neuroendocrine carcinoma (peach). Solid tumor cancer therapies are indicated by: radiation therapy (R); chemotherapy (C); hormonal therapy (H); surgery (S); and ?, unknown. Mean latency refers to the average years between the onsets of solid tumors and HMs, whereas the range refers to the minimum and maximum latencies present.

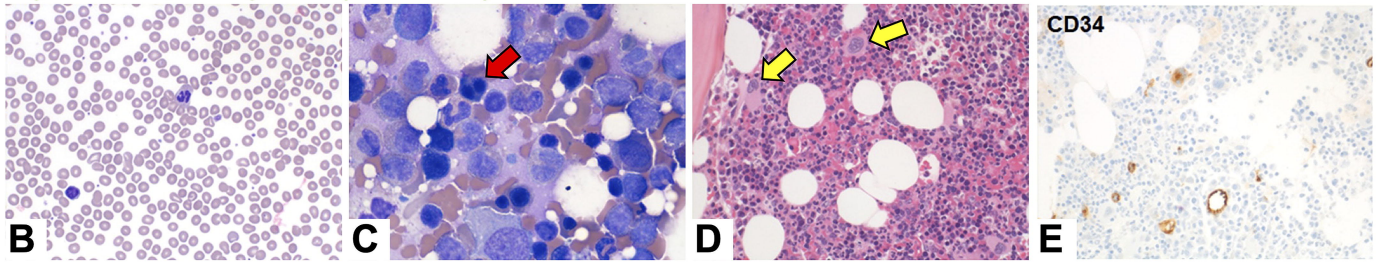
Figure 3. RNA-sequencing, cytokine arrays, and Luminex assays reveal inflammatory dysregulation in *DDX41*$^{var/+}$ patient-derived LCLs. (A) Principal component analysis (PCA) plot of RNA-sequencing data for *DDX41*$^{var/+}$ (n=5, purple) and *DDX41*^{WT} (n=3, green) LCLs. Noted clustering in gene expression is demonstrated between *DDX41*$^{P258L/+}$ and *DDX41*$^{A500Cfs*9/+}$, *DDX41*$^{A492Gfs*17/+}$ and *DDX41*$^{del\ ex12\text{-}17/+}$, and of *DDX41*^{WT} LCLs. **(B)** Volcano plot showing significantly (CI=95%) upregulated (red) and downregulated (blue) genes in *DDX41*$^{var/+}$ LCLs (n=5) compared to *DDX41*^{WT} LCLs (n=3). Genes with no statistically significant change are in grey. **(C)** Normalized enrichment plot of genes from 24 hallmark signaling pathways. Increases in overall gene expression in DDX41$^{var/+}$ LCLs from WT are in red, while decreases from WT are in blue. P-values were determined by Pearson's correlation. **(D)** Heat map of cytokine array data showing fold changes in pixel densities of increased cytokines in patient-derived *DDX41*$^{var/+}$ (purple) LCL-conditioned media compared to *DDX41*^{WT}. Fold changes range from 0.1 (blue) to $\geq$10 (red). **(E)** Heat map of commercial Luminex data showing fold changes in pixel densities of increased cytokines in patient-derived *DDX41*$^{var/+}$ (purple) LCL-conditioned media compared to *DDX41*^{WT}. Fold changes range from 0.1 (turquoise) to $\geq$2 (magenta). **(F-I)** Bar graphs showing data from a custom Luminex panel. P-values were determined using two-tailed t-tests with Welch's correction and confirm higher levels of **(F)** ANG, **(G)** CXCL13, **(H)** CXCL8, and **(I)** IL-9 in *DDX41*$^{var/+}$ (purple) LCL-conditioned media compared to *DDX41*^{WT} (green).

Figure 4. Testing the proposed mechanism of inflammatory dysregulation in germline *DDX41*$^{var/+}$ LCLs. **(A)** Western blots to quantify NF-κB (p65 subunit) in nuclear and cytoplasmic protein fractions from patient-derived *DDX41*$^{var/+}$ (purple) and *DDX41*^{WT} (green) LCLs. Histone H3 was used as a nuclear marker and loading control while GAPDH was used as a cytoplasmic marker and loading control. **(B)** Average NF-κB pixel densities in nuclear protein fractions from patient-derived *DDX41*$^{var/+}$ (purple) and *DDX41*^{WT} (green) LCLs normalized to Histone H3. Higher levels of NF-κB were detected in *DDX41*$^{var/+}$ LCLs compared to *DDX41*^{WT} ($p=0.008$) according to a two-tailed t-test with Welch's correction. **(C)** Average NF-κB pixel densities in cytoplasmic protein fractions from patient-derived *DDX41*$^{var/+}$ (purple) and *DDX41*^{WT} (green) LCLs normalized to GAPDH. No significant change in NF-κB was detected according to a two-tailed t-test with Welch's correction. **(D)** Visual summary of cytokine array, Luminex, and Western blot data. Cytokines whose levels were higher in *DDX41*$^{var/+}$ than in *DDX41*^{WT} LCL-conditioned media according to cytokine array and Luminex are shown in red. Increased activation and translocation of NF-κB (gold) is indicated by red upward arrows. Direct protein interactions are indicated by solid black arrows. Indirect protein interactions between mutant DDX41 (purple) and NF-κB are indicated by a dotted black arrow. Indirect activation of NF-κB by inflammatory cytokine signaling is indicated by a dotted red arrow. Proteins/receptors that were identified in literature but were not quantified are shown in grey. Created in https://BioRender.com.

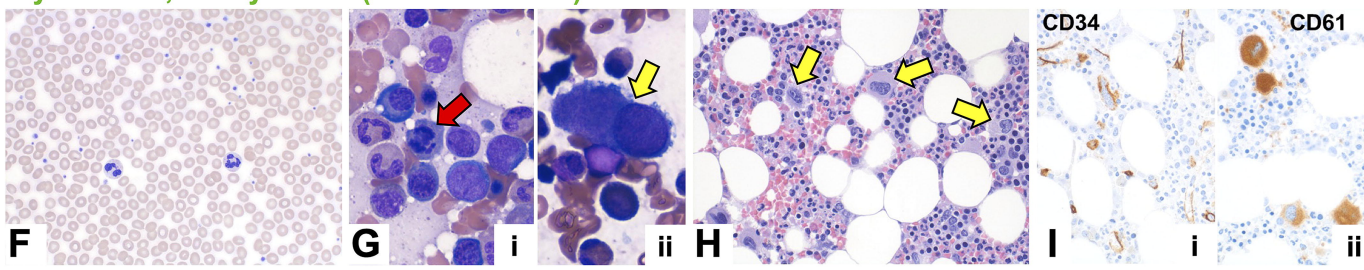
Figure 5. Inflammatory dysregulation in UK Biobank participants with likely germline *DDX41*^{LoF} variants. **(A)** Volcano plot showing proteins that are increased (red; CI=95%) or decreased (blue) in individuals with likely germline *DDX41*^{LoF} variants compared to WT controls. **(B)** Protein-protein interaction network showing proteins found to decrease in individuals with likely germline *DDX41*^{LoF} variants compared to WT controls. Only proteins with "high confidence" (0.700) or "highest confidence" (0.900) interactions (n=30) according to the STING database are shown. The level of significance with which proteins were found to decrease are indicated by color: $p<0.001$ (green), $p<0.01$ (light blue), or $p<0.05$ (dark blue). **(C)** Enrichment plot showing pathways enriched among the 114 proteins found to decrease in individuals with likely germline *DDX41*^{LoF} variants compared to WT controls according to the STRING database.



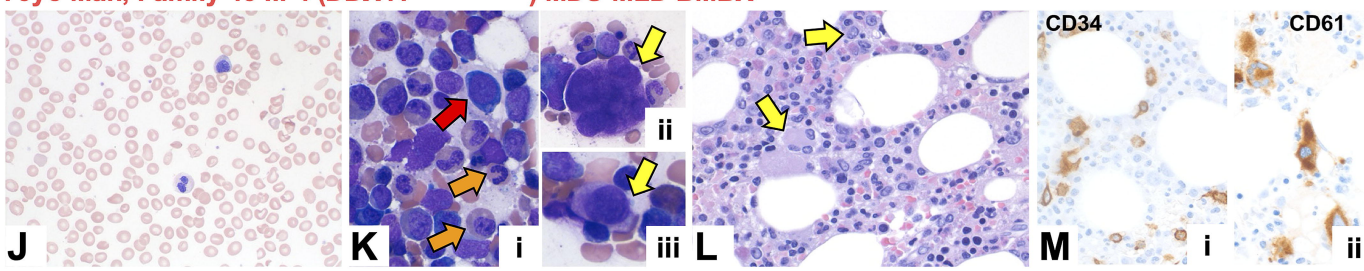
17yo Woman, Family 1 V-3 (*DDX41*$^{M1?/+}$) Baseline BMBX



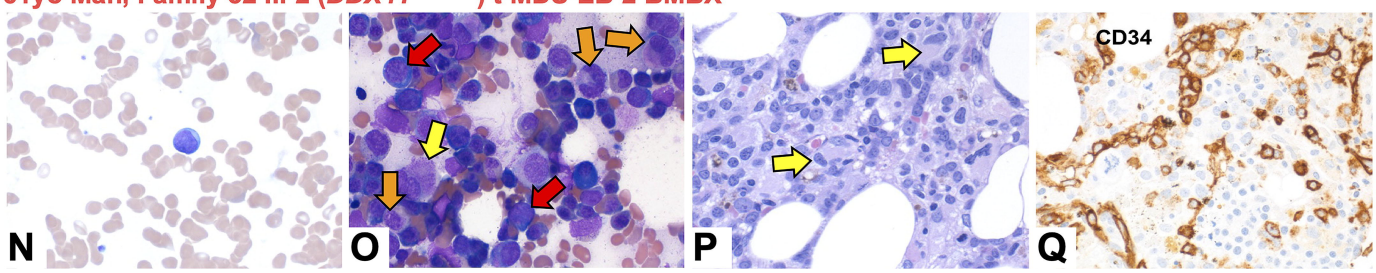
46yo Woman, Family 43 IV-1 (*DDX41*$^{del\ ex.\ 12\text{-}17/+}$) Baseline BMBX



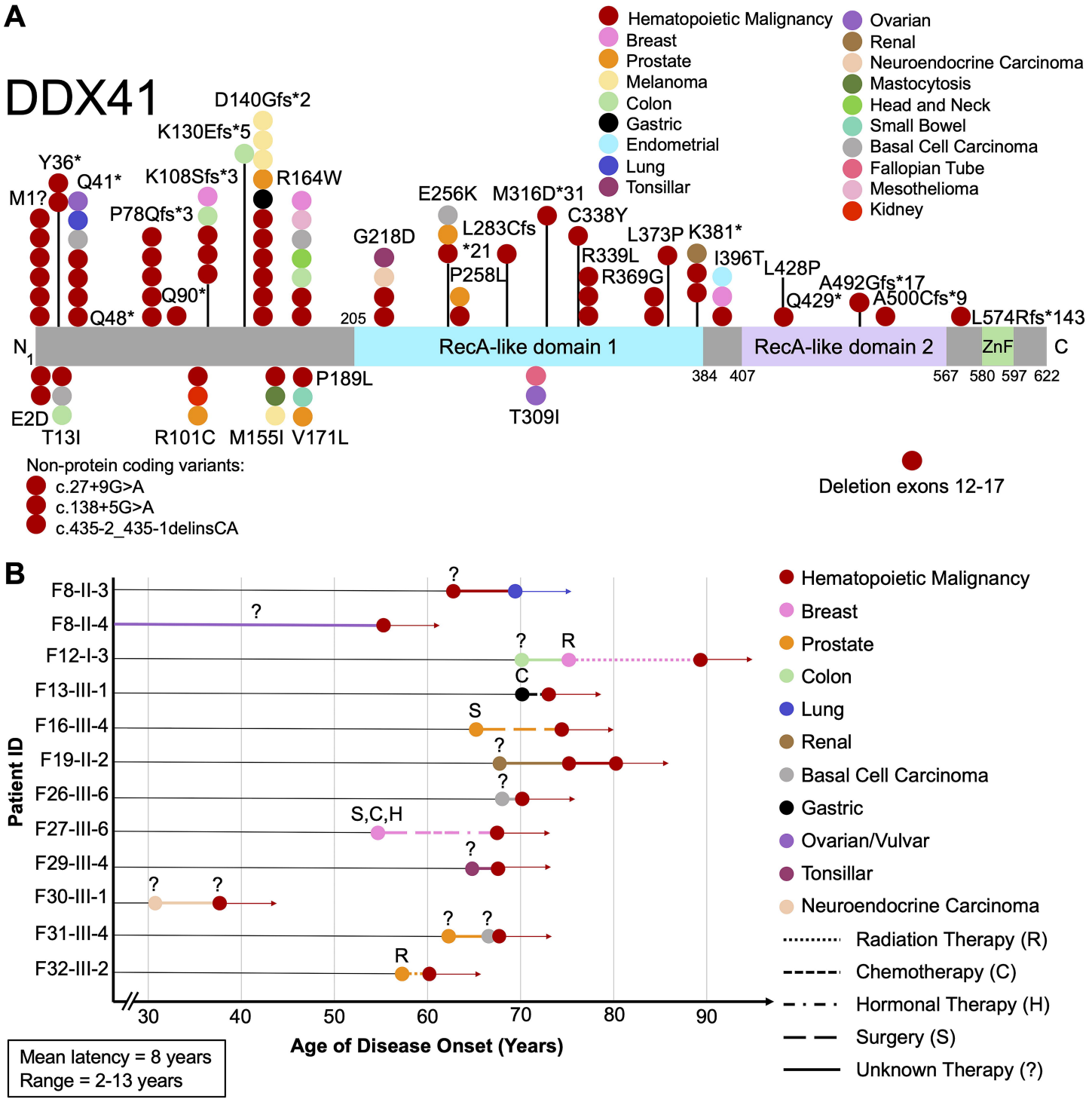
73yo Man, Family 43 III-4 (*DDX41*$^{del\ ex.\ 12\text{-}17/+}$) MDS-MLD BMBX

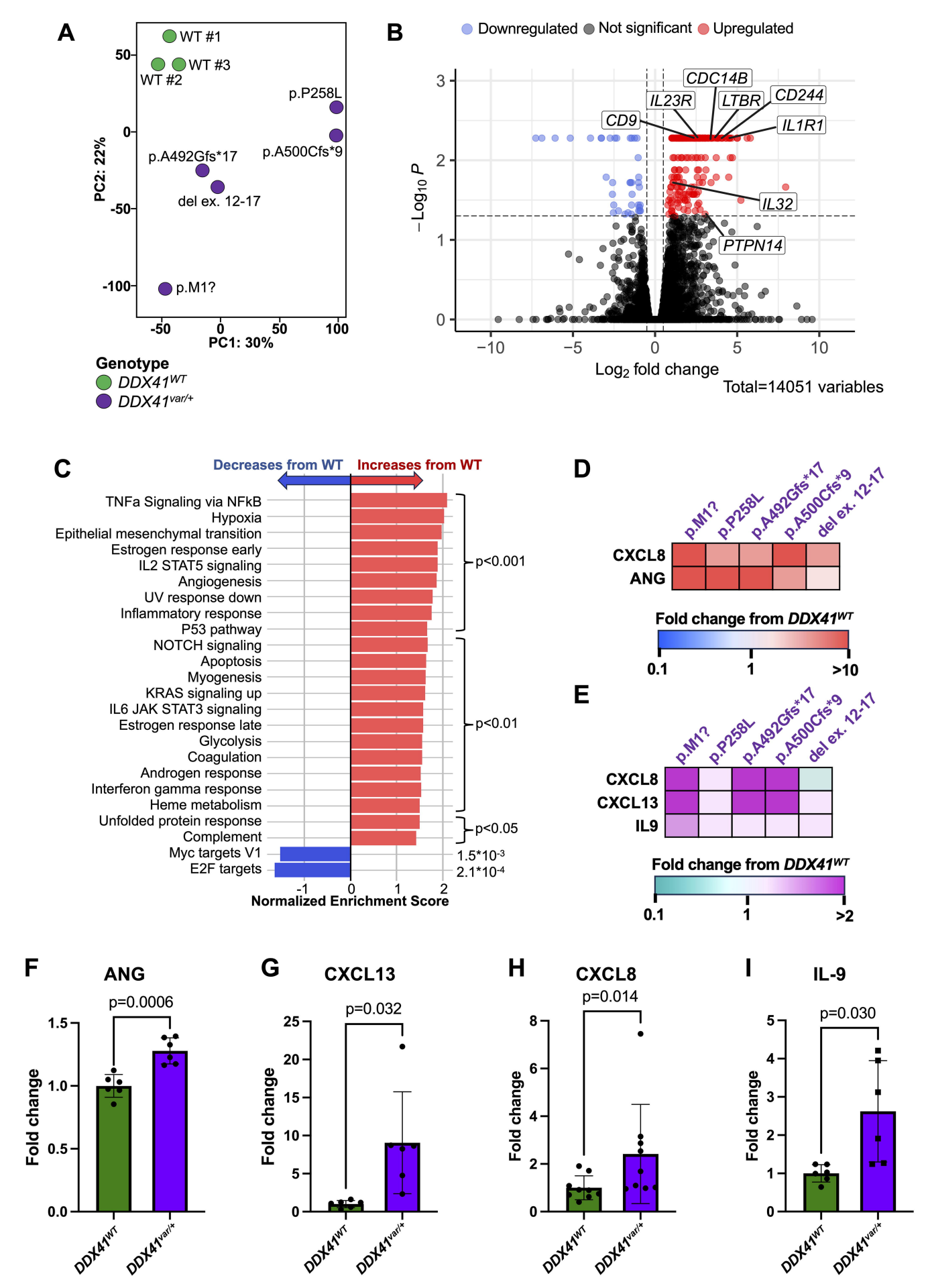


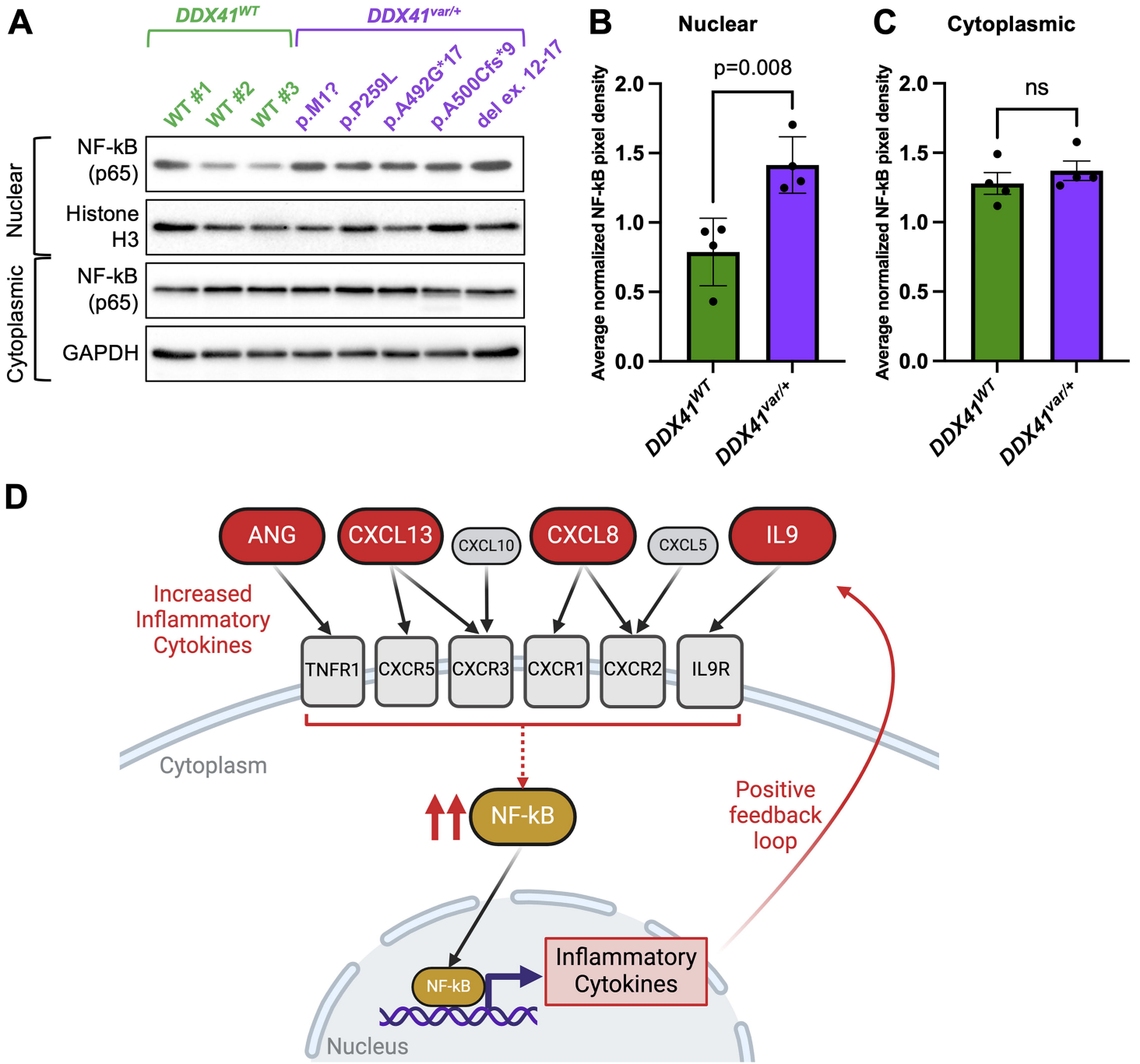
61yo Man, Family 32 III-2 (*DDX41*$^{P258L/+}$) t-MDS-EB-2 BMBX

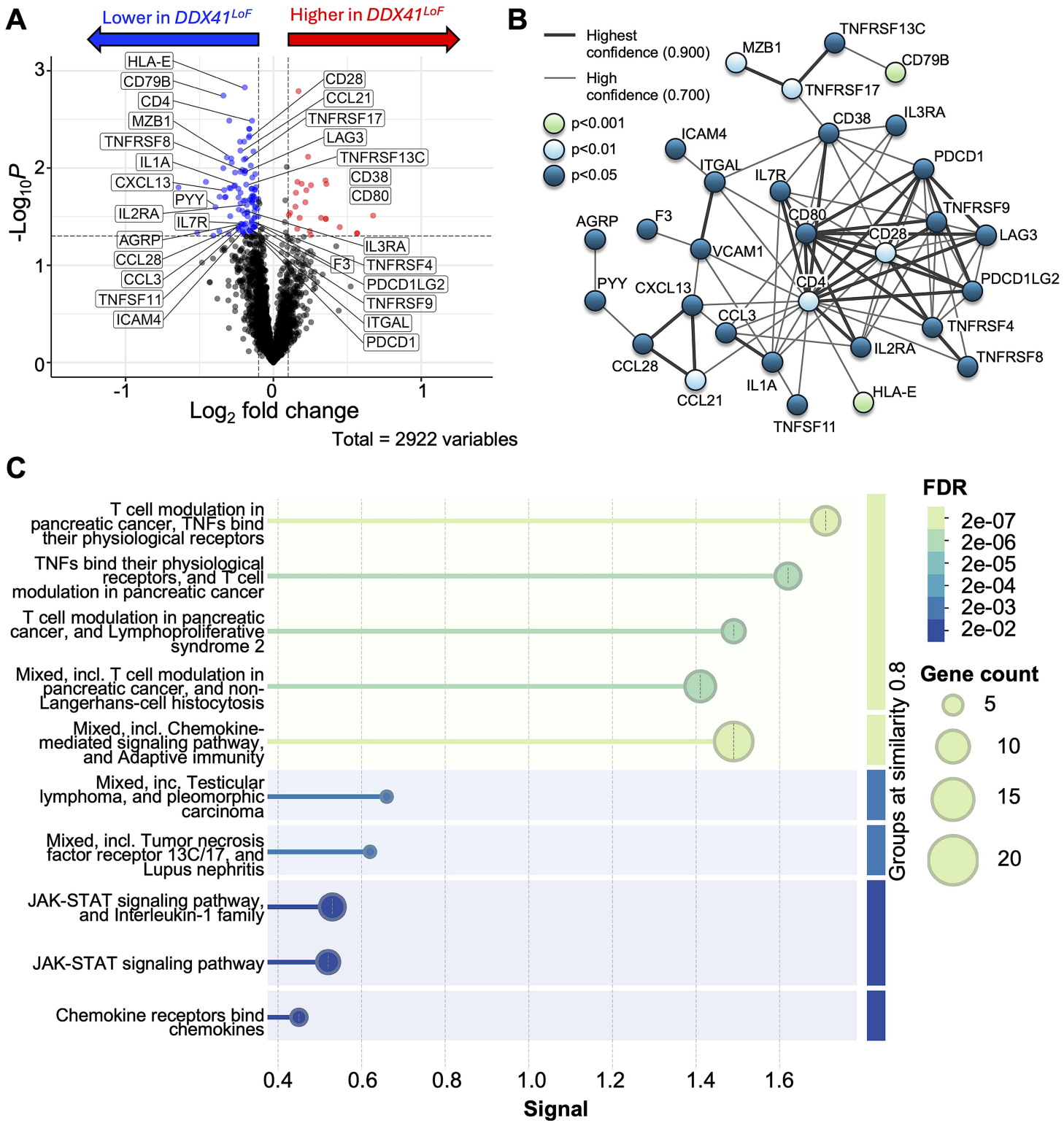


Peripheral Blood | BM Aspirate | BM Trephine | IHC on Trephine


A
DDX41
Hematopoietic Malignancy
Breast
Prostate
Melanoma
Colon
Gastric
Endometrial
Lung
Tonsillar
Ovarian
Renal
Neuroendocrine Carcinoma
Mastocytosis
Head and Neck
Small Bowel
Basal Cell Carcinoma
Fallopian Tube
Mesothelioma
Kidney
D140Gfs*2
K130Efs*5
Y36*
Q41*
K108Sfs*3
R164W
M1?
P78Qfs*3
E256K
M316D*31
C338Y
G218D
L283Cfs
*21
L373P
K381*
R339L
I396T
P258L
R369G
L428P
Q429*
A492Gfs*17
A500Cfs*9
L574Rfs*143
Q90*
Q48*
205
N
1
RecA-like domain 1
RecA-like domain 2
ZnF
C
384
407
567
580
597
622
P189L
E2D
T13I
R101C
M155I
V171L
T309I
Non-protein coding variants:
c.27+9G>A
c.138+5G>A
c.435-2_435-1delinsCA
Deletion exons 12-17
B
Patient ID
F8-II-3
F8-II-4
F12-I-3
F13-III-1
F16-III-4
F19-II-2
F26-III-6
F27-III-6
F29-III-4
F30-III-1
F31-III-4
F32-III-2
S,C,H
30
40
50
60
70
80
90
Age of Disease Onset (Years)
Mean latency = 8 years
Range = 2-13 years
Hematopoietic Malignancy
Breast
Prostate
Colon
Lung
Renal
Basal Cell Carcinoma
Gastric
Ovarian/Vulvar
Tonsillar
Neuroendocrine Carcinoma
Radiation Therapy (R)
Chemotherapy (C)
Hormonal Therapy (H)
Surgery (S)
Unknown Therapy (?)


A
WT #1
WT #3
WT #2
p.P258L
p.A500Cfs*9
p.A492Gfs*17
del ex. 12-17
p.M1?
PC2: 22%
PC1: 30%
Genotype
DDX41WT
DDX41var/+
B
Downregulated
Not significant
Upregulated
CDC14B
IL23R
LTBR
CD244
CD9
IL1R1
IL32
PTPN14
−Log10 P
Log2 fold change
Total=14051 variables
C
Decreases from WT
Increases from WT
TNFa Signaling via NFkB
Hypoxia
Epithelial mesenchymal transition
Estrogen response early
IL2 STAT5 signaling
Angiogenesis
UV response down
Inflammatory response
P53 pathway
NOTCH signaling
Apoptosis
Myogenesis
KRAS signaling up
IL6 JAK STAT3 signaling
Estrogen response late
Glycolysis
Coagulation
Androgen response
Interferon gamma response
Heme metabolism
Unfolded protein response
Complement
Myc targets V1
E2F targets
p<0.001
p<0.01
p<0.05
1.5*10-3
2.1*10-4
Normalized Enrichment Score
D
p.M1?
p.P258L
p.A492Gfs*17
p.A500Cfs*9
del ex. 12-17
CXCL8
ANG
Fold change from DDX41WT
0.1
1
>10
E
CXCL8
CXCL13
IL9
Fold change from DDX41WT
0.1
1
>2
F
ANG
p=0.0006
Fold change
G
CXCL13
p=0.032
H
CXCL8
p=0.014
I
IL-9
p=0.030
DDX41WT
DDX41var/+


A
DDX41WT
DDX41var/+
WT #1
WT #2
WT #3
p.M1?
p.P259L
p.A492G*17
p.A500Cfs*9
del ex. 12-17
Nuclear
NF-kB (p65)
Histone H3
Cytoplasmic
NF-kB (p65)
GAPDH
B
Nuclear
p=0.008
Average normalized NF-kB pixel density
2.0
1.5
1.0
0.5
0.0
DDX41WT
DDX41var/+
C
Cytoplasmic
ns
Average normalized NF-kB pixel density
2.0
1.5
1.0
0.5
0.0
DDX41WT
DDX41var/+
D
ANG
CXCL13
CXCL10
CXCL8
CXCL5
IL9
Increased Inflammatory Cytokines
TNFR1
CXCR5
CXCR3
CXCR1
CXCR2
IL9R
Cytoplasm
NF-kB
Positive feedback loop
NF-kB
Inflammatory Cytokines
Nucleus


A
Lower in DDX41LoF
Higher in DDX41LoF
HLA-E
CD79B
CD4
MZB1
TNFRSF8
IL1A
CXCL13
PYY
IL2RA
IL7R
AGRP
CCL28
CCL3
TNFSF11
ICAM4
CD28
CCL21
TNFRSF17
LAG3
TNFRSF13C
CD38
CD80
IL3RA
F3
TNFRSF4
PDCD1LG2
TNFRSF9
ITGAL
PDCD1
-Log10P
Log2 fold change
Total = 2922 variables
B
Highest confidence (0.900)
High confidence (0.700)
p<0.001
p<0.01
p<0.05
TNFRSF13C
MZB1
CD79B
TNFRSF17
CD38
IL3RA
ICAM4
PDCD1
ITGAL
IL7R
TNFRSF9
AGRP
F3
CD80
CD28
LAG3
VCAM1
PDCD1LG2
PYY
CXCL13
CD4
CCL3
TNFRSF4
IL2RA
TNFRSF8
CCL28
IL1A
HLA-E
CCL21
TNFSF11
C
T cell modulation in pancreatic cancer, TNFs bind their physiological receptors
TNFs bind their physiological receptors, and T cell modulation in pancreatic cancer
T cell modulation in pancreatic cancer, and Lymphoproliferative syndrome 2
Mixed, incl. T cell modulation in pancreatic cancer, and non-Langerhans-cell histocytosis
Mixed, incl. Chemokine-mediated signaling pathway, and Adaptive immunity
Mixed, inc. Testicular lymphoma, and pleomorphic carcinoma
Mixed, incl. Tumor necrosis factor receptor 13C/17, and Lupus nephritis
JAK-STAT signaling pathway, and Interleukin-1 family
JAK-STAT signaling pathway
Chemokine receptors bind chemokines
Groups at similarity 0.8
Signal
FDR
2e-07
2e-06
2e-05
2e-04
2e-03
2e-02
Gene count
5
10
15
20

Overall cancer risk in people with deleterious germline *DDX41* variants

Supplementary Materials

Supplementary Methods

Patients

All individuals signed written informed consent to participate in research approved by Institutional Review Boards at the University of Chicago and Northwestern University, conducted in accordance with the Declaration of Helsinki, and protected by National Institutes of Health Certificates of Confidentiality.

Germline Sequencing

Individuals with personal and/or family histories consistent with a deleterious germline *DDX41* variant or those with such alleles identified via tumor profiling[1] underwent clinical germline genetic testing (Supplementary Table 1). DNA was sequenced using an augmented whole exome sequencing platform[2] in the University of Chicago Genetic Services Laboratory (https://genes.uchicago.edu/clinical-genetics). DNA variants in 139 cancer-predisposing genes (Supplementary Table 2) were analyzed, including the 5'UTRs of *ANKRD26*, *DKC1*, *TERC,* and *TERT*, two *RTEL1* intronic regions (c.3724+78 and c.3724+139; NM_032957.4), and one *GATA2* intronic region (c.1017+572; NM_032638.4). DNA sequence reads were aligned using the UCSC human genome build Hg19 as a reference, and a custom bioinformatic pipeline capable of detecting single nucleotide variants and copy number variants was used to identify potential predisposition alleles (https://github.com/LucyGodley/Pipeline/blob/main/Variant_Calling/WES/hg/Automated/WES_Pipeline.sh).[3] Variants were curated according to the American College of Medical Genetics and Genomics/Association of Molecular Pathology.[4] Deleterious variants in cancer-causing genes were confirmed by Sanger sequencing.

Somatic Solid Tumor Sequencing

DNA derived from formalin-fixed, paraffin-embedded solid tumor tissue derived from eight patients with germline *DDX41*^{LOF} variants was sequenced via the OncoPlus next-generation panel, which includes *DDX41*.[5] Additional tumor-derived sequencing data from The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/) were acquired for eleven additional patients with truncating *DDX41* alleles that are likely to be germline based on the frequency with which such alleles are inherited.[6]

LCL Preparation

Lymphoblastoid cell lines (LCLs) were derived from peripheral blood B-cells from individuals with deleterious germline *DDX41*^{LOF} variants (*DDX41*$^{var/+}$), which were transformed using Epstein-Barr Virus cultured in standard LCL growth media (Roswell Park Memorial Institute (RPMI) 1640 Medium + 20% FBS + 1% penicillin/streptomycin + 1X GlutaMAX). *DDX41*^{WT} LCLs were purchased from the Coriell Institute for Medical Research (https://www.coriell.org/), which were derived using a virtually identical transformation protocol. *DDX41*^{WT} LCLs were derived from three individuals: a 44yo man; a 25yo man; and a 42yo woman.

Protein Isolation and Western Blotting

Whole-cell protein lysates were prepared from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs two days after passaging using RIPA buffer (150mM NaCl; 5mM EDTA, pH8.0; 20mM Tris, pH 7.5; 1.0% NP-40; 1% sodium deoxycholate; 0.1% SDS). Nuclear and cytoplasmic fractions were prepared from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs two days after passaging using the Pierce "NE-PER Nuclear and Cytoplasmic Extraction Reagents" kit (Thermo Fisher Scientific). A standard SDS-PAGE Western blotting protocol was performed to quantify total DDX41 (cs-15076; Cell Signaling Technology) in whole-cell lysates and NF-κB (p65 subunit, cs-8242; Cell Signaling Technology) in nuclear and cytoplasmic fractions.

RNA Sequencing

RNA-sequencing was performed at the University of Chicago Functional Genomics Laboratory, and data was analyzed using the Cufflinks pipeline (https://cole-trapnell-lab.github.io/cufflinks/manual/; Supplementary Figure 1). Genes of interest were validated using real-time qualitative reverse transcriptase polymerase chain reaction (qRT-PCR).

Measurement of cytokine levels

Quantification of 105 unique cytokines from conditioned LCL growth medium was performed using the "Proteome Profiler Human XL Cytokine Array Kit" (R&D Systems). Quantification of 65 unique cytokines (43 of which were also assessed in the cytokine arrays; Supplementary Figure 2) from conditioned LCL growth medium was performed using the "Human Magnetic Luminex Multiplex Cytokine/Chemokine Array Kit-65 Plex" (Creative

Biolabs). Quantification of transforming growth factor-β (TGF-β) was performed using the "Human/Mouse/Rat/Porcine/Canine TGF-beta 1 Quantikine ELISA" (R&D Systems). Levels of ANG, CXCL13, CXCL8, and IL-9 were confirmed using a custom "ProcartaPlex" Luminex panel (Thermo Fisher Scientific) and normalized to a GDF-15 internal control. Conditioned LCL growth media from *DDX41*^{WT} and *DDX41*$^{var/+}$ LCLs was 8X concentrated for all assays.

UK Biobank Proteomics Analysis

We compared proteomics data from blood plasma in a cohort of 49 individuals with deleterious, likely germline *DDX41* variants (cases) to 98 age and sex-matched controls available in the UK Biobank (https://biobank.ndph.ox.ac.uk/ukb/field.cgi?id=30900, Project ID 83200).[7] To ensure none of the selected participants (neither cases nor controls) had cancer, we used national cancer registry data (https://biobank.ndph.ox.ac.uk/ukb/label.cgi?id=100092) and "summary diagnosis" (ICD10) from health-related outcomes data (https://biobank.ndph.ox.ac.uk/ukb/field.cgi?id=41270) in the UK Biobank. Therefore, at the time their peripheral blood was collected, none of the individuals included had been diagnosed with cancer. Normalized Protein Expression (NPX) values from 2922 proteins were obtained from the UK Biobank (https://biobank.ndph.ox.ac.uk/ukb/coding.cgi?id=143&nl=1). While preprocessing, missing NPX values (n=45244/384290, 11.78%) were imputed using K Nearest Neighbor. Differential expression analysis was conducted using the limma package in R with Olink's protein NPX values as the outcome, and group (case vs. control), age, and sex as predictors (https://academic.oup.com/braincomms/article/4/4/fcac155/6608340?login=true#366642284). After multiple test correction using the Benjamini Hochberg method, no proteins passed the threshold of FDR-adjusted p value with 95% confidence. Differential expression analysis plots were generated using ggplot2 and the EnhancedVolcano package in R (https://bioconductor.org/packages/devel/bioc/vignettes/EnhancedVolcano/inst/doc/EnhancedVolcano.html). Protein interaction analysis was performed using STRING (https://string-db.org/) with the minimum required interaction score set to "high confidence" (0.700). Pathway enrichment analysis was performed using the STRING database, the Kyoto Encyclopedia of Genes and Genomes (KEGG,

https://www.genome.jp/kegg/pathway.html), and the DISEASES database (https://diseases.jensenlab.org/Search).

Supplementary Table 1. Comprehensive cohort of patients with deleterious germline *DDX41* variants

Family	Relationship to Proband	Pedigree ID	Age (y)	Sex	Diagnosis (Age of Diagnosis)	*DDX41* Germline Variant [NM_016222.4]	DDX41 Encoded Protein Variant [NP_057306.2]	*DDX41* Germline Variant Classification	Second Germline Variant Gene	Second Germline Variant*	Encoded Protein Variant†	Second Germline Variant Classification
1	Proband	III-13	67	F	AML (67)	c.3G>A	p.Met1?	P				
	Daughter	IV-13	45	F	none	c.3G>A	p.Met1?	P				
	Granddaughter	V-6	18	F	none	c.3G>A	p.Met1?	P				
	Granddaughter	V-5	21	F	none	c.3G>A	p.Met1?	P				
	Niece	IV-10	35	F	none	c.3G>A	p.Met1?	P				
	Grandniece	V-3	17	F	none	c.3G>A	p.Met1?	P				
	Grandniece	V-4	11	F	none	c.3G>A	p.Met1?	P				
2	Proband	III-2	47	M	none	c.3G>A	p.Met1?	P				
	Father	II-5	73	M	MDS (71)	c.3G>A	p.Met1?	P				
3	Proband	III-10	74	M	AML (73)	c.3G>A	p.Met1?	P				
	Son	IV-16	47	M	none	c.3G>A	p.Met1?	P				
	Son	IV-18	42	M	none	c.3G>A	p.Met1?	P				
	Niece	IV-5	58	F	none	c.3G>A	p.Met1?	P				
	Grandniece	V-2	37	F	none	c.3G>A	p.Met1?	P				
4	Proband	III-1	65	M	AML (65)	c.3G>A	p.Met1?	P				
5	Proband	II-12	52	M	AML (52)	c.3G>A	p.Met1?	P				
6	Proband	III-6	65	M	AML (65)	c.3G>A	p.Met1?	P				
	Brother	III-4	59	M	none	c.3G>A	p.Met1?	P				
	Sister	III-5	63	F	none	c.3G>A	p.Met1?	P				
7	Proband	III-1	73	M	MDS (73)	c.121C>T	p.Gln41*	P				
8	Proband	III-2	49	F	Basal cell carcinoma (30s)	c.121C>T	p.Gln41*	P				
	Mother	II-3	69	F	AML (63), Lung (69)	c.121C>T	p.Gln41*	P				
	Maternal Aunt	II-6	69	F	none	c.121C>T	p.Gln41*	P				
	Maternal Aunt	II-4	55	F	Ovarian, Vulvar, AML (55)	c.121C>T	p.Gln41*	P				
	Maternal Cousin	III-3	47	M	AML (47)	c.121C>T	p.Gln41*	P				
9	Proband	III-1	58	M	Pancytopenia cirrhosis	c.142C>T	p.Gln48*	P	*PALB2*	c.2938del	p.Ser980Alafs*10	P
						c.566C>T	p.Pro189Leu	VUS				
10	Proband	IV-14	57	F	CML (51), AML (54)	c.232_233insAA	p.Pro78Glnfs*3	P				
	Paternal Uncle	III-19	81	M	MDS (80), AML (80)	c.232_233insAA	p.Pro78Glnfs*3	P				
	Paternal Cousin	IV-20	46	M	HL	c.232_233insAA	p.Pro78Glnfs*3	P				
	Brother	IV-15	56	M	none	c.232_233insAA	p.Pro78Glnfs*3	P				
	Paternal Cousin	IV-8	?	M	none	c.232_233insAA	p.Pro78Glnfs*3	P				
	Paternal Cousin	IV-22	47	M	none	c.232_233insAA	p.Pro78Glnfs*3	P				
11	Proband	III-1	65	M	AML (64)	c.268C>T	p.Gln90*	P	*ATRX*	c.7219C>T	p.Arg2407*	P
12	Proband	II-7	62	M	AML (60)	c.323del	p.Lys108Serfs*3	P				
	Brother	II-4	66	M	AML (65)	c.323del	p.Lys108Serfs*3	P				
	Sister	II-1	68	F	none	c.323del	p.Lys108Serfs*3	P				
	Brother	II-3	55	M	none	c.323del	p.Lys108Serfs*3	P				
	Mother	I-3	90	F	Colon (70), Breast (75), AML (89)	c.323del	p.Lys108Serfs*3	P				
	Nephew	III-7	36	M	none	c.323del	p.Lys108Serfs*3	P				
13	Proband	III-1	72	F	Gastric (70), t-AML (72)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
14	Proband	III-17	76	M	AML (75)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Sister	III-10	84	F	Melanoma (78)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Niece	IV-4	62	F	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Nephew	IV-12	57	M	AML (56)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Nephew	IV-13	57	M	Melanoma	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Daughter	IV-16	54	F	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Grandniece	V-14	19	F	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Brother	III-19	73	M	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Brother	III-12	85	M	Melanoma	c.415_418dupGATG	p.Asp140Glyfs*2	P				
	Nephew	IV-6	65	M	none	c.415_418dupGATG	p.Asp140Glyfs*2	P				
15	Proband	III-2	75	F	CN-AML (70s)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
16	Proband	III-4	75	M	Prostate (64), AML with MDS changes (74)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
17	Proband	III-3	63	M	AML (63)	c.415_418dupGATG	p.Asp140Glyfs*2	P				
18	Proband	III-1	64	M	MDS (63)	c.946_947del	p.Met316Asp*31	P				
	Son	IV-1	32	M	none	c.946_947del	p.Met316Asp*31	P				
	Brother	III-2	63	M	none	c.946_947del	p.Met316Asp*31	P				
19	Proband	II-2	80	M	Renal (68), MDS (75), AML (80)	c.1141A>T	p.Lys381*	P				
20	Proband	II-3	79	M	MDS (75)	c.1285C>T	p.Gln429*	P				
21	Proband	II-2	65	F	MDS (63)	c.1496dup	p.Ala500Cysfs*9	P				
22	Proband	III-1	54	M	none	c.108T>A	p.Tyr36*	LP				
	Father	II-1	?	M	MDS	c.108T>A	p.Tyr36*	LP				
	Brother	III-2	61	M	MDS (61)	c.108T>A	p.Tyr36*	LP				
23	Proband	III-1	69	M	Colon (69)	c.386dup	p.Lys130Glufs*5	LP				
24	Proband	III-1	67	M	CN-MDS (67)	c.435-2_435-1delinsCA	p.?	LP	*CHEK2*	c.470T>C	p.Ile200Thr	P
25	Proband	III-1	81	F	Mesothelioma (76)	c. 490C>T	p.Arg164Trp	LP				
26	Proband	III-6	76	F	Basal cell carcinoma (68), Colon (68), MPN/MDS overlap syndrome (70)	c. 490C>T	p.Arg164Trp	LP	*APC*	c.3920T>A	p.Ile1307Lys	LP
						c.38C>T	p.Thr13Ile	VUS				
27	Proband	III-6	69	F	Breast (54), AML (67)	c. 490C>T	p.Arg164Trp	LP				
	Brother	III-7	67	M	Head and neck (54)	c. 490C>T	p.Arg164Trp	LP				
	Son	IV-1	?	M	none	c. 490C>T	p.Arg164Trp	LP				
	Nephew	IV-3	31	M	none	c. 490C>T	p.Arg164Trp	LP				
28	Proband	III-6	58	F	Ovarian (53)	c. 490C>T	p.Arg164Trp	LP	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	P
29	Proband	III-4	66	M	Tonsillar (64), t-AML (66)	c.653G>A	p.Gly218Asp	LP				
30	Proband	III-1	37	F	Neuroendocrine carcinoma (31), CMML-2 (37)	c.653G>A	p.Gly218Asp	LP	*ATM*	c.2921+1G>A	p.?	P
31	Proband	III-4	67	M	Prostate (62), Basal Cell Carcinoma (66), MDS (67)	c.766G>A	p.Glu256Lys	LP	*CDKN2A*	c.9_32dup	p.Ala4_Pro11dup	LP
32	Proband	III-2	66	M	Prostate (57), MDS-EB-2 (60)	c.773C>T	p.Pro258Leu	LP				
33	Proband	III-3	56	F	MDS (54)	c.847delC	p.Leu283Cysfs*21	LP				
34	Proband	III-5	65	M	MDS, AML	c.1013G>A	p.Cys338Tyr	LP				
35	Proband	III-26	54	M	MDS (50)	c.1016G>T	p.Arg339Leu	LP				
	Brother	III-27	52	M	MDS (51)	c.1016G>T	p.Arg339Leu	LP				
	Paternal Aunt	II-7	87	F	MDS (87)	c.1016G>T	p.Arg339Leu	LP				
	Paternal Uncle	II-10	83	M	none	c.1016G>T	p.Arg339Leu	LP				
36	Proband	III-2	65	M	MDS and LGL (63)	c.1105C>G	p.Arg369Gly	LP				
	Sister	III-3	62	F	AML (62)	c.1105C>G	p.Arg369Gly	LP				
37	Proband	II-3	65	F	AML (65)	c.1118T>C	p.Leu373Pro	LP				
38	Proband	III-1	41	F	Breast (33)	c.1187T>C	p.Ile396Thr	LP	*BRCA2*	c.6174delT	p.Phe2058LeufsTer12	P
	Mother	II-4	52	F	NHL (38)	c.1187T>C	p.Ile396Thr	LP				
	Maternal Uncle	II-7	65	M	none	c.1187T>C	p.Ile396Thr	LP				
39	Proband	IV-1	70	F	Endometrial (68)	c.1187T>C	p.Ile396Thr	LP				
40	Proband	IV-I	17	F	Aplastic anemia (13)	c.1283T>C	p.Leu428Pro	LP				
41	Proband	III-3	73	M	AML (71)	c.1474dup	p.Ala492Glyfs*17	LP				
42	Proband	III-1	69	M	MDS (68)	c.1721del	p.Leu574Arg*fs143	LP				
43	Proband	III-4	74	M	MDS (73)	c.?	Del. Exons 12-17	LP				
	Daughter	IV-1	46	F	none	c.?	Del. Exons 12-17	LP				
44	Proband	III-1	63	M	CML (62), chronic phase PMF (62)	c.6G>T	p.Glu2Asp	VUS				
45	Proband	II-3	62	F	AML (62)	c.27+9G>A	p.?	VUS	*CHEK2*	c.1283C>T	p.Ser428Phe	P
46	Proband	II-3	62	M	AML (62)	c.138+5G>A	p.Gly?Ala	VUS				
47	Proband	I-1	75	M	Kidney, Prostate, MDS	c.301C>T	p.Arg101Cys	VUS				
48	Proband	III-1	73	M	Systemic mastocytosis (72), Melanoma (73)	c.465G>A	p.Met155Ile	VUS				
49	Proband	III-5	55	F	HL (54)	c.465G>A	p.Met155Ile	VUS				
50	Proband	II-2	73	M	AML	c.511G>C	p.Val171Leu	VUS				
51	Proband	III-2	77	M	NHL (67), Small bowel (68), Prostate (72)	c.511G>C	p.Val171Leu	VUS				
52	Proband	III-2	59	F	Fallopian tube (58), Ovarian (67)	c.926C>T	p.Thr309Ile	VUS				

Abbreviations used: AML, acute myeloid leukemia; CML, chronic myeloid leukemia; CN-AML, cytogenically normal acute myeloid leukemia; F, female; M, male; ID, identification; LGL, large granular lymphocyte; LP, likely-pathogenic; MDS, myelodysplastic syndrome; MDS-EB-2, myelodysplastic syndrome with excess blasts; MPN, myeloproliferative neoplasm; NHL, non-Hodgkin's lymphoma; P, Pathogenic; P#, pedigree number; VUS, variant of uncertain significance; y, years

*These numberings are given according to: *APC* (NM_000038.6), *ATM* (NM_000051.4), *ATRX* (NM_000489.6), *BRCA1* (NM_007294.4), *BRCA2* (NM_000059.4), *CDKN2A* (NM_000077.5), *CHEK2* (NM_007194.4), *PALB2* (NM_024675.3)

†These numberings are given according to: APC (NP_000029.2), ATRX (NP_000480.3), BRCA1 (NP_009225.1), BRCA2 (NP_000050.3) CDKN2A (NP_000068.1), CHK2 (NP_009125.1), PALB2 (NP_078951.2)

Supplementary Table 2. Genes assessed using augmented whole exome sequencing

AIP	*GPC3*	*RBBP6*
ALK	*GREM1*	*RBM8A*
ANKRD26	*GSN*	*RECQL4*
APC	*HOXB13*	*RET*
APOA1	*HRAS*	*RTEL1*
APOA2	*IKZF1*	*RUNX1*
ARID1A	*ITK*	*SAMD9*
ATM	*JAK2*	*SAMD9L*
AXIN2	*KDM1A*	*SDHA*
BAP1	*KIT*	*SDHAF2*
BARD1	*LYZ*	*SDHB*
BLM	*MAGT1*	*SDHC*
BMPR1A	*MAX*	*SDHD*
BRCA1	*MBD4*	*SH2B3*
BRCA2	*MECOM*	*SMAD4*
BRIP2	*MEN1*	*SMARCA4*
BTK	*MET MITF*	*SMARCB1*
CARD11	*MLH1*	*SMARCE1*
CASP10	*MPL*	*SRP72*
CASR	*MRTFA*	*STAT3*
CBL	*MSH2*	*STK1*
CD27	*MSH3*	*SUFU*
CD40LG	*MSH6*	*TERC*
CD70	*MUTYH*	*TERT*
CDC73	*NAF1*	*TET2*
CDH1	*NBN*	*TMEM127*
CDK4	*NF1*	*TNFRSF9*
CDKN1B	*NF2*	*TP53*
CDKN1C	*NPAT*	*TSC1*
CDKN2A	*NPM1*	*TSC2*
CEBPA	*NTHL1*	*TTR*
CHEK2	*PAB2*	*UNC13D*
CSF3R	*PAX5*	*UP45*
CST3	*PDGFRA*	*VHL*
CTLA4	*PGM3*	*WAS*
CTNNA1	*PHOX2B*	*WRN*
CTPS1	*PIK3CD*	*WT1*
DDX41	*PMS2*	*ZNF431*
DICER1	*POLD1*	
DIS3	*POLE*	
DIS3L2	*POT1*	
DOCK8	*PRKAR1A*	
EGFR	*PTCH1*	
EPCAM	*PTEN*	
ERCC6L2	*PTPN11*	
ETV6	*RAD50*	
FGA	*RAD51C*	
FH	*RAD51D*	
FLCN	*RASGRP1*	
GATA2	*RB1*	

Supplementary Table 3. Pathologic descriptions of blood and BM biopsies in individuals with germline deleterious *DDX41* variants at baseline or with HMs

	Family	Relationship to Proband (ID)	Age at biopsy (y)	Diagnosis at biopsy	Peripheral Blood	Core Biopsy	Aspirate Smear	Staining	
								Reticulin	Iron
Baseline	1	Niece (IV-10)	36	Baseline	- Slight left shift of granulocytes - Rare circulating bands - Extremely rare metamyelocytes	Normocellular (60%)	- Slight left shift towards immature forms - Megakaryocytes are smaller and hypolobated - Plasma cells are slightly increased (5.6%) with extremely rare small cells and possible Dutcher body inclusions in the nuclei	Normal	Slightly decreased histiocytic iron
	1	Grandniece (V-3)	17	Baseline	No data	No data	- Mild dysplastic changes in erythroid and megakaryocytic lineages	No data	No data
	3	Son (IV-16)	51	Baseline	- Occasional neutrophils show nuclear excrescences or chromatin hypercondensation - Minimal anisocytosis - Occasional large hypogranular platelets	Normocellular (40-50%)	- Focal shift towards immaturity and focal mild megaloblastoid feature - Some hypolobated/immature megakaryocytes	Normal	Normal
	8	Proband (III-2)	49	Baseline	- Platelets are slightly increased	Normocellular	- Some small hypolobated megakaryocytes	Normal	Increased histiocytic iron
	10	Brother (IV-15)	55	Baseline	Normal	Normocellular (40%)	- Some (<10%) smaller and hypolobated megakaryocytes	Normal	Decreased histiocytic iron
	28	Proband (III-6)	58	Baseline	- Myelocytes, metamyelocytes and band forms - Red cells have Howell-Jolly bodies and poikilocytosis due to prior surgical removal of spleen	Normocellular (40-50%)	- Rare cells with erythrophagocytosis	Normal	Normal
	38	Maternal Uncle (II-7)	65	Baseline	- Some large platelets	- Normocellular (30%) - Subcortical bone	Normal	Normal	Normal
	43	Daughter (IV-1)	46	Baseline	- Occasional neutrophils show nuclear excrescences or chromatin hypercondensation - Occasional reactive and large granular lymphocytes - Focal mild macrocytic erythrocytes with shift towards immaturity - Some dyspoietic megakaryocytes with immature nuclei with multiple separate nuclear lobes, hyperchromatic nuclei or small hypolobated/immature forms	Normocellular (40-50%)	- Focal mild macrocytic erythrocytes with shift towards immaturity, occasional irregular nuclear outlines consistent with "stress" dyserythropoiesis - Small, dyspoietic, hypolobated/immature megakaryocytes	Focally mild (grade 1 of 3) increase in reticulin fibrosis	Decreased storage iron
Malignant	4	Proband (III-1)	69	AML	- Pancytopenia - Macrocytic anemia with significant anisopoikilocytosis including fragmented RBCs and tear-drop cells. Significant RBC polychromasia. - Megakaryocytes increased with dysplasia - Platelets are reduced with hypogranulation - Increased monocytes	Hypocellular (~25%)	- Erythroid and megakaryocytic hyperplasia - Granulocytic hypoplasia	Moderately increased (MF-2, ~25%)	Could not be assessed
	6	Proband (III-6)	65	AML	- Red cells show moderate anisopoikilocytosis with scattered stubby elliptocytes, teardrop cells, and macrocytes with polychromasia - Rare circulating blasts	Hypocellular (0-30%, overall 20%)	- Dysplastic, small, hypolobated megakaryocytes - Megaloblastic erythroid precursors with dysplastic changes - Decreased maturing myeloid component including neutrophils. Instead, several pockes within interstitium comprise blastic cells or maturing myeloid precursors. - Clusters of lymphocytes and plasma cells with edematous stroma	Normal	Rare sideroblasts are present
	7	Proband (III-1)	73	MDS	- Pancytopenia severe neutropenia (ANC 0.5 K/uL), moderate anemia (HGB 9.8 g/dL), severe thrombocytopenia - Mild dyspoietic neutrophils in the form of abnormal nuclear segmentation, chromatin hypercondensation, hypogranular cytoplasm, and some toxic granulation. - Red cells are mildly hypochromic and show moderate anisopoikilocytosis, including ovalocytes, elliptocytes, teardrop cells and occasional fragments. - Mild polychromasia and occasional NRBCs - Some large and occasional giant platelets	Variably cellular (<5-50%, overall 30-40%)	- Increase in blasts, scattered interstitially with variable distribution (10-20% to focally 20-30%) - Immature and maturing erythroid precursors with megaloblastoid features and evidence of dyserythropoiesis in the form of irregular nuclear outline, nuclear budding, cytoplasmic vacuolization - Granulopoiesis reduced with shift towards immaturity and reduced dyspoietic maturation - Megakaryocytes are markedly increased and dysplastic with clustering, mostly small hypolobated/immature	Mild to moderate (grade 1-2 of 3) increase in reticulin fibrosis	No data
	9	Proband (III-1)	56	Baseline, Pancytopenia Cirrhosis	- Left-shifted myelopoiesis - Erythroid hyperplasia with many vacuolated erythroid precursors - Megakaryocytic dysplasia - Mild polyclonal plasmacytosis **[often associated with inflammatory diseases such as autoimmune or infections]**	Normocellular (~50%)	- Marked inflammatory changes in the bone marrow - Mild dyserythropoiesis and dysmegakaryopoiesis	No data	Reduced storage iron
	10	Proband (IV-14)	52	CML, AML	- Increased blasts - Reduced megakaryocytes - Reduced erythropoiesis	Hypocellular (10-15%)	- 10-15% blasts - Shift towards immaturity in granulocytic lineage - Reduced megakaryocytes	No data	No data
	10	Paternal uncle (III-19)	80	AML	- Red cells show mild anisopoikilocytosis with a few microcytes, macrocytes, polychromasia, occasional elliptocytes, and rare teardrop cells	Hypercellular (40%)	- 21% blasts - Megaloblastoid erythroid precursors	No data	No stainable or sideroblastic iron
	12	Proband (II-7)	60	AML	- Leukopenia - Thrombocytopenia - Neutropenia - Red cells are macrocytic with moderate anisopoikilocytosis - Marked proliferation of erythroid precursors - A few small, hypolobated megakaryocytes	Normocellular (50%)	- Erythroid precursors with dyspoietic and megaloblastoid features	No data	Rare ring sideroblasts are present
	13	Proband (III-1)	72	t-MDS RAEB-2 (history of chemo)	- Pancytopenia - Reduced leukocytes - Some neutrophils with toxic granulation, and some with other dysplastic changes including hypogranulation, abnormal nuclear segmentation and chromatin patterns - Mild normocytic anemia - RBCs show mild anisocytosis with occasional spherocytes and rare teardrop cells	Hypocellular (~15%)	- Increased blasts (~14%) - Increased erythropoiesis with full spectrum maturation and significant dysplasia including megaloblastoid changes, nuclear irregularities and rare multinucleated forms - Decreased megakaryocytes with dysplasia including hypolobated nuclei widely separate nuclear lobes and occasional micromegakaryocytes	Normal	Adequate storage iron
	15	Proband (III-2)	69	CN-MDS	- Leukopenia - Absolute neutropenia - Mild anisocytosis and increased polychromasia - Occational circulating blasts	Hypercellular (variable from <5% to 20-30%)	- 11% blasts: an interstitial infiltrate of small blasts in clusters between islands of erythroid precursors - Granulopoiesis is reduced, shifted toward immaturity	No data	No data
	16	Proband (III-4)	73	AML	- Leukopenia with absolute neutropenia - Macrocytic anemia - Moderate thrombocytopenia - Blasts have round/oval nuclei, dispersed chromatin, and scant blue agranular cytoplasm - RBCs show anisopoikilocytosis with macro-ovalocytes, rare dacryocytes, fragmented forms, and some polychromasia	Variable cellularity (20-40%)	- Occasional small, dyspoietic megakaryocytes - Erythropoiesis shows dyspoietic features including nuclear-cytoplasmic dyssynchrony, irregular nuclear borders, and rare nuclear budding	No data	Normal
	17	Proband (III-3)	63	AML	- Circulating blasts - Granulocytic dysplasia characterized by abnormal chromatin clumping and abnormal nuclear condensation - Normocytic anemia with mild anisopoikilocytosis characterized by macroovalocytes, and occasional microcytes - Mild RBC polychromasia	Normocellular (~30%)	- Marginally increased blasts (4.4%) - Many small hypolobated megakaryocytes indicating significant dysplasia - Significant left-shift in granulocytes with markedly increased myelocytes - Mild erythroid hyperpasia with megaloblastoid maturation, occasional nuclear irregularities and rare bi-nucleation	Focal mild increase in reticulin fibrosis, MF-1	Increased storage iron
	21	Proband (II-2)	64	MDS	- Numerous macroovalocytes - Circulating blasts	Normocellular (30%)	- 12-15% blasts - Erythroid precursors appear megaloblastoid - Small hypolobated dysplastic megakaryocytes present	No data	No data
	24	Proband (III-1)	67	MDS REAB-2	- Mild neutropenia, some neutrophils show toxic granulation, are pale, larger, and hypogranular with hyper-condensed chromatin - Occasional circulating blasts - Polychromatophilic RBCs	Hypocellular (15%)	- Both erythroid and myeloid lineages show shift towards immaturity - Erythropoiesis appears megaloblastoid as judged from the pronormoblasts - Megakaryocytes are reduced, and some are dysplastic with widely separated nuclear lobes	Patchy increase in reticulin fibrosis (grade 1/3)	- Increase in storage iron - Granular appearance
	26	Proband (III-6)	75	MDS/MPN	- Normocytic anemia (11.0 g/dL) - Thrombocytosis (613 K/uL)	Hypercellular (45%)	- Granulocytes show abnormal nuclear segmentation, numerous pseudo-Pelger-Huet forms, nuclear excrescencies, and some forms with hypogranulated cytoplasm - Erythroid cells show significant dysplasia including megaloblastoid changes, nuclear irregularities and occasional multinucleated forms - Increased megakaryocytes with numerous small, mono-lobated and some hypolobated forms indicative of dysmegakaryopoiesis	Mild focal increase in reticulin fibrosis, MF-0-1	Adequate storage iron
	29	Proband (III-4)	65	t-AML (TP53 mut and complex karyotype), low-grade B-cell lymphoproliferative disorder	- Pancytopenia - Occasional (~4%) circulating blasts - Blasts have irregular nuclear contours, fine chromatin, distinct nucleoli, and a small amount of cytoplasm - Rare segmented neutrophils - Reduced RBCs, some with anisocytosis, some elliptocytes and teardrop cells	Hypercellular (~55%)	- Decreased erythroid cells, some show dyserythropoiesis - Decreased megakaryocytes, some small hypolobated	Moderate reticulin fibrosis, MF-2	Increased storage iron
	30	Proband (III-1)	36	t-MN (history of chemo, t(11;16)(q23;p13) translocation)	- Monocytosis (64%) - Macrocytic anemia - RBCs show marked anisopoikilocytosis including occasional tear-drop cells - Thrombocytopenia - Granulocytic dysplasia	Hypocellular (~30%)	- Mature neutrophils markedly reduced and show significant granulocytic dysplasia - Mild to moderate erythroid dysplasia including megaloblastoid changes, nuclear irregularities and occasional bi-nucleated forms - Marked megakaryocytic dysplasia, some are small hypolobated	Mild to moderate reticulin fibrosis, MF-1-2	Could not be assessed
	31	Proband (III-4)	67	t-MDS (morphology consistent with therapy-related)	- Increased megakaryocytes, many are small dyspoietic hypolobated or with separated nuclear lobes, occasional micromegakaryocytes - Mild leukopenia with moderate neutropenia and mild lymphopenia - Rare circulating blasts - Moderate anemia, RBCs are macrocytic and show moderate anisopoikilocytosis, including macro-ovalocytes, microcytes, elliptocytes, and occasional spherocytes	Normocellular (30-40%)	- Predominance of erythropoiesis with megaloblastoid features, focal shift towards immaturity, evidence of dyserythropoiesis in the form of irregular nuclear outlines, binucleation and cytoplasmic vacuolization - Megakaryocytes are increased with many small dyspoietic hypolobated/immature forms or with separated nuclear lobes, occasional micromegakaryocytes	- Mild to focally moderate (grade 1-2 of 3) increase in reticulin fibrosis	- Increased iron - Occasional ring sideroblasts
	32	Proband (III-2)	61	t-MDS-EB-2	- Occasional dysplastic neutrophils with hypogranulation and abnormal nuclear segmentation, and pseudo-Pelger-Huet nuclei - Anemia, RBCs show marked anisocytosis with macro-ovalocytes, occasional spherocytes, elliptocytes, and teardrop cells - Increased polychromasia	Hypocellular (~10%)	- Increased blasts (~15%) - Occasional granulocytes show dysplastic changes including hyposegmented nuclei, abnormal chromatin patterns and rare hypogranulation - Erythroid cells show dysplastic changes including megaloblastoid maturation, occasional nuclear irregularities and bi-/multi-nucleation - Megakaryocytes are reduced, with dysplasia including hypolobated nuclei, widely separate nuclear lobes and micromegakaryocytes	Moderate increase in reticulin fibrosis, MF-2	Could not be assessed
	34	Proband (III-5)	62	t-MDS (t(11;16)(q23;p13) translocation)	- Moderate macrocytic anemia - Dyspoietic neutrophils in the form of abnormal nuclear segmentation with pseudo Pelgar-Huet change, chromatin hypercondensation, nuclear excrescences, and hypogranular cytoplasm. Some show toxic granulation. - Mild lymphopenia - RBCs are macrocytic and show moderate anisocytosis, including macro-ovalocytes, microcytes, and occasional red cell fragments - Mild thrombocytopenia, platelets show anisocytosis, including some large and occasional giant platelets	Hypocellular (10-20%)	- Dysplastic megakaryocytes including some with separated nuclear lobes, many hypolobated forms, and micromegakaryocytes with frequent clustering - Erythropoiesis with megaloblastoid features - Decreased granulopoiesis with dysplastic maturation - Evidence of Intrasinusoidal hematopoiesis	- Mild (grade 1 of 3) increase in reticulin fibrosis	No data
	41	Proband (III-3)	71	AML	- Significant pancytopenia - Acrocytic hypochromic anemia - Mild anisopoikilocytosis - Reduced platelets - Rare circulating blasts	Hypocellular (5-10%)	- Increased blasts, some with irregular nuclear membranes, high nuclear cytoplasmic ratio, prominent nucleoli and scant cytoplasm - Left shifted granulopoiesis - Rare blasts, granulocyte precursors, and erythroid precursors present	- No increase in reticulin fibrosis	- No marrow stroma or spicules to assess for storage iron - Too few erythroid precursors to assess for ring sideroblasts
	43	Proband (III-4)	73	MDS-MLD	No data	No data	- Significant dysgranulopoiesis - 4.6% blasts - Significant dyserythropoiesis - Significant dysmegakaryopoiesis	No data	No data

Abbreviations used: ANC, absolute neutrophil count; BM, bone marrow; HGB, hemoglobin; ID, identification; MF, marrow fibrosis; NRBCs, nucleated red blood cells; RBCs, red blood cells; y, years

Supplementary Table 4. Individuals with germline *DDX41*^{LoF} and other cancer-risk alleles

Relationship to Proband	Pedigree ID	Sex	Age, y	Diagnosis (Age of Diagnosis)	Second Germline Variant Gene	Second Germline Variant*	Encoded Protein Variant†	Classification
***DDX41* P/LP**								
Proband	F9-III-1	M	58	Thrombocytopenia	*PALB2*	c.2938del	p.Ser980Alafs*10	P
Proband	F11-III-1	M	65	AML (64)	*ATRX*	c.7219C>T	p.Arg2407*	LP
Proband	F24-III-1	M	67	CN-MDS (67)	*CHEK2*	c.470T>C	p.Ile200Thr	P
Proband	F26-III-6	F	76	Basal cell carcinoma (68), MPN/MDS overlap syndrome (70)	*APC*	c.3920T>A	p.Ile1307Lys	LP
Proband	F28-III-6	F	58	Ovarian (53)	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	P
Proband	F30-III-1	F	37	Neuroendocrine carcinoma (31), t-AML (37)	*ATM*	c.2921+1G>A	p.?	P
Proband	F31-III-4	M	67	Prostate (62), MDS (67)	*CDKN2A*	c.9_32dup	p.Ala4_Pro11dup	LP
Proband	F38-III-1	F	41	Breast (33)	*BRCA2*	c.6174delT	p.Phe2058LeufsTer12	P
***DDX41* VUS**								
Proband	F45-II-3	F	62	AML (62)	*CHEK2*	c.1283C>T	p.Ser428Phe	P

Abbreviations used: AML, acute myeloid leukemia; CN-MDS, cytogenetically normal myelodysplastic syndrome; F, family; ID, identification; LP, likely pathogenic; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; P, pathogenic; P#, pedigree number; VUS, variant of uncertain significance; y, years

*These numberings are given according to: *APC* (NM_000038.6), *ATM* (NM_000051.4), *ATRX* (NM_000489.6), *BRCA1* (NM_007294.4), *BRCA2* (NM_000059.4), *CDKN2A* (NM_000077.5), *CHEK2* (NM_007194.4), *PALB2* (NM_024675.3)

†These numberings are given according to: APC (NP_000029.2), ATRX (NP_000480.3), BRCA1 (NP_009225.1), BRCA2 (NP_000050.3), CDKN2A (NP_000068.1), CHEK2 (NP_009125.1), GATA2 (NP_116027.2), PALB2 (NP_078951.2)

Supplementary Table 5. Classifications of second cancer-risk alleles

Pedigree ID	Second Germline Variant Gene	Second Germline Variant*	Encoded Protein Variant†	Classification	DNA Source	Test Type	VAF; Germline confirmation	Justification
F9-III-1	*PALB2*	c.2938del	p.Ser980Alafs*10	P	Bone marrow	OncoPlus large tumor panel (NGS)	Confirmed germline in cultured skin fibroblasts	P in ClinVar; Clinical report
F11-III-1	*ATRX*	c.7219C>T	p.Arg2407*	LP			Confirmed germline	LP in ClinVar
F24-III-1	*CHEK2*	c.470T>C	p.Ile200Thr	P			Confirmed germline	P in ClinVar
F26-III-6	*APC*	c.3920T>A	p.Ile1307Lys	LP	Peripheral blood	OncoPlus large tumor panel (NGS)	48%; Confirmed germline	LP in ClinVar; Clinical report
F28-III-6	*BRCA1*	c.68_69delAG	p.Glu23Valfs*17	P	Bone marrow	OncoPlus large tumor panel (NGS)	46%; Confirmed germline	P in ClinVar; Reviewed by expert panel
F30-III-1	*ATM*	c.2921+1G>A	p.?	P	Bone marrow	OncoPlus large tumor panel (NGS)	49%; Confirmed germline	P in ClinVar; Clinical report
F31-III-4	*CDKN2A*	c.9_32dup	p.Ala4_Pro11dup	LP	Skin biopsy	Hereditary Leukemia and Breast Cancer Panel (NGS)	Confirmed germline	LP in Clinical report; P/LP in ClinVar
F38-III-1	*BRCA2*	c.6174delT	p.Phe2058LeufsTer12	P	Right pleura; formalin-fixed, paraffin-embedded	OncoPlus large tumor panel (NGS)	56%; Confirmed germline	P in ClinVar; Reviewed by expert panel
F45-II-3	*CHEK2*	c.1283C>T	p.Ser428Phe	P				P in ClinVar

Abbreviations used: F, family; P, pathogenic; LP, likely pathogenic; VAF, variant allele frequency

*These numberings are given according to: *APC* (NM_000038.6), *ATM* (NM_000051.4), *ATRX* (NM_000489.6), *BRCA1* (NM_007294.4), *BRCA2* (NM_000059.4), *CDKN2A* (NM_000077.5), *CHEK2* (NM_007194.4), *PALB2* (NM_024675.3)

†These numberings are given according to: APC (NP_000029.2), ATRX (NP_000480.3), BRCA1 (NP_009225.1), BRCA2 (NP_000050.3), CDKN2A (NP_000068.1), CHEK2 (NP_009125.1), GATA2 (NP_116027.2), PALB2 (NP_078951.2)

Supplementary Table 6. FPKM RNA-sequencing values indicating gene expression in *DDX41*$^{var/+}$ and *DDX41*^{WT} LCLs for proteins of interest

Gene	*DDX41* ^{WT}			*DDX41* $^{var/+}$					P value*	Significant† (yes/no)	Validation with qRT-PCR (fold change from WT)
	WT #1	WT #2	WT #3	M1?	P258L	A492G*17	A500C*9	del ex. 12-17			
CD244	0.273343	0.269191	0.285173	11.0895	1.66513	0.774687	0.953599	0.685519	5.00E-05	yes	1.531
CD9	2.25758	7.70813	1.90579	69.2145	3.17566	12.9506	14.8167	5.7882	5.00E-05	yes	2.886
CDC14B	5.85656	2.67411	2.26175	7.17762	86.3545	6.16464	85.5129	5.57933	5.00E-05	yes	1.702
IL1R1	0.438136	0.581984	1.52523	49.0788	1.66037	2.10244	9.29089	6.67852	5.00E-05	yes	2.685
IL23R	0.0295062	1.05441	0.335415	9.57457	0.513941	0.320671	0.74782	3.37672	5.00E-05	yes	5.443
IL32	13.369	43.0563	14.4911	139.772	13.4532	26.6972	7.69567	249.089	1.00E-04	yes	1.915
LTBR	0.481455	0.718212	1.19857	23.8029	3.93722	2.87089	15.654	2.87813	5.00E-05	yes	9.553
PTPN14	0.107992	0.254362	0.203035	0.0191269	1.74213	2.31123	0.844935	2.86304	0.00085	yes	7.788
ANG	0.0766067	0	0.0800762	0.136627	0.138466	0.13552	0	0	1	no	
CXCL13	0	0	0	0	0	0	0.0748012	0.0771039	1	no	
CXCL8	0	0.309606	0	3.54681	0.177275	0.276612	0.050492	0.453417	0.24035	no	
DDX41	91.4954	101.097	97.9534	66.87	70.5655	51.5405	45.3021	84.5139	0.0575	no	
IL9	0	0	0	0	0	0	0	0	1	no	
NFKB1	61.2399	69.3384	66.4465	136.892	66.4874	88.2016	73.7998	95.4516	0.0543	no	
NFKB2	70.8146	116.882	80.716	205.606	63.6811	103.725	140.523	106.256	0.10435	no	
REL	5.93962	4.56755	5.27166	16.9442	3.532	10.2362	4.44955	8.30849	0.10055	no	
RELA	71.8171	69.5421	68.9387	76.3011	85.5456	79.914	76.0734	97.7434	0.4517	no	
RELB	13.8668	16.8349	11.4696	26.1377	12.8202	20.1112	23.1998	23.5245	0.0563	no	

Abbreviations used: var, variant

*P values were determined using a Pearson's correlation

†Confidence interval=95%

Supplementary Table 7. UK Biobank participants used in proteomics analysis

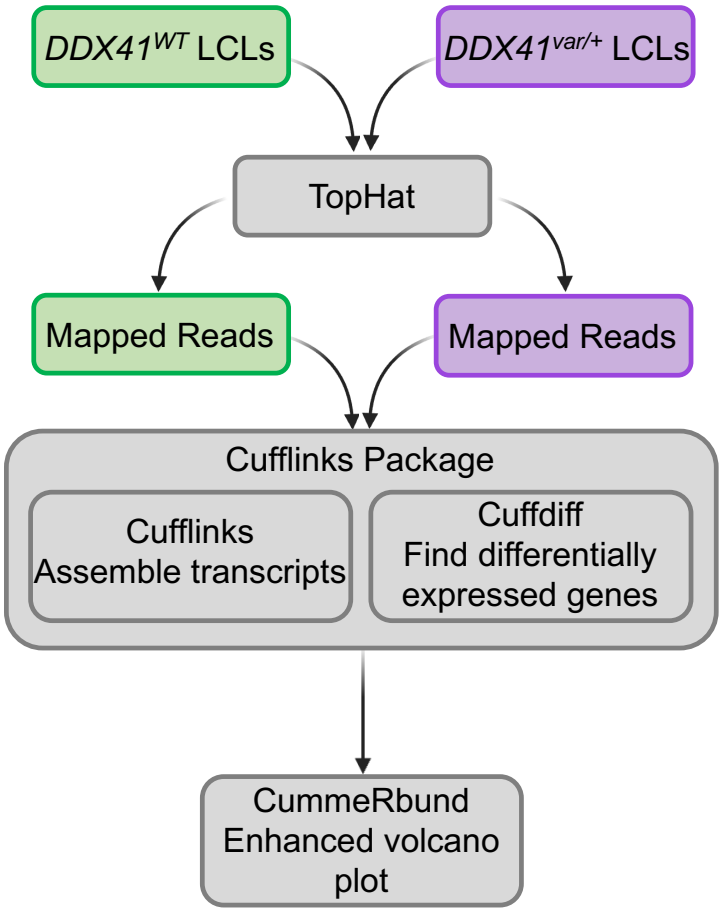
Individuals with Likely Germline P/LP *DDX41* Variants					Corresponding WT Controls		
Case #	Age at Recruitment	Sex	*DDX41* Likely Germline Variant [NM_016222.4]	DDX41 Encoded Protein Variant [NP_057306.2]	Control #	Age at Recruitment	Sex
1	40	Female	c.415_418dupGATG	p.Asp140Glyfs*2	1	40	Female
					2	40	Female
2	41	Male	c.415_418dupGATG	p.Asp140Glyfs*2	3	41	Male
					4	41	Male
3	41	Female	c.3G>A	p.Met1?	5	41	Female
					6	41	Female
4	43	Male	c.415_418dupGATG	p.Asp140Glyfs*2	7	43	Male
					8	43	Male
5	44	Male	c.1187T>C	p.Ile396Thr	9	44	Male
					10	44	Male
6	45	Female	c.3G>A	p.Met1?	11	45	Female
					12	45	Female
7	46	Male	c.1187T>C	p.Ile396Thr	13	46	Male
					14	46	Male
8	46	Female	c.415_418dupGATG	p.Asp140Glyfs*2	15	46	Female
					16	46	Female
9	47	Female	c.946_947del	p.Met316Asp*31	17	47	Female
					18	47	Female
10	47	Male	c.3G>A	p.Met1?	19	47	Male
					20	47	Male
11	48	Male	c.3G>A	p.Met1?	21	48	Male
					22	48	Male
12	48	Female	c.415_418dupGATG	p.Asp140Glyfs*2	23	48	Female
					24	48	Female
13	48	Female	c.3G>A	p.Met1?	25	48	Female
					26	48	Female
14	50	Female	c.3G>A	p.Met1?	27	50	Female
					28	50	Female
15	50	Female	c.121C>T	p.Gln41*	29	50	Female
					30	50	Female
16	52	Female	c.3G>A	p.Met1?	31	52	Female
					32	52	Female
17	52	Male	c.946_947del	p.Met316Asp*31	33	52	Male
					34	52	Male
18	53	Male	c.3G>A	p.Met1?	35	53	Male
					36	53	Male
19	54	Male	c.1586_1587del	p.Thr529Argfs*12	37	54	Male
					38	54	Male
20	54	Male	c.415_418dupGATG	p.Asp140Glyfs*2	39	54	Male
					40	54	Male
21	55	Female	c.1187T>C	p.Ile396Thr	41	55	Female
					42	55	Female
22	56	Female	c.415_418dupGATG	p.Asp140Glyfs*2	43	56	Female
					44	56	Female
23	56	Male	c.157G>A	p.Gly173Arg	45	56	Male
					46	56	Male
24	56	Male	c.415_418dupGATG	p.Asp140Glyfs*2	47	56	Male
					48	56	Male
25	57	Female	c.3G>A	p.Met1?	49	57	Female
					50	57	Female
26	58	Female	c.3G>A	p.Met1?	51	58	Female
					52	58	Female
27	58	Male	c.415_418dupGATG	p.Asp140Glyfs*2	53	58	Male
					54	58	Male
28	58	Male	c.3G>A	p.Met1?	55	58	Male
					56	58	Male
29	59	Male	c.3G>A	p.Met1?	57	59	Male
					58	59	Male
30	59	Female	c.121C>T	p.Gln41*	59	59	Female
					60	59	Female
31	60	Male	c.157G>A	p.Gly173Arg	61	60	Male
					62	60	Male
32	60	Female	c.415_418dupGATG	p.Asp140Glyfs*2	63	60	Female
					64	60	Female
33	60	Male	c.121C>T	p.Gln41*	65	60	Male
					66	60	Male
34	61	Male	c.3G>A	p.Met1?	67	61	Male
					68	61	Male
35	61	Male	c.3G>A	p.Met1?	69	61	Male
					70	61	Male
36	61	Male	c.3G>A	p.Met1?	71	61	Male
					72	61	Male
37	63	Male	c.415_418dupGATG	p.Asp140Glyfs*2	73	63	Male
					74	63	Male
38	64	Female	c.415_418dupGATG	p.Asp140Glyfs*2	75	64	Female
					76	64	Female
39	64	Female	c.415_418dupGATG	p.Asp140Glyfs*2	77	64	Female
					78	64	Female
40	64	Female	c.415_418dupGATG	p.Asp140Glyfs*2	79	64	Female
					80	64	Female
41	65	Female	c.3G>A	p.Met1?	81	65	Female
					82	65	Female
42	65	Female	c.415_418dupGATG	p.Asp140Glyfs*2	83	65	Female
					84	65	Female
43	67	Male	c.415_418dupGATG	p.Asp140Glyfs*2	85	67	Male
					86	67	Male
44	67	Female	c.3G>A	p.Met1?	87	67	Female
					88	67	Female
45	67	Female	c.415_418dupGATG	p.Asp140Glyfs*2	89	67	Female
					90	67	Female
46	67	Male	c.157G>A	p.Gly173Arg	91	67	Male
					92	67	Male
47	68	Female	c.946_947del	p.Met316Asp*31	93	68	Female
					94	68	Female
48	68	Female	c.415_418dupGATG	p.Asp140Glyfs*2	95	68	Female
					96	68	Female
49	69	Male	c.3G>A	p.Met1?	97	69	Male
					98	69	Male

Abbreviations used: LP, likely-pathogenic; P, pathogenic

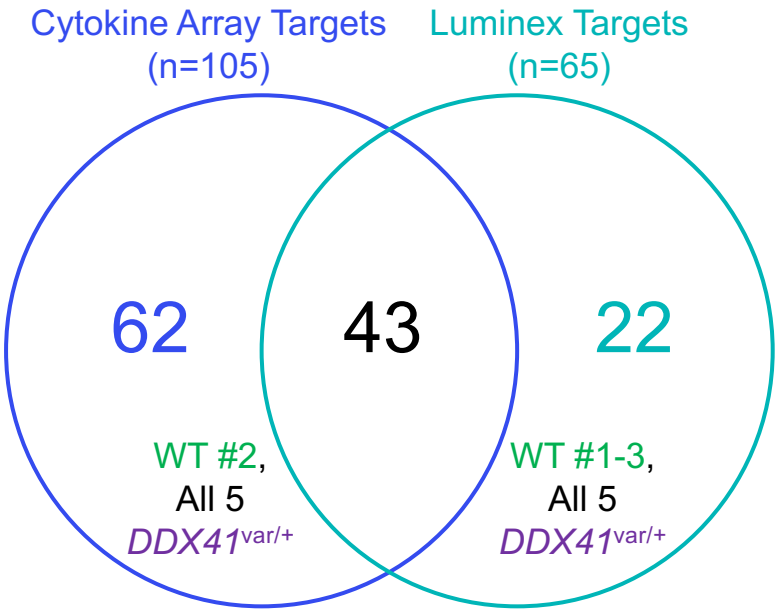
Supplementary Table 8. Summary of UK Biobank participants with likely germline *DDX41*^{LoF} variants

DDX41 Likely Germline Variant [NM_016222.4]	DDX41 Encoded Protein Variant [NP_057306.2]	*DDX41* Germline Variant Classification	Number of UK Biobank Participants
c.3G>A	p.Met1?	P	8
c.121C>T	p.Gln41*	P	3
c.415_418dupGATG	p.Asp140Glyfs*2	P	8
c.157G>A	p.Gly173Arg	P/LP	3
c.946_947del	p.Met316Asp*31	P	3
c.1187T>C	p.Ile396Thr	LP	3
c.1586_1587del	p.Thr529Argfs*12	P	1

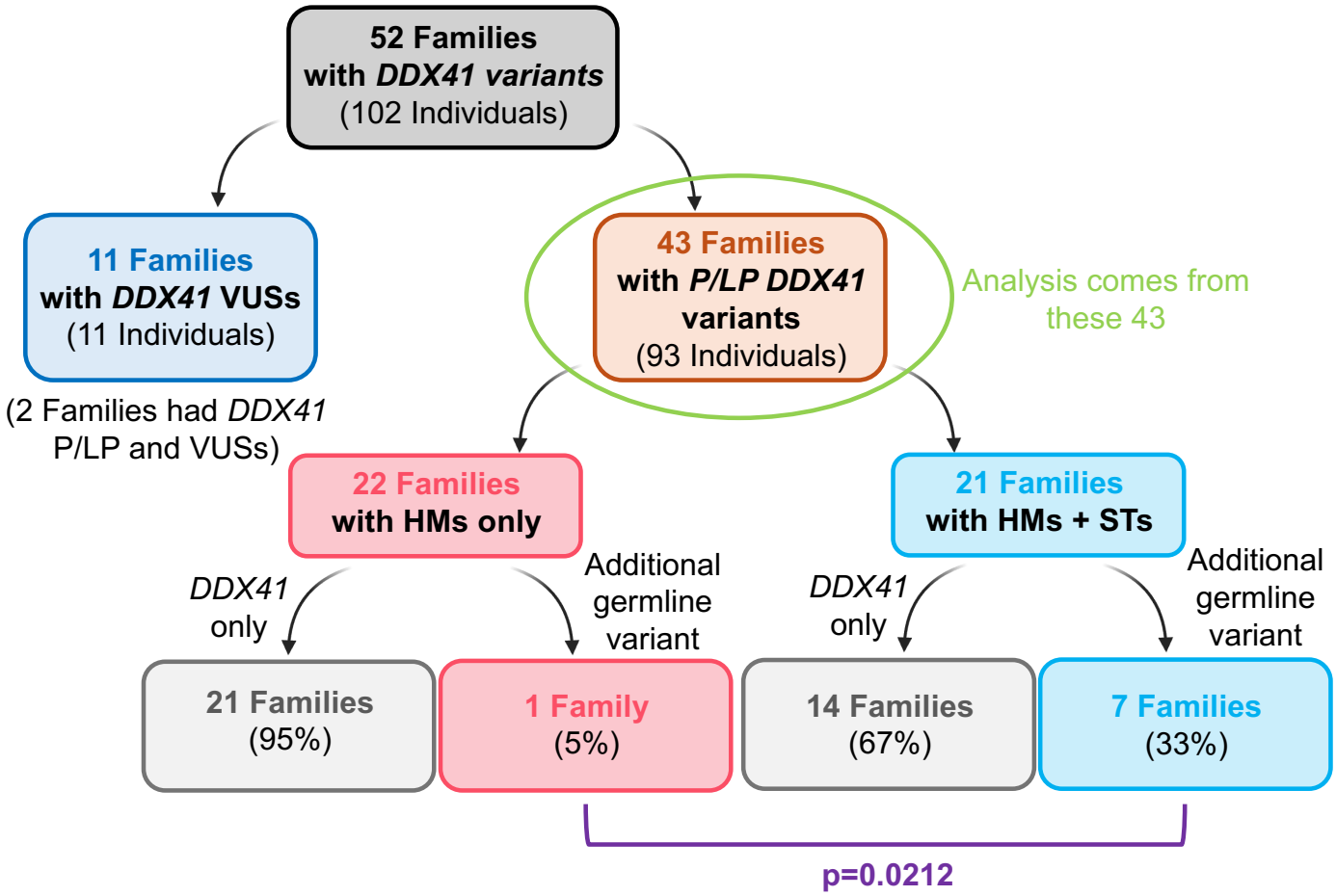
Abbreviations used: LP, likely-pathogenic; P, pathogenic



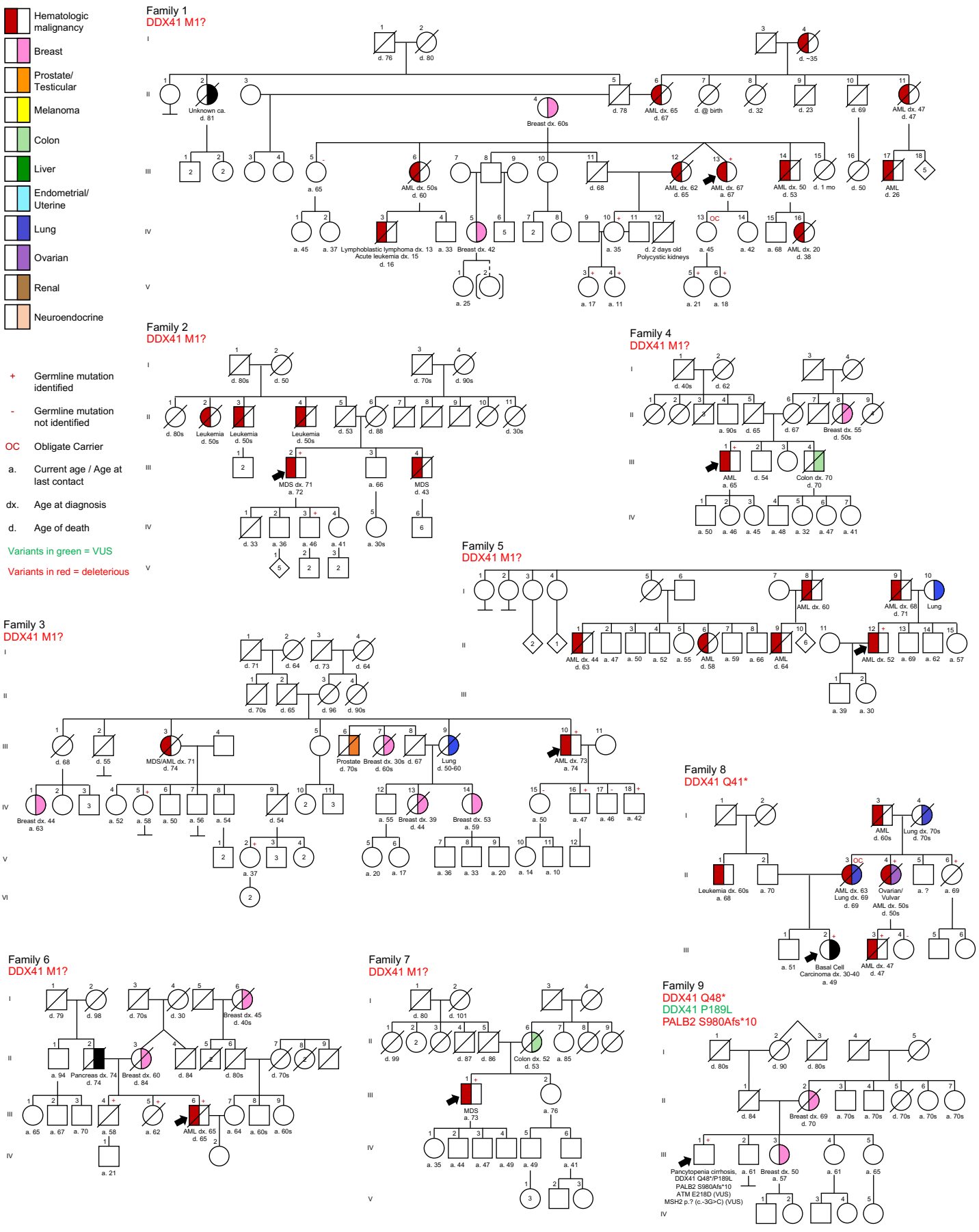
Supplementary Figure 1. Cufflinks pipeline used to analyze RNA-sequencing data. Data from *DDX41*^{WT} LCLs (green) and from patient-derived *DDX41*$^{var/+}$ LCLs (purple) is shown. Packages used to input data are indicated in gray.

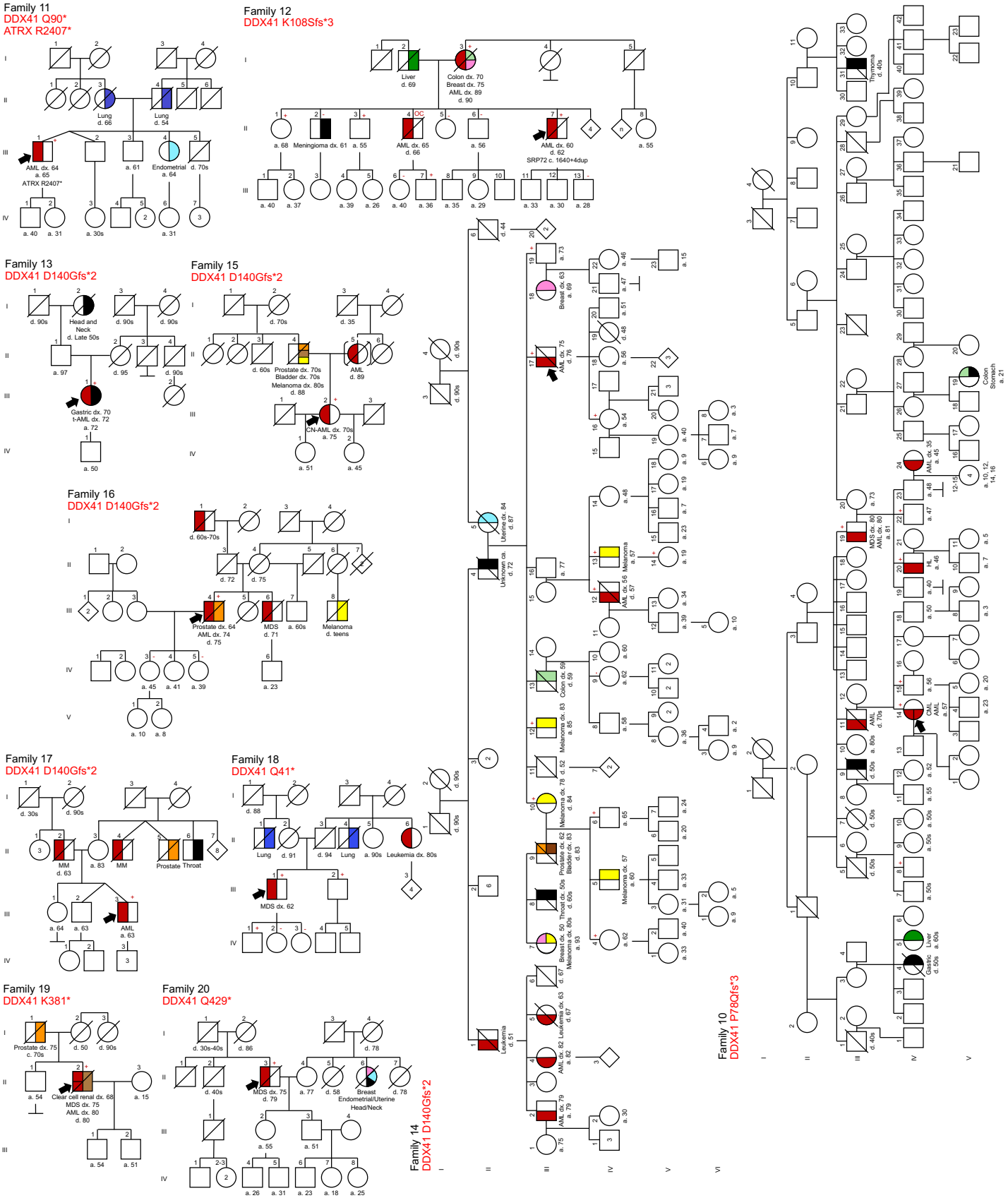


Supplementary Figure 2. Venn diagram of inflammatory cytokines assessed by cytokine arrays and Luminex. The number of inflammatory cytokines assessed by cytokine arrays only (blue), Luminex only (teal), and by both cytokine arrays and Luminex (black) are shown. Levels of inflammatory cytokines were measured in conditioned media from *DDX41*^{WT} (green) and patient-derived *DDX41*$^{var/+}$ (purple) LCLs.

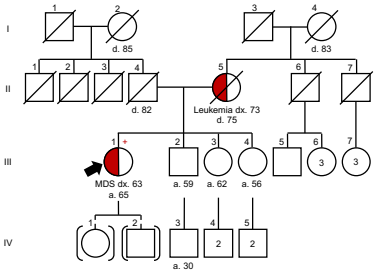


Supplementary Figure 3. Summary of our 52-family cohort. Our cohort consists of 52 families with germline *DDX41* variants of any classification (dark grey). Eleven of these families had germline *DDX41* variants of uncertain significance (VUSs, dark blue). Forty-three of these families had deleterious (P/LP) germline *DDX41* variants (orange) and were used for most of our analyses (light green). Twenty-two of those 43 families had hematopoietic malignancies (HMs) only (pink). The rest (21) had HMs and solid tumors (STs, light blue), defined as those with a history of solid tumors in ≥15% of primary relatives of the proband including the proband. Families with HMs and STs were significantly more likely to have additional germline variants in other cancer-associated genes (p=0.0212).

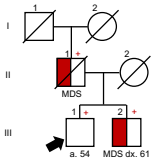

Hematologic malignancy
Breast
Prostate/ Testicular
Melanoma
Colon
Liver
Endometrial/ Uterine
Lung
Ovarian
Renal
Neuroendocrine
+ Germline mutation identified
- Germline mutation not identified
OC Obligate Carrier
a. Current age / Age at last contact
dx. Age at diagnosis
d. Age of death
Variants in green = VUS
Variants in red = deleterious
Family 1
DDX41 M1?
Family 2
DDX41 M1?
Family 3
DDX41 M1?
Family 4
DDX41 M1?
Family 5
DDX41 M1?
Family 6
DDX41 M1?
Family 7
DDX41 M1?
Family 8
DDX41 Q41*
Family 9
DDX41 Q48*
DDX41 P189L
PALB2 S980Afs*10


Family 11
DDX41 Q90*
ATRX R2407*
Lung d. 66
Lung d. 54
AML dx. 64
a. 65
ATRX R2407*
a. 61
Endometrial
a. 64
d. 70s
a. 40
a. 31
a. 30s
a. 31
Family 12
DDX41 K108Sfs*3
Liver d. 69
Colon dx. 70
Breast dx. 75
AML dx. 89
d. 90
a. 68
Meningioma dx. 61
a. 55
AML dx. 65
d. 66
a. 56
AML dx. 60
d. 62
SRP72 c. 1640+4dup
a. 55
a. 40
a. 37
a. 39
a. 26
a. 40
a. 36
a. 35
a. 29
a. 33
a. 30
a. 28
Family 13
DDX41 D140Gfs*2
d. 90s
Head and Neck
d. Late 50s
a. 97
d. 95
d. 90s
Gastric dx. 70
t-AML dx. 72
a. 72
a. 50
Family 15
DDX41 D140Gfs*2
d. 70s
a. 35
d. 60s
Prostate dx. 70s
Bladder dx. 70s
Melanoma dx. 80s
d. 88
AML
d. 89
CN-AML dx. 70s
a. 75
a. 51
a. 45
d. 90s
Family 16
DDX41 D140Gfs*2
d. 60s-70s
d. 72
d. 75
Prostate dx. 64
AML dx. 74
d. 75
MDS
d. 71
a. 60s
Melanoma
d. teens
a. 45
a. 41
a. 39
a. 23
a. 10
a. 8
Family 17
DDX41 D140Gfs*2
d. 30s
d. 90s
MM
d. 63
a. 83
MM
Prostate
Throat
a. 64
a. 63
AML
a. 63
Family 18
DDX41 Q41*
d. 88
Lung
d. 91
d. 94
Lung
a. 90s
Leukemia dx. 80s
MDS dx. 62
Family 19
DDX41 K381*
Prostate dx. 75
c. 70s
d. 50
d. 90s
a. 54
Clear cell renal dx. 68
MDS dx. 75
AML dx. 80
d. 80
a. 15
a. 54
a. 51
Family 20
DDX41 Q429*
d. 30s-40s
d. 86
d. 78
d. 40s
MDS dx. 75
d. 79
a. 77
d. 58
Breast
Endometrial/Uterine
Head/Neck
d. 78
a. 55
a. 51
a. 26
a. 31
a. 23
a. 18
a. 25
Family 14
DDX41 D140Gfs*2
d. 44
Uterine dx. 84
d. 87
Unknown ca.
d. 72
Leukemia
d. 51
Breast dx. 73
Breast dx. 69
a. 69
AML dx. 75
d. 76
a. 54
Melanoma
a. 57
AML dx. 56
d. 57
Colon dx. 59
d. 59
Melanoma dx. 83
a. 85
Melanoma dx. 78
d. 84
d. 52
Prostate dx. 62
Bladder dx. 83
Throat dx. 50s
d. 60s
Breast dx. 50
Melanoma dx. 80s
a. 93
Melanoma dx. 67
a. 60
Leukemia dx. 63
d. 67
AML dx. 82
a. 82
AML dx. 79
a. 79
a. 75
a. 30
Family 10
DDX41 P78Qfs*3
Thymoma
d. 40s
MDS dx. 80
AML dx. 80
a. 81
HL
a. 46
AML
d. 70s
CML
AML
a. 57
Colon
Stomach
a. 21
AML dx. 35
a. 45
a. 10, 12,
14, 16
Liver
a. 60s
Gastric
d. 50s

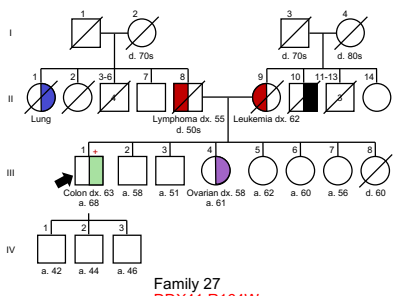
Family 21
DDX41 A500Cfs*9



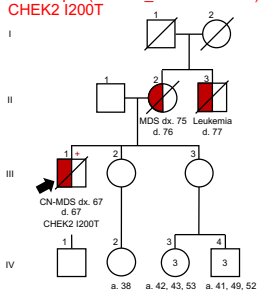
Family 22
DDX41 Y36*



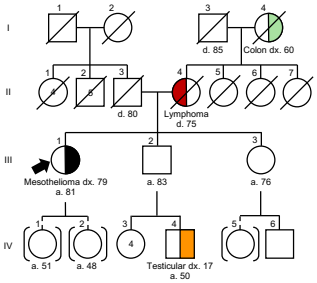
Family 23
DDX41 K130Efs*5



Family 24
DDX41 p.? (c.435-2_435-1delinsCA)
CHEK2 I200T



Family 25
DDX41 R164W



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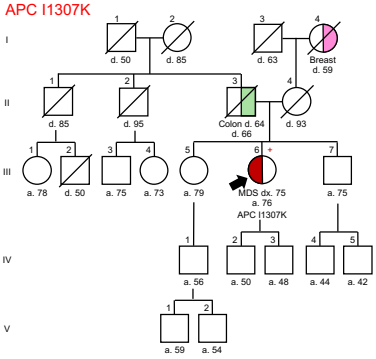
DDX41 R164W
APC I1307K



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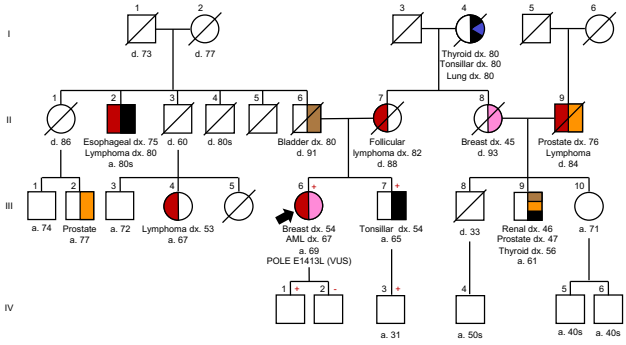
DDX41 R164W



Family 28
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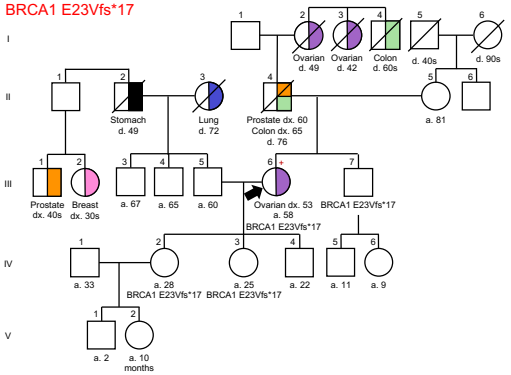
BRCA1 E23Vfs*17



Family 29
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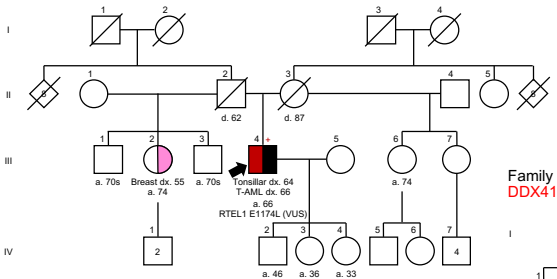
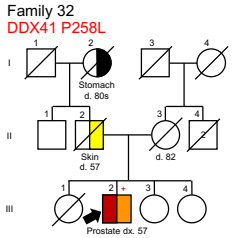


Family 32
DDX41 P258L



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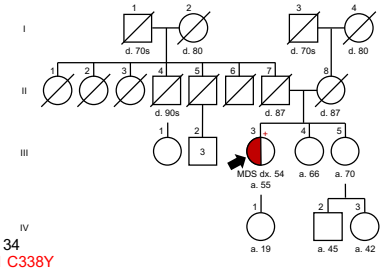
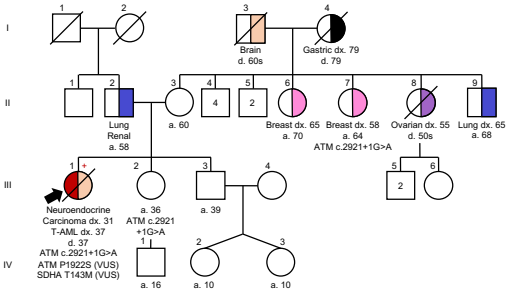


Family 30
DDX41 G218D
ATM p.? (c.2921+1G>A)



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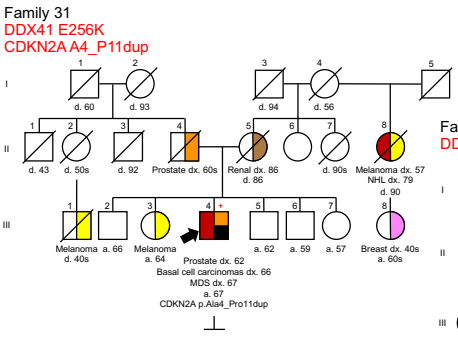
DDX41 E256K
CDKN2A A4_P11dup



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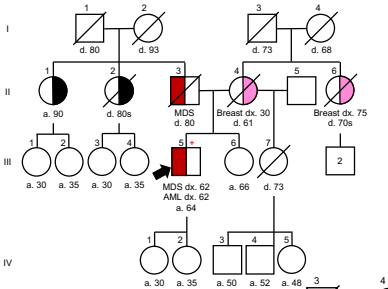
DDX41 C338Y



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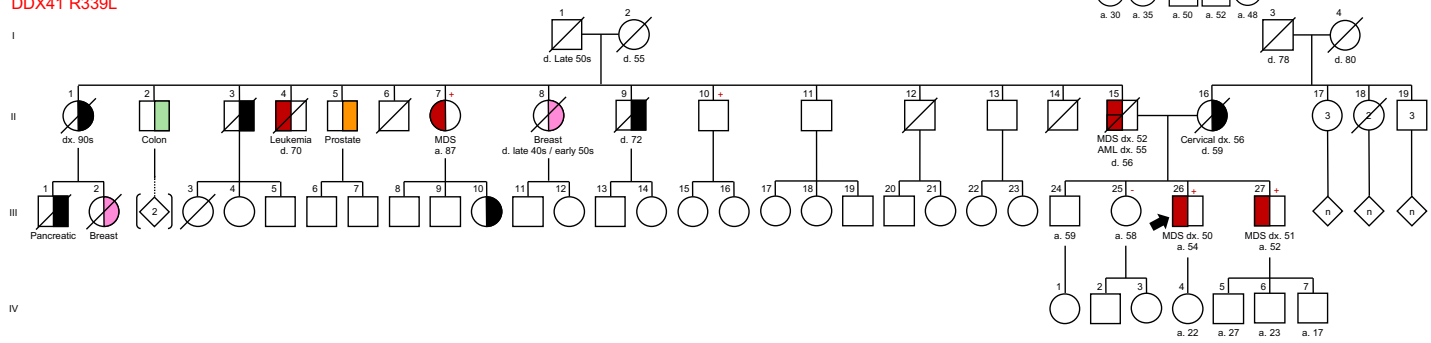
DDX41 R339L



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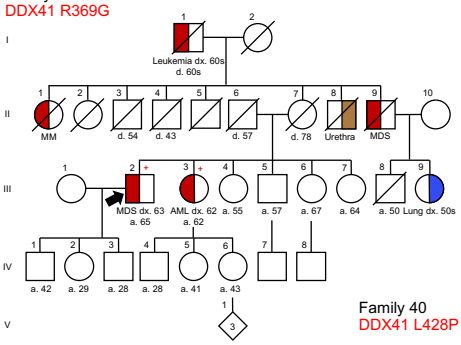
DDX41 R369G



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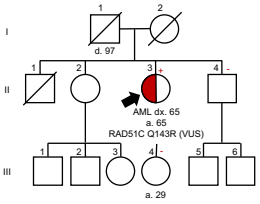
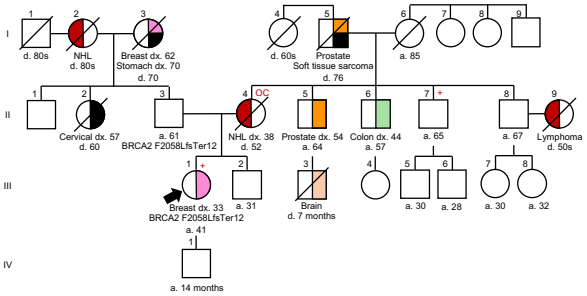
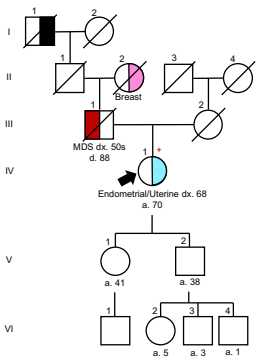
DDX41 L373P



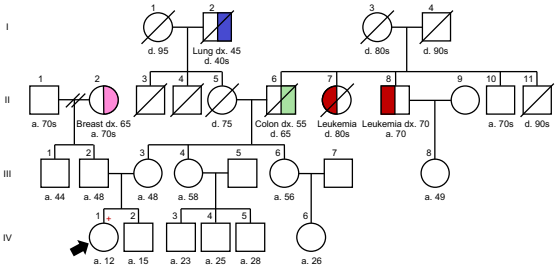
Family 38
DDX41 I396T
BRCA2 F2058LfsTer12



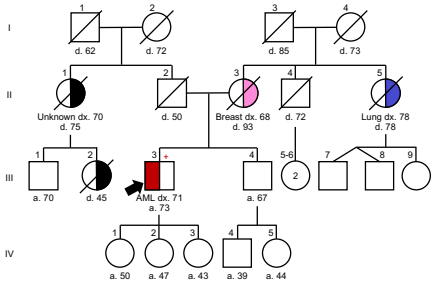
Family 39
DDX41 I396T



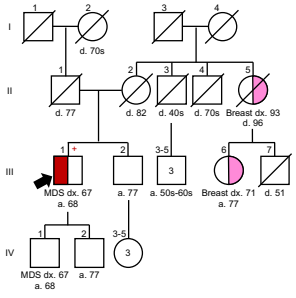
Family 40
DDX41 L428P



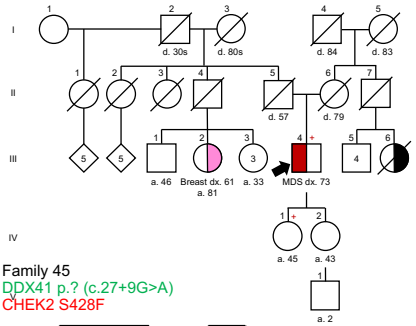
Family 41
DDX41 A492Gfs*17



Family 42
DDX41 L574Rfs*143



Family 43
Deletion exons 12-17



Family 44
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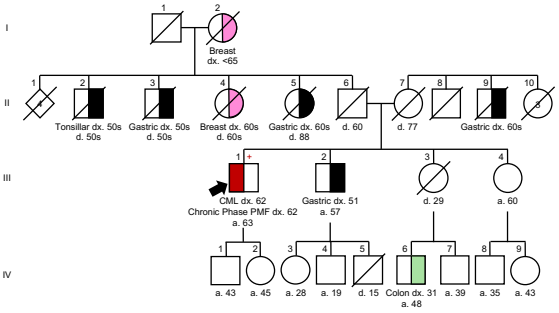
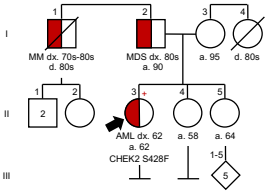
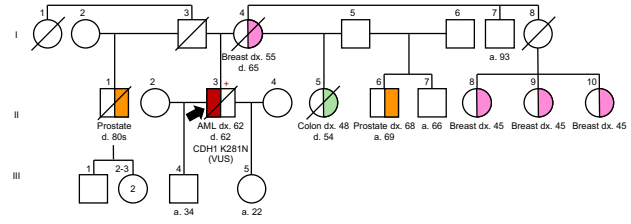


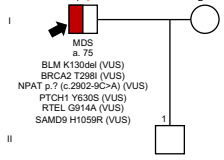
Family 45
DDX41 p.? (c.27+9G>A)
CHEK2 S428F



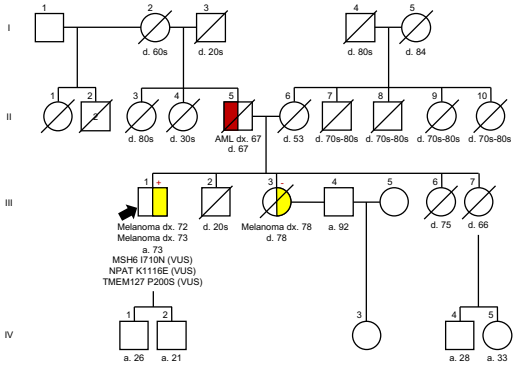
Family 46
DDX41 p.? (c.138+5G>A)



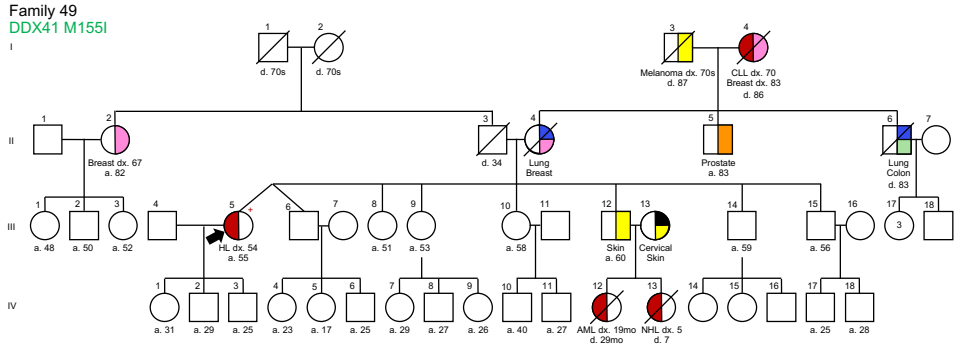
Family 47
DDX41 R101C



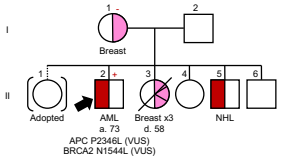
Family 48
DDX41 M155I

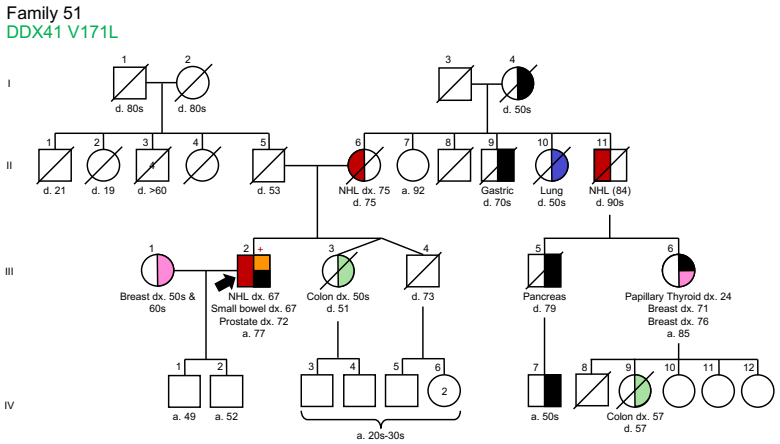


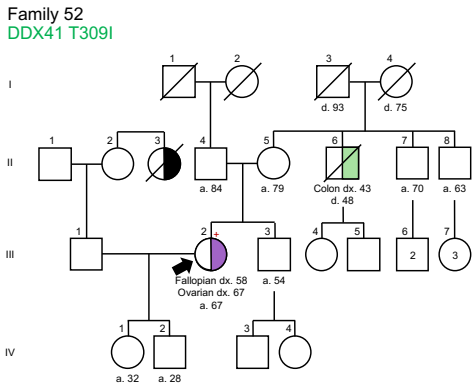
Family 49
DDX41 M155I



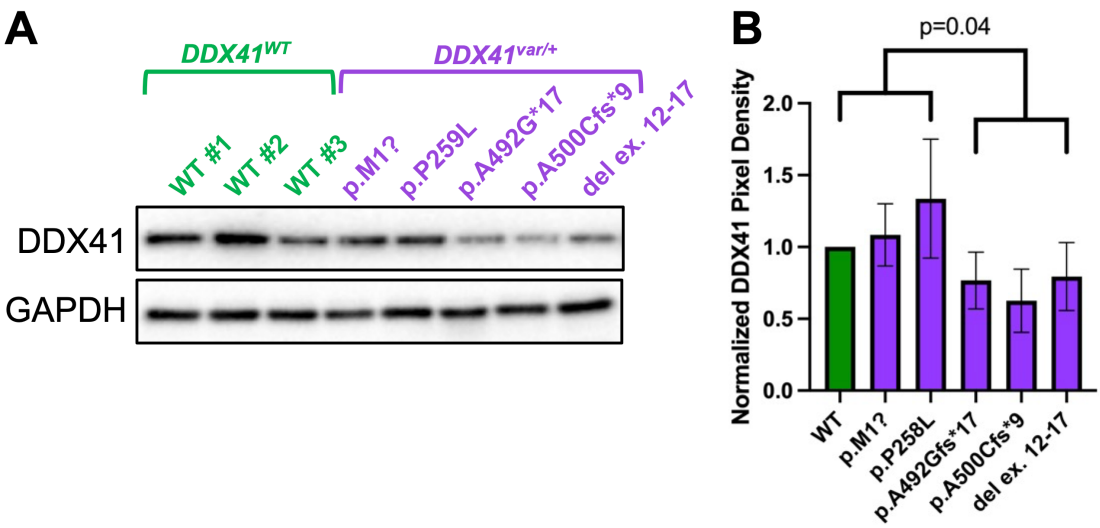
Family 50
DDX41 V171L



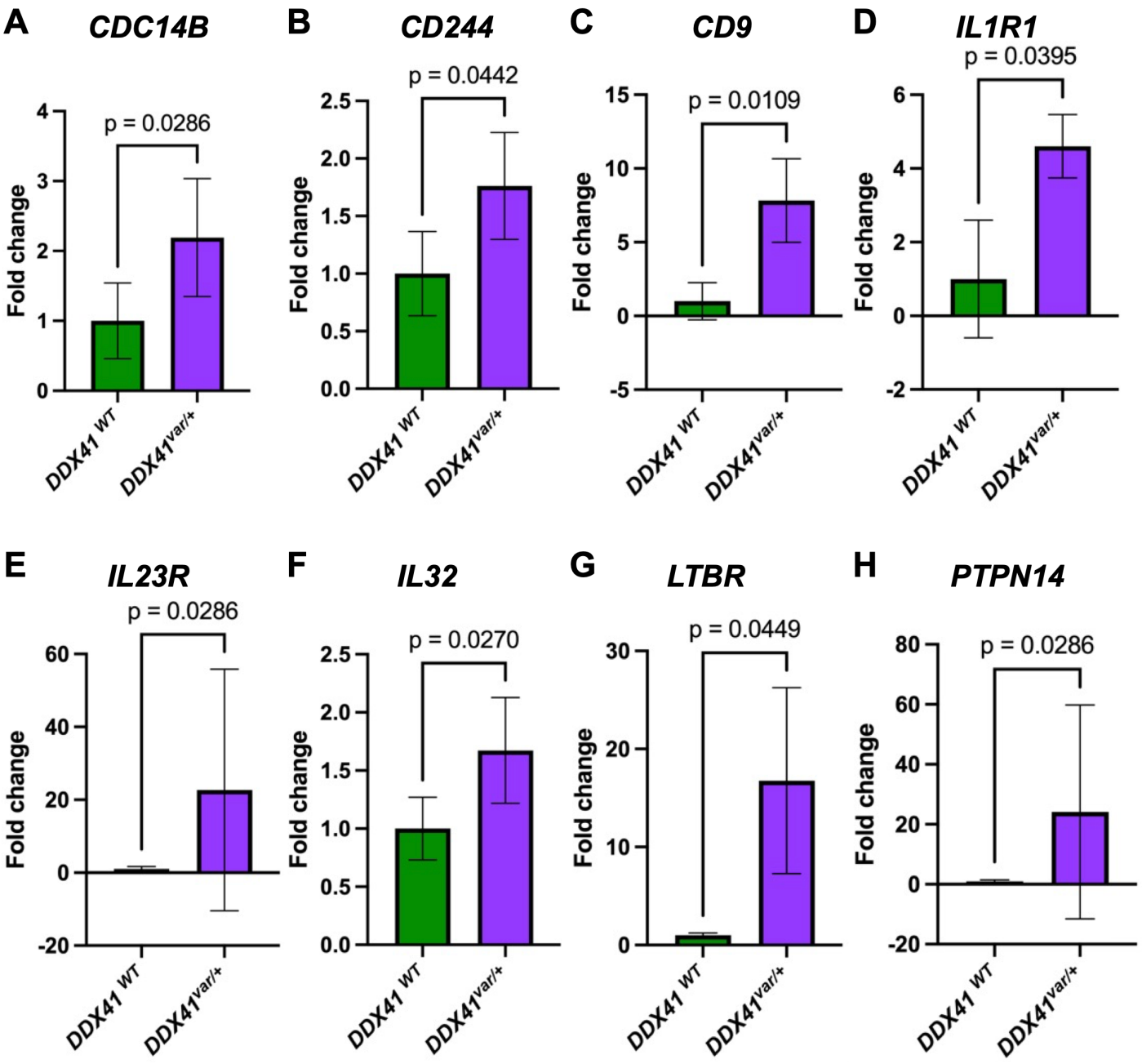




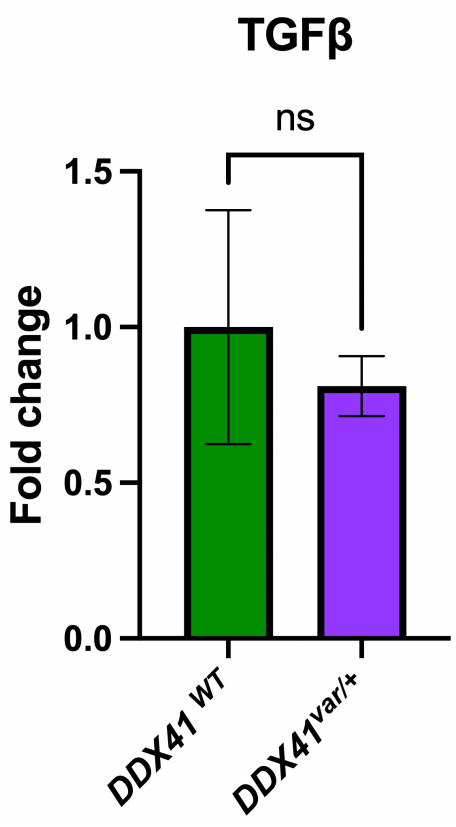
Supplementary Figure 4. Family pedigrees representing our comprehensive cohort of families with germline *DDX41*^{LoF} alleles. Squares represent males, circles represent females, and diamonds indicate that the sex is unknown. All family members that we have knowledge of are shown, regardless of genotype or presence of disease. A "+" sign indicates an individual who has tested positive for the familial DDX41 variant, whereas a "-" sign indicates an individual who has tested negative for the familial DDX41 variant. "OC" indicates that someone is an obligate carrier of the familial variant. "a." indicates the individual's age, and "d." and a strikethrough indicates that the individual is deceased, with the age at time of death indicated. Dark red denotes individuals with HM(s). Solid tumors such as breast (pink), prostate (orange), melanoma (yellow), colon (light green), liver (dark green), endometrial (light blue), lung (dark blue), ovarian (purple), renal (brown), and neuroendocrine (peach) are shown. Age of diagnosis is given after "dx." if it is known.



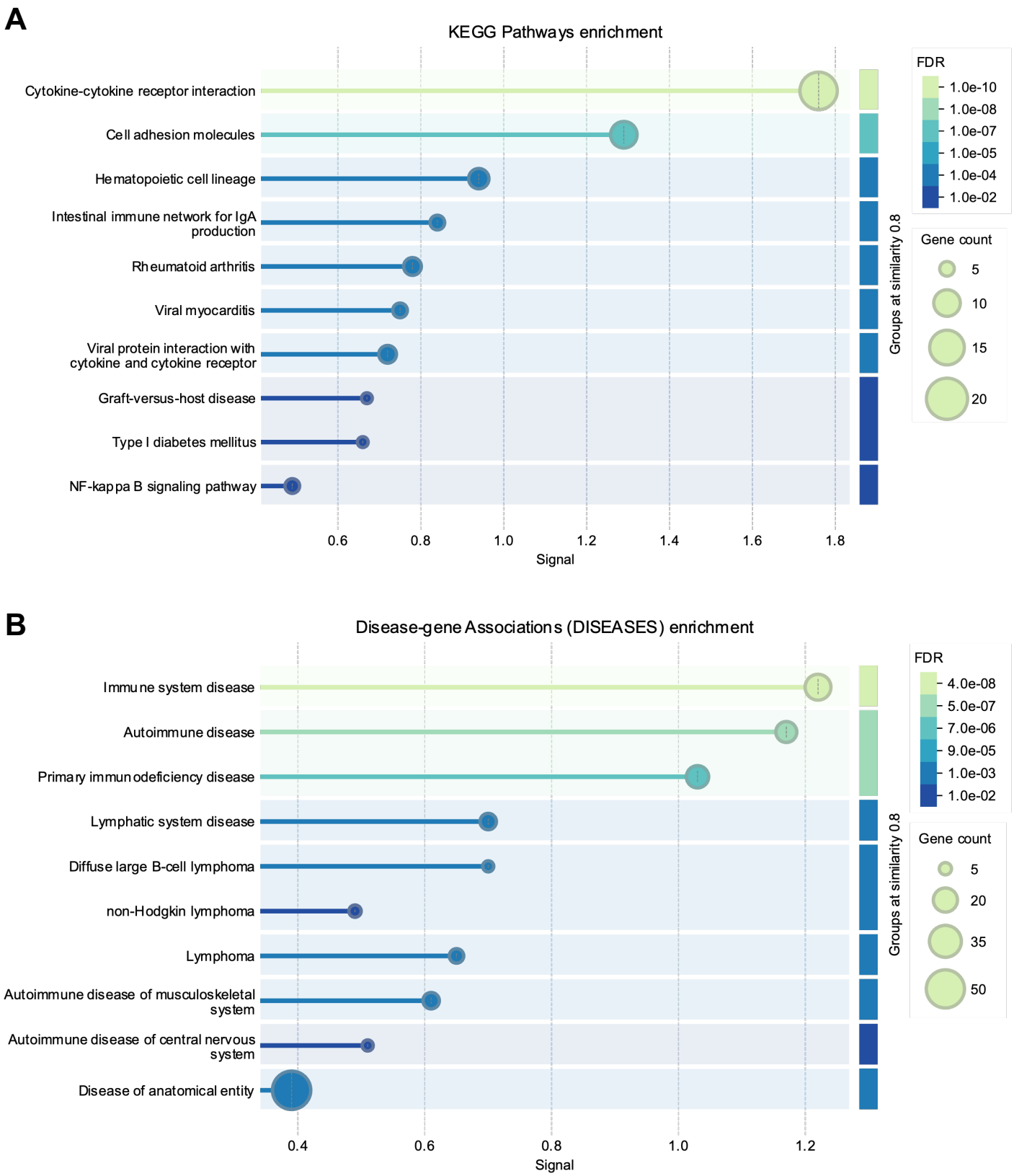
Supplementary Figure 5. DDX41 protein levels in patient-derived LCLs with different *DDX41* alleles. (A) Western blots for total DDX41 in whole cells lysates from *DDX41*^{WT} (n=3, green) and *DDX41*$^{var/+}$ (n=5, purple) patient-derived LCLs. GAPDH was used as a loading control. **(B)** Bar plot of average normalized DDX41 pixel densities in *DDX41*^{WT} (n=3, green) and *DDX41*$^{var/+}$ (n=5, purple) patient-derived LCLs. DDX41 levels were lower in LCLs with frameshift deletions (A492Gfs*17, A500Cfs*9, and del ex. 12-17) in *DDX41* than in those with other mutations (M1?, and P258L) or those with wild type *DDX41* alleles (p=0.04).



Supplementary Figure 6. RT-qPCR results to validate RNA-sequencing findings. (A-H) Fold changes in gene expression are shown in *DDX41*^{WT} (green) and *DDX41*$^{var/+}$ (purple) patient-derived LCLs. P-values were determined using two-tailed t-tests with Welch's correction and confirm increased expression of **(A)** *CDC14B*, **(B)** *CD244*, **(C)** *CD9*, **(D)** *IL1R1*, **(E)** *IL23R*, **(F)** *IL32*, **(G)** *LTBR*, and **(H)** *PTPN14* in *DDX41*$^{var/+}$ LCLs.



Supplementary Figure 7. Quantification of TGF-β by ELISA. (A) Average concentrations of TGF-β (pg/mL) in conditioned media from *DDX41*^{WT} LCLs (n=3, green) and *DDX41*$^{var/+}$ LCLs (n=5, purple). No significant change in TGF-β levels was detected as determined by a two-tailed t-test with Welch's correction (p=0.38).



Supplementary Figure 8. Associated pathways and diseases of proteins found to decrease in individuals with likely germline *DDX41*^{LoF} variants. (A) Results of KEGG pathway enrichment analysis and **(B)** disease-gene association analysis (based on the DISEASES database) of 114 proteins found to decrease in the context of likely germline *DDX41*^{LoF} alleles compared to WT controls. **(A-B)** Plots were generated using STRING (https://version11.string-db.org/).

Supplementary References

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