Supplemental Material

Prevalence and clinical expression of germline predisposition to myeloid neoplasms in adults with marrow hypocellularity

Supplemental Methods

Sample collection and cell separation

Peripheral blood granulocytes and T-lymphocytes were isolated by standard density gradient centrifugation, followed by red blood cell lysis with hypotonic solution and immunomagnetic selection on MiniMACS separation columns using anti-CD15 and anti-CD3 antibody (Miltenyi Biotec, Bergisch Gladbach, Germany), as previously reported.[1,2] Genomic DNA was extracted by following standard protocols for human tissue. Buccal epithelial cells were isolated from mouthwash samples, and genomic DNA was purified by following standard protocols for human tissue.

Germline mutation analysis

Genomic DNA was analyzed for germline mutations through next generation sequencing, using a capture-based approach. DNA Prep with Enrichment technology (Illumina, San Diego, CA, USA) was used for library preparation and target enrichment, according to the manufacturer's protocol. A broad range of input DNA (50-500 ng) was tagmented by using bead-linked transposomes (eBLT), and obtained DNA fragment libraries were PCR amplified incorporating two unique, library

specific, pre-paired 10 bp indexes. A pre-enrichment pooling of 12 libraries was performed before to the following steps. A custom panel of 6120 biotinylated probes (80 bp each) was used to capture the 552 genomic regions of interest, belonged to 60 genes reported in peer-reviewed literature as consistently mutated in congenital disorders predisposing to myeloid neoplasm. On the basis of the mutational distribution, the full gene or specific exonic and/or intronic regions were selected for sequencing, for a cumulative target region size of 540 kb. Streptavidin magnetic beads (SMB) were used to capture biotinylated probes, enriching the fragment libraries within the regions of interest. Enriched libraries were PCR amplified, purified with AMPure XP beads (Beckman Coulter, California, USA), and normalized to the same concentration using a fluorescence-based quantification procedure (Qubit dsDNA BR assay kit; Thermo Fisher Scientific, Massachusetts, USA). The average fragment size of around 350 bp was evaluated using a Fragment Analyzer System and the NGS Fragment Kit (Agilent Technologies, California, USA). Pooled libraries were 2x150 paired-end sequenced on a HiSeq2500 sequencer (Illumina, San Diego, CA, USA). Average depth of coverage across the targeted regions was around 500X.

Data analysis was performed using a bioinformatics workflow that starts from standard FASTQ files generated after demultiplexing and performs reads mapping, realignment, variant calling and variant filtering. Sequence reads were initially aligned to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler aligner.[3] The Genome Analysis Toolkit

(www.broadinstitute.org/gatk/) was later used to cleanup reads and make alignment data more reliable for the variant calling (GATK data cleanup best practice): SNVs and small INDELs were identified using HaplotypeCaller.[4] Manual revision of the BAM files was performed on the *SBDS* gene and its pseudogene *SBDSP1*. Data were delivered in form of Variant Call Format (VCF) file, after filtering variants with coverage <20X and less than 5 supporting reads. The resulting variants were analyzed using the Expert Variant Interpreter (eVai), a web-tool developed by enGenome to interpret genomic variants generated by NGS experiments, according to the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP) guidelines and Clinical Genome Resource (ClinGen) disease-associated gene specifications.[5,6] The software initially enriches all variants reported in a VCF files with information obtained from more than 20 omics resources at variant, gene, protein domain and disease level (e.g. public databases as dbSNP, gnomAD, ClinVar, MedGen or functional prediction tools for coding, splicing and noncoding variants as PaPI, SIFT, PolyPhen-2, dbscSNV and DANN). For each variant, eVai combines the annotation information and supporting evidences, and automatically apply ACMG/AMP criteria to pre-classify variants according to their pathogenicity (pathogenic, likely pathogenic, variant of unknown significance, likely benign and benign). All variants were then manually curated before final classification according to the ACMG/AMP guidelines. Manual curation included: review of the literature to identify different pathogenic/likely pathogenic missense change(s) at the same amino acid residue as the variant detected; literature review to

identify functional assays/studies supporting a damaging effect of the variant on protein function; search of protein, conserved domains and functional site databases (UniProt, NCBI-Conserved Domains, PhosphoSitePlus), as well as protein 3D structure database (RCSB PDB), to investigate variant localization in hot spot regions, functional sites/domains, or protein active site; implementation of eight functional prediction tools for coding, splicing and noncoding variants (SIFT, PolyPhen-2, MutationTaster, LRT, Provean, DANN, PaPI, dbscSNV); correlation with hematologic and extra-hematologic patient's phenotype and family history.

Somatic mutation analysis

Genomic DNA was analyzed for somatic mutations through next-generation sequencing, using an amplicon-based approach. TruSight Myeloid Sequencing Panel (Illumina, San Diego, CA, USA) was used for library preparation, according to the manufacturer's protocol. The probe set targeted 15 full genes and 39 hot spot mutation regions across 568 amplicons of 250 bp in length designed against the human GRCh37/hg19 reference genome, for a total genomic content of 141 kb. The probe pool was hybridized to 250 ng of genomic DNA upstream and downstream of each region of interest. An extension-ligation reaction extended across the selected region followed by a ligation step. The resulting templates were amplified by PCR and two unique library specific indexes were incorporated. The resulting libraries were normalized to the same

concentration using a fluorescence-based quantification procedure (Qubit dsDNA HS assay kit; Thermo Fisher Scientific, Massachusetts, USA), enabling pooling of libraries. Pooled DNA libraries were loaded onto the cBot System for cluster generation followed by 2x250 paired-end sequencing on a HiSeq2500 sequencer (Illumina, San Diego, CA, USA).

Data analysis was performed using a bioinformatics workflow that starts from standard FASTQ files generated after demultiplexing and performs reads mapping, realignment, variant calling and variant filtering. Sequence reads were initially aligned to the human reference genome (GRCh37/hg19) using the Burrows-Wheeler aligner.[3] The Genome Analysis Toolkit (www.broadinstitute.org/gatk/) was then used to cleanup reads and make alignment data more reliable for the variant calling (GATK data cleanup best practice): SNVs and small INDELs were identified using Mutect2 and Scalpel, respectively.[7,8] Data were delivered as a VCF file, after filtering variants with coverage <30X and less than 10 supporting reads. Functionally annotated variants were then filtered based on the information retrieved from public databases of polymorphisms, i.e. dbSNP, 1000 Genomes, and ESP6500. The remaining variants were considered as candidate somatic mutations and were finally tagged as oncogenic, based on the information derived from peer-reviewed literature, the Catalog of Somatic Mutations in Cancer (COSMIC; http://cancer.sanger.ac.uk/cancergenome/projects/cosmic), and *in silico* variant effect predictors

i.e. SIFT, PolyPhen-2, Provean, Mutation Taster, as well as the inclusion of the mutated aminoacid in a conserved/functional protein domain.

Statistical analysis

Numerical variables were summarized by median and range; categorical variable were described with count and relative frequency (%) of subjects in each category. Comparison of numerical variables between groups was carried out using a nonparametric approach (Mann-Whitney test or Kruskall Wallis ANOVA). Comparison of the distribution of categorical variables in different groups was performed with either the Fisher exact test or the $\chi 2$ test. Uni- and multivariable regression analyses and ordered logistic regression were carried out to evaluate the mutually adjusted associations of genetic and clinical variables.

Survival analyses were performed with the Kaplan-Meier method. Multivariate survival analyses were performed by means of Cox proportional hazards regression. The cumulative incidence (CI) of disease progression was estimated with a competing risk approach, considering death for any cause as a competing event. The comparison of CI curves was carried out using the Pepe-Mori test, whereas the effect of quantitative covariates was estimated by applying the Fine-Gray regression model.[9,10]

The association between gene mutations and diagnosis was described in terms of sensitivity, specificity, positive predictive value and negative predictive value.

Statistical analyses were performed using Stata SE 16.1 (StataCorp LP, College Station, TX, http://www.stata.com) and R 3.6.2 (https://www.r-project.org) software.

Results

Clinical phenotype of patients with genotype consistent with congenital syndrome or disorder

Fanconi anemia

Fanconi Anemia (FA) genes (*FANCA*, *FANCG*) were mutated in four of 27 patients with causative genotype (15%). Three patients received a diagnosis of MDS, and one had a provisional diagnosis of ICUS. The median age at the time of diagnosis was 43 years. Three patients showed physical abnormalities attributable to FA, and two had diverse solid tumors (head and neck squamous cell carcinoma and gynecologic cancers). One patients had a family history of hematologic disorders and solid tumors. The majority of these patients (3 of 4) showed cytogenetic abnormalities including del(17p) and complex karyotype. One patient carried somatic mutations in *ASXL1*, *EZH2*, *STAG2* and *TET2*. In addition, two patients harboured a single heterozygous mutation in *FANCD1*(*BRCA2*) which was suggested as a predisposing factor to hematological malignancies.[11,12] These patients received a provisional diagnosis of ICUS and one of them showed a hypopituitarism.

GATA2-deficiency syndrome

Germline *GATA2* mutations were observed in three of 27 patients with causative and likely causative genotype (11%). Two of them received a diagnosis of myeloid neoplasm (MDS and AML), while one received a diagnosis of ICUS. The median age at diagnosis was 24 and two patients had a family history of hematologic or solid tumor. Acquired oncogenic alterations in *NPM1* and *STAG2* were observed in two patients.

RASopathy syndromes

RASopathy genes (*NF1*, *PTPN11*) were mutated in two patients of 27 patients with causative genotype (7.4%). One patient showed a clinical phenotype within the spectrum of manifestations associated with Noonan syndrome (short stature, amenorrhea, idiopathic liver fibrosis), in the absence of a related family history. The patient received a provisional diagnosis of ICUS, consistent with the absence of concurrent somatic mutations. The second patient showed neurological manifestations, history of cardiac malformation and gastrointestinal stromal tumor (GIST) consistent with a diagnosis of neurofibromatosis-1. The hematological phenotype was characterized by of absolute and relative monocytosis to configure CMML-0 diagnosis (bone marrow blasts <5%), associated with loos of Y chromosome (60% of metaphases),[14] and somatic mutations in *RAF1* and *TET2*.

Severe congenital neutropenia

Germline mutations in Severe Congenital Neutropenia (SCN) genes (*ELANE*, *CSF3R*) occurred in two of 27 patients with causative and likely causative genotype (7.4%). One patient received a diagnosis of MDS, and one patient of ICUS. None of them showed a relevant extra-hematological phenotype, while one had a family history for hematologic disorders. In addition, one patient was heterozygous mutated for *CSF3R,* which was recently suggested as a predisposing factor to hematological malignancies.[13] This patient received a diagnosed of MDS/MPN with somatic gene mutations in *JAK2*, *U2AF1* and *ZRSR2,* and had a prostate cancer.

Diamond-Blackfan anemia

Germline mutations in Diamond-Blackfan Anemia (DBA) genes (*GATA1*, *RPS26*) were detected in two of 27 patients with causative genotype (7.4%). Both patients were provisionally classified as ICUS without somatic gene mutations. Interestingly, PV2292 had a sister previously diagnosed with primary myelofibrosis, who was then found heterozygous carrier of the same *GATA1* c.-19-2A>G variant detected in her brother.

RUNX1-related familial platelet disorder

A germline frameshift mutation in *RUNX1* was detected in a patient consistent with a diagnosis of *RUNX1*-related familial platelet disorder (FPD). The patient was referred for absolute monocytosis, and rapidly progressed to acute leukemia. The analysis of the pedigree then revealed the presence of the same mutation in one sibling, who received a diagnosis of chronic myelomonocytic leukemia (CMML-1, WHO 2017), subsequently progressing to AML.

Shwachman-Diamond syndrome

A compound heterozygosity was detected in a patient for the two most common mutations in Shwachman-Diamond syndrome-associated gene *SBDS*, i.e. p.(K62*) and c.258+2T>C. This proband showed a moderate-severe neutropenia associated with short stature, without hematologic or genetic sign of clonal evolution. Interestingly, the proband's brother was found to have a mild isolated neutropenia and to carry both *SBDS* p.(K62*) and c.258+2T>C variants.

SAMD9/*SAMD9L*-associated predisposition

SAMD9 and *SAMD9L* mutations have been described in MIRAGE syndrome and Ataxia Pancytopenia syndrome.[15-17] Recently, Nagata *et al*. reported a cohort of adult MDS/BMF carried well characterized LOF *SAMD9*/*SAMD9L* germline mutations, associated with an increased cell proliferation, not subject to somatic reversion and accompanied by additional somatic second

hits resulting in a late onset of adult myeloid neoplasm.[18] Differently to infant GOF variants which are predominantly localized at *SAMD9/SAMD9L* central and C-terminal regions, most adult LOF mutations clustered at N-terminal, suggesting a different mechanism of action (Supplemental Figure 8).

We identified eight patients (2%) harboring germline VUS in *SAMD9* or *SAMD9L* (Supplemental Table 4). Almost all the detected *SAMD9/SAMD9L* germline variants localized at N-terminal and central region of both genes (Supplemental Figure 8).[18] *SAMD9* p.(Q862E) and p.(E866Q) were observed in *cis* in a patient, in combination with somatic *RUNX1* p.(R207Q) and del(7q), while *SAMD9L* p.(T832fs) was identified in two cases.

Three patients carrying *SAMD9/SAMD9L* germline variants showed markedly reduced age-adjusted BM cellularity, with mild or absent dysplasia; in two cases, these findings were suggestive of AA, while in one the concurrent detection of del(7q) led to a diagnosis of hypoplastic MDS. One patient received a diagnosis of MDS with excess blasts associated with somatic mutations of *ASXL1*, *DNMT3A* and *IDH2*, one patient was diagnosed with MDS/MPN with somatic mutation of *JAK2*, while one patient was diagnosed with AML with somatic *ASXL1*, *NRAS* and *WT1* mutations. Finally, two patients received a provisional diagnosis of ICUS.

DHX34-associated predisposition

DHX34 has been recently reported as a novel locus involved in inherited forms of myeloid neoplasm. So far, four heterozygous LOF mutations have been reported in patients with hypoplastic MDS/AML and monosomy 7 with an autosomal dominant inheritance pattern.[19] Germline VUS in *DHX34* were detected in 13 patients (3.2%) (Supplemental Figure 9; Supplemental Table 4). Five of them received a diagnosis of myeloid neoplasm, while the remaining was diagnosed with ICUS, CCUS or AA. In two cases a concomitant renal disease possibly related to germline mutations of *DHX34* was documented. In our cohort we identified 11 new missense variants and the two new truncating variants *DHX34* p.(E627*) and p.(H1086fs).

Supplemental Table 1. Core panel of genes sequenced for germline variants in the study cohort.

Gene	Regions*	Syndrome / Disorder	NCBI ID	Locus
ANKRD26	5'utr	*ANKRD26*-mutated thrombocytopenia	22852	10p12.1
CBL	exons 8,9	RASopathy, Noonan syndrome (NS)-like disorder	867	11q23.3
CEBPA	all exon	*CEBPA*-mutated AML	1050	19q13.11
CSF3R	all exons	severe congenital neutropenia (SCN)	1441	1p34.3
CTC1	all exons	telomere biology disorder (TBD)	80169	17p13.1
DDX41	all exons	*DDX41*-associated predisposition	51428	5q35.3
DHX34	all exons	*DHX34*-associated predisposition	9704	19q13.32
DKC1	all exons	telomere biology disorder (TBD)	1736	Xq28
DNAJC21	all exons	Shwachman-Diamond syndrome (SDS)	134218	5p13.2
ELANE	all exons, 5'utr	severe congenital neutropenia (SCN)	1991	19p13.3
ERCC6L2	exons 5-19	inherited bone marrow failure syndrome (IBMFS)	375748	9q22.32
ETV6	all exons	*ETV6*-mutated thrombocytopenia	2120	12p13.2
FANCA	all exons	Fanconi anemia (FA)	2175	16q24.3
FANCB	all exons	Fanconi anemia (FA)	2187	Xp22.2
FANCC	all exons	Fanconi anemia (FA)	2176	9q22.32
FANCD1 (BRCA2)	all exons	Fanconi anemia / hereditary breast and ovarian cancer syndrome (FA/HBOC)	675	13q13.1
FANCD2	all exons	Fanconi anemia (FA)	2177	3p25.3
FANCE	all exons	Fanconi anemia (FA)	2178	6p21.31
FANCF	all exon	Fanconi anemia (FA)	2188	11p14.3
FANCG	all exons	Fanconi anemia (FA)	2189	9p13.3
FANCI	all exons	Fanconi anemia (FA)	55215	15q26.1
FANCJ (BRIP1)	all exons	Fanconi anemia (FA)	83990	17q23.2
G6PC3	all exons	severe congenital neutropenia (SCN)	92579	17q21.31
GATA1	all exons, 5'utr	Diamond-Blackfan anemia (DBA)	2623	Xp11.23
GATA2	all exons, intron 5	*GATA2*-deficiency syndrome	2624	3q21.3
GFI1	all exons	severe congenital neutropenia (SCN)	2672	1p22.1
HAX1	all exons	severe congenital neutropenia (SCN)	10456	1q21.3
JAGN1	all exons	severe congenital neutropenia (SCN)	84522	3p25.3
KRAS	all exons	RASopathy, Noonan syndrome (NS)	3845	12p12.1
MECOM	exons 5-17	radioulnar synostosis with amegakaryocytic thrombocytopenia (RUSAT)	2122	3q26.2

MPL	all exons	congenital amegakaryocytic thrombocytopenia (CAMT)	4352	1p34.2
NF1	all exons	RASopathy, neurofibromatosis (NF)	4763	17q11.2
NHP2	exon 4	telomere biology disorder (TBD)	55651	5q35.3
NOP10	exon 2	telomere biology disorder (TBD)	55505	15q14
NRAS	all exons	RASopathy, Noonan syndrome (NS)	4893	1p13.2
PARN	all exons	telomere biology disorder (TBD)	5073	16p13.12
PTPN11	all exons	RASopathy, Noonan syndrome (NS)	5781	12q24.13
RAF1	exons 5-17	RASopathy, Noonan syndrome (NS)	5894	3p25.2
RBM8A	all exons, 5'utr, intron 1	thrombocytopenia-absent radius (TAR) syndrome	9939	1q21.1
RIT1	all exons	RASopathy, Noonan syndrome (NS)	6016	1q22
RPL5	all exons	Diamond-Blackfan anemia (DBA)	6125	1p22.1
RPL11	all exons	Diamond-Blackfan anemia (DBA)	6135	1p36.11
RPL35A	exons 3,4	Diamond-Blackfan anemia (DBA)	6165	3q29
RPS10	all exons	Diamond-Blackfan anemia (DBA)	6204	6p21.31
RPS17	all exons	Diamond-Blackfan anemia (DBA)	6218	15q25.2
RPS19	all exons, 5'utr	Diamond-Blackfan anemia (DBA)	6223	19q13.2
RPS24	all exons	Diamond-Blackfan anemia (DBA)	6229	10q22.3
RPS26	all exons	Diamond-Blackfan anemia (DBA)	6231	12q13.2
RTEL1	all exons	telomere biology disorder (TBD)	51750	20q13.33
RUNX1	all exons	*RUNX1*-related familial platelet disorder (FPD)	861	21q22.12
SAMD9	exon 3	*SAMD9/9L*-associated predisposition	54809	7q21.2
SAMD9L	exon 5	*SAMD9/9L*-associated predisposition	219285	7q21.2
SBDS	all exons	Shwachman-Diamond syndrome (SDS)	51119	7q11.21
SOS1	all exons	RASopathy, Noonan syndrome (NS)	6654	2p22.1
SRP72	exons 1,6,10	inherited bone marrow failure syndrome (IBMFS)	6731	4q12
TERC	all exon	telomere biology disorder (TBD)	7012	3q26.2
TERT	all exons	telomere biology disorder (TBD)	7015	5p15.33
TINF2	exon 6	telomere biology disorder (TBD)	26277	14q12
TP53	all exons	Li-Fraumeni syndrome (LFS)	7157	17p13.1
WAS	exons 7-12	severe congenital neutropenia (SCN)	7454	Xp11.23

*All regions reported included extra 5′ and 3′ flanking regions of 12 nucleotides

Supplemental Table 2. Core panel of genes sequenced for somatic mutations in the study cohort.

Gene	Pathway	NCBI ID	Position
ABL1	signal transduction	25	9q34.12
ASXL1	histones/chromatin modification	171023	20q11.21
ATRX	histones/chromatin modification	546	Xq21.1
BCOR	histones/chromatin modification	54880	Xp11.4
BCORL1	histones/chromatin modification	63035	Xq26.1
BRAF	signal transduction	673	7q34
CALR	signal transduction	811	19p13.13
CBL	signal transduction	867	11q23.3
CBLB	signal transduction	868	3q13.11
CBLC	signal transduction	23624	19q13.32
CDKN2A	tumor suppression	1029	9p21.3
CEBPA	transcription regulation	1050	19q13.11
CSF3R	signal transduction	1441	1p34.3
CUX1	transcription regulation	1523	7q22.1
DNMT3A	DNA methylation	1788	2p23.3
ETV6	transcription regulation	2120	12p13.2
EZH2	histones/chromatin modification	2146	7q36.1
FBXW7	signal transduction	55294	4q31.3
FLT3	signal transduction	2322	13q12.2
GATA1	transcription regulation	2623	Xp11.23
GATA2	transcription regulation	2624	3q21.3
GNAS	signal transduction	2778	20q13.32
HRAS	signal transduction	3265	11p15.5
IDH1	DNA methylation	3417	2q34
IDH2	DNA methylation	3418	15q26.1
IKZF1	transcription regulation	10320	7p12.2
JAK2	signal transduction	3717	9p24.1
JAK3	signal transduction	3718	19p13.11
KDM6A	histones/chromatin modification	7403	Xp11.3
KIT	signal transduction	3815	4q12
KMT2A	histones/chromatin modification	4297	11q23.3
KRAS	signal transduction	3845	12p12.1
MPL	signal transduction	4352	1p34.2
MYD88	signal transduction	4615	3p22.2
NOTCH1	signal transduction	4851	9q34.3
NPM1	transcription regulation	4869	5q35.1
NRAS	signal transduction	4893	1p13.2
PDGFRA	signal transduction	5156	4q12
PHF6	transcription regulation	84295	Xq26.2

PTEN	signal transduction	5728	10q23.31
PTPN11	signal transduction	5781	12q24.13
RAD21	cohesin complex	5885	8q24.11
RUNX1	transcription regulation	861	21q22.12
SETBP1	histones/chromatin modification	26040	18q12.3
SF3B1	RNA splicing	23451	2q33.1
SMC1A	cohesin complex	8243	Xp11.22
SMC3	cohesin complex	9126	10q25.2
SRSF2	RNA splicing	6427	17q25.1
STAG2	cohesin complex	10735	Xq25
TET2	DNA methylation	54790	4q24
TP53	tumor suppression	7157	17p13.1
U2AF1	RNA splicing	7307	21q22.3
WT1	tumor suppression	7490	11p13
ZRSR2	RNA splicing	8233	Xp22.2

Supplemental Table 3. List of pathogenic/likely pathogenic (P/LP) germline mutations detected in the study cohort.

		GERMLINE										SOMATIC				Family history	
Patient ID	**Age/ Sex**	**Gene**	**VAF** Myeloid	**VAF** Germline	**Mutation**	**Zigosity**	**Inheritance**	**Syndrome / Disorder**	**ACMG/AMP classification (criteria)**	**ClinVar classification**	**PMID reference**	**Gene**	**VAF**	**Mutation**	**Extra-hematologic phenotype**	**Cancer Hem/Solid**	**Other**
PV1410	70/M	***CSF3R***	0.43	0.46	c.437_438delCA; p.(P146fs)	Het	AR	SCN	LP (PVS1, PM2)	-	This paper	***JAK2*** ***U2AF1*** ***ZRSR2***	0.10 0.12 0.15	c.1849G>T; p.(V617F) c.470A>G; p.(Q157R) c.515G>T; p.(C172F)	Prostate cancer	-/-	-
PV1185	51/F	***CSF3R*** ***CSF3R***	0.40 0.40	0.48 0.45	c.1474+1G>C; p.? c.1698G>A; p.(W566*)	Het Het	AR	SCN	LP (PVS1, PM2) LP (PVS1, PM2)	-	This paper	WT			Connective tissue disease treated with methotrexate	-/-	-
PV1395	82/M	***DDX41***	0.46	0.48	c.3G>A; p.(M1?)†	Het	AD	*DDX41*-associated predisposition	P (PVS1, PM3, PP5)	P/LP	30963592 31484648 35671390 36322930 36455200	***DDX41***	0.11	c.962C>T; p.(P321L)	Hyperthyroidism treated with radio-metabolic therapy	-/-	-
PV1583	56/F	***DDX41***	0.48	0.50	c.3G>A; p.(M1?)†	Het	AD	*DDX41*-associated predisposition	P (PVS1, PM3, PP5)	P/LP	30963592 31484648 35671390 36322930 36455200	***SF3B1***	0.02	c.1876A>G; p.(N626D)	-	-/-	-
PV1336	59/M	***DDX41***	0.48	0.48	c.142C>T; p.(Q48*)†	Het	AD	*DDX41*-associated predisposition	P (PVS1, PM2, PP5)	P/LP	30963592 36322930 36455200	WT			-	-/-	-
PV1475	66/M	***DDX41***	0.45	0.48	c.305_306delAA; p.(K102fs)†	Het	AD	*DDX41*-associated predisposition	P (PVS1, PM2, PP5)	P/LP	36322930 36455200	WT			-	-/-	-
PV10015	62/M	***DDX41***	0.48	0.45	c.305_306delAA; p.(K102fs)†	Het	AD	*DDX41*-associated predisposition	P (PVS1, PM2, PP5)	P/LP	36322930 36455200	WT			Colon cancer treated with chemotherapy	-/+	-
PV1178	57/M	***DDX41***	0.49	0.40	c.620T>C; p.(I207T)†	Het	AD	*DDX41*-associated predisposition	LP (PM1, PM2, PM3, PP3)	-	36322930 36455200	***DDX41***	0.01	c.1574G>A; p.(R525H)	-	-/-	-
PV1581	64/M	***DDX41***	0.46	0.48	c.620T>C; p.(I207T)†	Het	AD	*DDX41*-associated predisposition	LP (PM1, PM2, PM3, PP3)	-	36322930 36455200	WT			Esophageal cancer	-/-	-
PV693	49/M	***DDX41***	0.49	0.50	c.653G>A; p.(G218D)†	Het	AD	*DDX41*-associated predisposition	LP (PM1, PM2, PP3, PP5)	LP/VUS	33585199 35671390 36322930 36455200	***ASXL1*** ***STAG2***	0.29 0.35	c.4127_4128insG; p.(P1377fs) c.1701_1702insT; p.(A568fs)	-	-/-	-
PV1591	66/M	***DDX41***	0.48	0.49	c.653G>A; p.(G218D)†	Het	AD	*DDX41*-associated predisposition	LP (PM1, PM2, PP3, PP5)	LP/VUS	33585199 35671390 36322930 36455200	WT			Cardiomyopathy	-/-	-
PV2607	65/M	***DDX41***	0.49	0.46	c.773C>T; p.(P258L)†	Het	AD	*DDX41*-associated predisposition	LP (PM1, PM2, PP3, PP5)	LP	30407884 33850299 35671390 36322930 36455200	WT			Chronic gastritis HP+; anti-parietal cell antibodies (APCA) positive	-/-	-
PV583	73/M	***DDX41***	0.46	0.48	c.775T>C; p.(Y259H)	Het	AD	*DDX41*-associated predisposition	LP (PM1, PM2, PM3, PP3, BP4)	-	This paper	***DDX41***	0.11	c.1570G>A; p.(G524R)	-	-/-	-
PV554	60/F	***DDX41***	0.44	0.50	c.799C>T; p.(R267W)†	Het	AD	*DDX41*-associated predisposition	LP (PM1, PM2, PM5, PP3)	-	27721487 36322930 36455200	***ASXL1*** ***ATRX*** ***BCOR***	0.38 0.14 0.12	c.1934_1935insG; p.(G646fs) c.3667G>T; p.(E1223*) c.2514_2515insC; p.(K839fs)	-	-/-	-

Supplemental Table 3. (Continued)

		GERMLINE										SOMATIC				Family history	
Patient ID	**Age/ Sex**	**Gene**	**VAF** Myeloid	**VAF** Germline	**Mutation**	**Zigosity**	**Inheritance**	**Syndrome / Disorder**	**ACMG/AMP classification (criteria)**	**ClinVar classification**	**PMID reference**	**Gene**	**VAF**	**Mutation**	**Extra-hematologic phenotype**	**Cancer Hem/Solid**	**Other**
PV2480	25/M	***DNAJC21***	0.53	0.50	c.1729_*1delTAGA; p.(Ter577fs)	Het	AR	SDS	LP (PVS1 strong, PM2, PM4)	-	This paper	WT			Intellectual disability	-/-	-
PV1546	35/M	***ELANE***	0.50	0.52	c.659G>A; p.(R220Q)	Het	AD	SCN	LP (PM1, PM2, PP2, PP3, PP5, BP4)	P	10581030 23463630 25427142	WT			Mild splenomegaly	+/-	Brother with isolated neutropenia
PV2613	54/F	***FANCA***	0.50	0.50	c.79+1G>C; p.?	Het	AR	FA	P (PVS1, PM2, PP4, PP5)	LP	This paper	WT			Short stature, triangular face, small head café au lait spots, hypertrichosis, learning disabilities; spinocellular cell and genital carcinoma; positive DEB test	+/-	Father with low platelet counts died of intracranial bleeding
		FANCA	0.49	0.44	c.3971C>T; p.(P1324L)	Het			LP (PS3, PP3, PP4, PP5, BS1)	P/LP	12444097 17924555 23973728						
PV2443	37/F	***FANCA***	0.99	0.97	c.1115_1118delTTGG; p.(V372fs)‡	Hom	AR	FA	P (PVS1, PM2, PP5, BS1)	P	17924555 22778927 23613520	WT			Short stature; intrauterine fetal death (VII month); hepatic steatosis	-/-	-
PV1047	45/M	***FANCA***	1.00	0.98	c.3420delC; p.(N1140fs)	Hom	AR	FA	LP (PVS1, PM2)	-	This paper	***ASXL1*** ***EZH2*** ***STAG2*** ***TET2***	0.45 0.94 0.50 0.22	c.1927dupG; p.(G642fs) c.2069G>A; p.(R690H) c.1452_1453insTGGGA; (p.M484fs) c.4761dupT; p.(I1588fs)	-	-/-	-
PV1515	45/F	***FANCD1*** (***BRCA2***)	0.44	0.48	c.7871A>G; p.(Y2624C)	Het	AR/AD	FA/HBOC	LP (PS3, PM1 supporting, PM2, PP3)	LP/VUS	16758124 25682074 29884841	WT			-	-/-	-
PV1949	25/M	***FANCD1*** (***BRCA2***)	0.46	0.52	c.9676delT; p.(Y3226fs)	Het	AR/AD	FA/HBOC	P (PVS1, PM2, PP5)	P/LP	22720145 27225819 31209999	WT			Hypopituitarism	-/-	-
PV30044	50/M	***FANCD2***	0.48	0.45	c.2036delC; p.(P679fs)	Het	AR	FA	LP (PVS1, PM2)	-	This paper	***DNMT3A***	0.10	c.1969delG; p.(V657fs)	-	+/-	-
PV10061	41/F	***FANCG***	1.00	0.98	c.1183_1192delGAGGTGTTTT; (p.E395fs)	Hom	AR	FA	P (PVS1, PM2, PP4, PP5, BS1)	P	11093276 12552564	WT			Short stature intellectual disability, congenital right hearing loss, horseshoe kidney; polyabortivity; squamous cell carcinoma; positive DEB test	+/+	-
PV2292	32/M	***GATA1***	0.97	0.98	c.-19-2A>G; p.?‡	Hem	XLR	DBA	LP (PS3, PM2, PP3)	-	26713410	WT			-	+/-	-
PV2663	29/F	***GATA2***	0.50	0.46	c.988C>T; p.(R330*)	Het	AD	*GATA2*-deficiency syndrome	P (PVS1, PS3, PM2, PP3, PP5)	P	23223431 24227816 25239263 33417088	WT			Hashimoto's thyroiditis and papillary carcinoma; chronic gastritis; obesity; early menarche	-/+	-
PV2274	23/F	***GATA2***	0.56	0.54	c.1018-1G>A; p.?	Het	AD	*GATA2*-deficiency syndrome	P (PVS1, PM2, PP4, PP5)	LP	21670465 24345756 24227816	***NPM1***	NA	c.860_863dup; p.(W288fs)	Long lasting peripheral blood cytopenia; recurrent respiratory infections; B and NK lymphocytes deficiency	-/-	-
PV1264	24/F	***GATA2***	0.52	0.42	c.1192C>T; p.(R398W)	Het	AD	*GATA2*-deficiency syndrome	P (PS3, PM1, PM2, PM5, PP3, PP5)	P/LP	21670465 23223431 24345756	***STAG2***	0.04	c.3034C>T; p.(R1012*)	-	+/-	-
PV2661	45/M	***NF1***	0.47	0.44	c.3113+2T>A; p.?	Het	AD	NF	P (PVS1, PM2, PP4)	P	This paper	***RAF1*** ***TET2***	0.25 0.22	c.785A>C; p.(N262T) c.2746C>T; p.(Q916*)	Moderate-severe cognitive deficit, aortic stenosis and hypertension, short stature; GIST in Imatinib therapy	-/-	-

Supplemental Table 3. (Continued)

		GERMLINE										SOMATIC				Family history	
Patient ID	Age/ Sex	Gene	VAF Myeloid	VAF Germline	Mutation	Zigosity	Inheritance	Syndrome / Disorder	ACMG/AMP classification (criteria)	ClinVar classification	PMID reference	Gene	VAF	Mutation	Extra-hematologic phenotype	Cancer Hem/Solid	Other
PV2662	44/F	***PTPN11***	0.51	0.51	c.1403C>T; p.(T468M)	Het	AD	NS	LP (PS3, PM5, PP2, PP3, PP4)	P/LP	16358218 21784453 32164556	WT			Short stature, amenorrhea at age 25, hepatic fibrosis, portal hypertension and esophageal varices	-/-	-
PV2664	38/M	***RPS26***	0.51	0.53	c.1A>G; p.(M1?)	Het	AD	DBA	P (PVS1, PS1, PM2, PP5)	P	20116044 28102861 28280134	WT			Steroid diabetes	-/+	-
PV1774	52/F	***RUNX1***	0.50	NA	c.166_196delTTGCCGCTGGGCGCCCCGGACGCCGGCGCTG; p.(L56fs)‡	Het	AD	*RUNX1*-related FPD	LP (PVS1, PM2)	P§	This paper	WT			Essential tremor since age 29; low count platelets since 1985	+/-	Father died of lung fibrosis
PV2117	24/F	***SBDS***	0.48	0.40	c.183_184TA>CT; p.(K62*)‡	Het	AR	SDS	P (PVS1, PS3, PP4, PP5)	P/LP	12496757 28102861 30413969	WT			Short stature, intellectual disability/low IQ; pancreatic insufficiency; knee chondropathy	-/-	Brother with isolated neutropenia
		SBDS	0.54	0.52	c.258+2T>C; p.?‡	Het			P (PVS1, PS3, PP4, PP5)								
PV1174	38/F	***SBDS***	0.40	0.48	c.258+2T>C; p.?	Het	AR	SDS	P (PVS1, PS3, PP5)	P/LP	12496757 28102861 30413969	WT			-	-/-	-
PV2281	70/F	***SBDS***	0.41	0.40	c.258+2T>C; p.?	Het	AR	SDS	P (PVS1, PS3, PP5)	P/LP	12496757 28102861 30413969	***TET2***	0.38	c.2268_2269del; p.(756_757del)	Breast cancer radiotreated; small lymphocytic lymphoma	-/-	-
PV2346	47/M	***SBDS***	0.41	0.41	c.258+2T>C; p.?	Het	AR	SDS	P (PVS1, PS3, PP5)	P/LP	12496757 28102861 30413969	***PHF6*** ***SRSF2***	0.93 0.47	c.820C>T; p.(R274*) c.284C>T; p.(P95L)	-	-/-	-
PV2360	47/M	***SBDS***	0.40	0.40	c.258+2T>C; p.?	Het	AR	SDS	P (PVS1, PS3, PP5)	P/LP	12496757 28102861 30413969	WT			-	-/+	-

F, female; M, male; VAF, variant allele frequency; NA, not available; Het, heterozygous; Hom, homozygous; Hem, hemizygous; AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive; SCN, severe congenital neutropenia; SDS, Shwachman-Diamond syndrome; FA, Fanconi anemia; DBA, Diamond-Blackfan anemia; NF, neurofibromatosis; NS, Noonan syndrome; FPD, familial platelet disorder; P/LP, pathogenic/likely pathogenic; VUS, variant of unknown significance; WT, wild-type; GIST, gastrointestinal stromal tumor; Hem, hematopoietic.

For *DDX41* germline variants classification the pathogenic moderate criterion PM2 was applied to variants with a Genome Aggregation Database (gnomAD) population frequency less than the two most frequent known pathogenic variants p.(M1I) and p.(D140fs) (both with gnomAD frequency of 0.008%).[20] For *DDX41* the pathogenic moderate criterion PM3 was used in a modified manner to account for the known mechanism of *DDX41* somatic second hit in affected individuals. This criterion was applied to the germline variant when a second somatic pathogenic variant was also present in an affected patient in our study.[20,21]

†*DDX41* variants recently reported as pathogenic/likely pathogenic by Makishima *et al.* Blood 2022, and Makishima *et al.* Blood 2023.[22,23]

‡*FANCA* p.(V372fs) variant was detected in a healthy son with VAF 0.43. *GATA1* c.-19-2A>G variant was detected in an affected sibling with VAF 0.50. *RUNX1* p.(L56fs) variant was detected in an affected sibling with VAF 0.54. *SBDS* p.(K62*) and c.258+2T>C variants were both detected in an affected sibling with VAF 0.48 and 0.40, respectively.

§Submitted to ClinVar by our Lab.

Supplemental Table 4. List of germline variants of unknown significance (VUS) detected in the study cohort.

Patient ID	Age/Sex	Gene	Germline Variant	Zigosity	Inheritance	Congenital syndrome/disorder	ACMG/AMP classification (criteria)	ClinVar classification	PMID reference	Diagnosis	Extra-hematologic phenotype	Family history
PV907	62/F	***CBL***	c.1199T>C; p.(M400T)†	Het	AD	NS-like disorder	VUS (PM2, PP2, PP3)	VUS	-	ICUS	-	-
PV1523	67/F	***CEBPA***	c.1018G>A; p.(G340S)	Het	AD	*CEBPA*-mut AML	VUS (PP3, BP1)	VUS	14726504 18768433 29146883	MDS-EB-1	+	-
PV10042	80/F	***CSF3R***	c.355G>A; p.(A119T)	Het	AR	SCN	VUS (PS3, PP3)	VUS	33108454	ICUS	+	-
PV1870	30/M	***CTC1***	c.1177C>T; p.(R393W)	Het	AR	TBD	VUS (PM2, PP3)	VUS	-	AA	-	-
PV2308	33/F	***CTC1***	c.2404G>T; p.(G802C)	Het	AR	TBD	VUS (PM2, PP3)	-	-	ICUS	+	-
PV2058	40/F	***CTC1***	c.2887C>G; p.(P963A)	Het	AR	TBD	VUS (PM2, PP3)	-	-	ICUS	+	+
PV894	69/F	***CTC1***	c.3317C>A; p.(S1106Y)	Het	AR	TBD	VUS (PM2, PP3)	VUS	-	ICUS	-	-
PV1061	40/F	***CTC1***	c.3604C>T; (p.R1202*)	Het	AR	TBD	VUS (PVS1 strong, BP6)	VUS/LB	30891747 34426522	CCUS	+	-
PV927	59/M	***DDX41***	c.644+5G>C; p.?†	Het	AD	*DDX41*-associated predisposition	VUS (PM2, PM3, PP3)	-	35671390	MDS-EB-2	-	-
PV653	84/M	***DDX41***	c.992_994delAGA; p.(K331del)†	Het	AD	*DDX41*-associated predisposition	VUS (PM3, PM4)	-	31484648 35671390	MDS-EB-1	-	-
PV1336	58/M	***DHX34***	c.128T>C; p.(F43S)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PM2, PP3)	-	-	MDS-EB-2	-	-
PV2435	71/F	***DHX34***	c.904C>T; p.(R302W)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3)	-	-	AA	+	+
PV1146	53/F	***DHX34***	c.1202A>G; p.(Y401C)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3)	-	-	MDS-EB-1	-	+
PV1377	54/F	***DHX34***	c.1403C>T; p.(P468L)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3)	VUS	-	ICUS	+	-
PV1871	57/M	***DHX34***	c.1684C>T; p.(R562W)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3)	-	-	AA	-	-
PV1020	70/M	***DHX34***	c.1742C>G; p.(A581G)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3)	VUS	-	MDS-SLD	+	-
PV10035	60/M	***DHX34***	c.1847C>T; p.(S616L)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3)	-	-	AA	-	-
PV1757	74/F	***DHX34***	c.1879G>T; p.(E627*)	Het	AD	*DHX34*-associated predisposition	VUS (PS3 moderate)	-	-	MDS/MPN	-	-
PV1754	69/F	***DHX34***	c.2152C>T; p.(R718C)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3)	VUS	-	ICUS	+	+
PV2095	52/M	***DHX34***	c.2716C>T; p.(L906F)	Het	AD	*DHX34*-associated predisposition	VUS (PM2, PP2, PP3)	-	-	ICUS	-	-
PV2376	76/M	***DHX34***	c.2845C>T; p.(R949C)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3, BP4)	VUS	31785789	ICUS	+	-
PV2212	50/F	***DHX34***	c.2999G>A; p.(R1000Q)	Het	AD	*DHX34*-associated predisposition	VUS (PP2, PP3)	-	-	CCUS	+	+
PV1169	77/M	***DHX34***	c.3255_3256delCC; p.(H1086fs)	Het	AD	*DHX34*-associated predisposition	VUS (PM2)	-	-	MDS-MLD	+	-
PV2474	52/F	***DKC1***	c.838A>C; p.(S280R)	Het	XLR	TBD	VUS (PS3, PP2, PP3, BS3, BP6)	VUS/LB/B	11379875 20008900 27622320	ICUS	+	+
PV1846	30/F	***DNAJC21***	c.253A>G; p.(S85G)	Het	AR	SDS	VUS (PM2, PP3, BP1)	VUS	-	ICUS	-	+
PV1757	73/F	***DNAJC21***	c.438G>A; p.(T146T)	Het	AR	SDS	VUS (PM2, PP3, BS1)	VUS	-	MDS/MPN	-	-
PV2555	52/M	***DNAJC21***	c.1628T>C; p.(F543S)	Het	AR	SDS	VUS (PM2, PP3, BP1)	VUS	-	ICUS	+	-
PV10035	60/M	***ELANE***	c.323G>C; p.(G108A)	Het	AD	SCN	VUS (PM2, PP2, PP3, BP4)	-	-	AA	-	-
PV687	66/F	***ELANE***	c.556G>A; p.(V186I)	Het	AD	SCN	VUS (PP2, BP4)	VUS	19775295 25427142 31321910	MDS-EB-1	-	+

Supplemental Table 4. (Continued)

Patient ID	Age/Sex	Gene	Germline Variant	Zigosity	Inheritance	Congenital syndrome/disorder	ACMG/AMP classification (criteria)	ClinVar classification	PMID reference	Diagnosis	Extra-hematologic phenotype	Family history
PV1754	69/F	***ERCC6L2***	c.3376G>A; p.(V1126I)	Het	AR	IBMFS	VUS (PM2, PP3, BP1)	-	-	ICUS	+	+
PV1628	67/M	***FANCA***	c.1360-5C>G; p.?†	Hom	AR	FA	VUS (PM2, PP3)	-	-	MDS-U	+	-
PV1729	69/F	***FANCA***	c.1404G>C; p.(K468N)	Het	AR	FA	VUS (PM2, PP3, BS1)	VUS	-	CCUS	+	-
PV10066	39/M	***FANCA***	c.3062T>G; p.(L1021W)	Het	AR	FA	VUS (PM2, PP3)	VUS	-	AA	-	-
PV1568	73/M	***FANCA***	c.3376C>G; p.(L1126V)	Het	AR	FA	VUS (PM2, PP3)	-	-	MDS-EB-2	-	+
PV2574	29/F	***FANCA***	c.3384_3386dupGGA; p.(Q1128_D1129insE)†	Het	AR	FA	VUS (PM2, PM4)	-	9371798	MDS-EB-2	-	-
PV2574	29/F	***FANCA***	c.3782T>C; p.(F1261S)†	Het	AR	FA	VUS (PM2, PP3)	-	-	MDS-EB-2	-	-
PV1898	50/M	***FANCC***	c.1294C>T; p.(P432S)	Het	AR	FA	VUS (PM2, PP3, BP1)	VUS	-	ICUS	-	-
PV2381	51/F	***FANCD1 (BRCA2)***	c.9052_9057delAGTAAA; p.(S3018_K3019del)	Het	AR/AD	FA/HBOC	VUS (PM2, PM4, BP6)	VUS/LB	21918853	ICUS	+	+
PV1310	35/F	***FANCD2***	c.805A>C; p.(K269Q)	Het	AR	FA	VUS (PM2, PP3, BS1)	VUS	-	AML	+	-
PV1633	80/M	***FANCD2***	c.1147G>T; p.(V383L)	Het	AR	FA	VUS (PM2, BP4)	-	-	MDS-EB-2	+	-
PV2501	60/M	***FANCD2***	c.1203G>T; p.(R401S)	Het	AR	FA	VUS (PM1 supporting, PM2, PP3)	-	-	MDS-MLD	-	-
PV10036	54/M	***FANCD2***	c.1757C>T; p.(A586V)	Het	AR	FA	VUS (PM2, PP3, BS1)	VUS	-	AA	-	-
PV1316	48/M	***FANCD2***	c.1757C>T; p.(A586V)	Het	AR	FA	VUS (PM2, PP3, BS1)	VUS	-	AA	-	-
PV1633	80/M	***FANCD2***	c.3868G>C; p.(V1290L)	Het	AR	FA	VUS (PM2, PP3)	-	-	MDS-EB-2	+	-
PV2615	47/M	***FANCD2***	c.4189G>A; p.(E1397K)	Het	AR	FA	VUS (PM2, PP3, PP4, BS1)	VUS	-	ICUS	+	+
PV1816	58/M	***FANCF***	c.648_650delTCG; p.(R217del)	Het	AR	FA	VUS (PM2, PM4)	-	-	AA	-	-
PV1915	47/M	***FANCI***	c.625G>C; p.(E209Q)	Het	AR	FA	VUS (PM2, PP3, BS1)	VUS	29891941	ICUS	+	+
PV2481	26/F	***FANCI***	c.939_941delTCT; p.(L314del)	Het	AR	FA	VUS (PM2, PM4, BS1)	VUS	-	ICUS	-	-
PV2381	51/F	***FANCI***	c.1412C>G; p.(P471R)	Het	AR	FA	VUS (PM2, PP3, BS1)	VUS	30303537 34861889	ICUS	+	+
PV2460	71/M	***FANCI***	c.1759A>G; p.(I587V)	Het	AR	FA	VUS (PM2, PP3, BS1)	-	-	MDS-SLD	+	-
PV1146	53/F	***FANCI***	c.3521C>G; p.(T1174R)	Het	AR	FA	VUS (PM2, PP3, BS1)	VUS	-	MDS-EB-1	-	+
PV2008	24/F	***FANCJ (BRIP1)***	c.139C>G; p.(P47A)	Het	AR	FA	VUS (PS3, PP3, PP5, BS1, BP6)	P/VUS/LB/B	21345144 25374583 28104920	ICUS	-	-
PV1182	44/F	***FANCJ (BRIP1)***	c.139C>G; p.(P47A)	Het	AR	FA	VUS (PS3, PP3, PP5, BS1, BP6)	P/VUS/LB/B	21345144 25374583 28104920	ICUS	-	-
PV894	69/F	***FANCJ (BRIP1)***	c.550G>T; p.(D184Y)	Het	AR	FA	VUS (PS3 moderate, PP3, BS1)	VUS	30230034	ICUS	-	-
PV1395	81/M	***FANCJ (BRIP1)***	c.550G>T; p.(D184Y)	Het	AR	FA	VUS (PS3 moderate, PP3, BS1)	VUS	30230034	MDS-MLD	+	-
PV1727	44/F	***FANCJ (BRIP1)***	c.1012G>A; p.(E338K)	Het	AR	FA	VUS (PM2, PP3)	VUS	26921362	CCUS	+	-
PV1211	68/M	***FANCJ (BRIP1)***	c.2087C>T; p.(P696L)	Het	AR	FA	VUS (PM2, PP3, BS1)	VUS	26921362 31882575	MDS-EB-2	-	-
PV1915	48/M	***GATA1***	c.740G>A; p.(R247H)	Hem	XLR	DBA	VUS (PM2, PP2, PP3, BP6)	LB	-	ICUS	+	+

Supplemental Table 4. (Continued)

Patient ID	Age/Sex	Gene	Germline Variant	Zigosity	Inheritance	Congenital syndrome/disorder	ACMG/AMP classification (criteria)	ClinVar classification	PMID reference	Diagnosis	Extra-hematologic phenotype	Family history
PV1848	38/F	***GATA2***	c.308C>T; p.(A103V)†	Het	AD	*GATA2*-deficiency syndrome	VUS (PM2, PP3)	VUS	-	MDS-U	+	-
PV1850	31/F	***GFI1***	c.233G>C; p.(S78T)	Het	AD	SCN	VUS (PP2, PP3)	VUS	-	AA	-	-
PV1942	40/M	***GFI1***	c.416A>G; p.(Y139C)	Het	AD	SCN	VUS (PP2, PP3)	-	-	ICUS	+	+
PV2370	47/F	***JAGN1***	c.433G>A; p.(V145I)	Het	AR	SCN	VUS (PM2, PP2, BP4)	VUS	-	MDS-MLD	-	-
PV2663	29/F	***KRAS***	c.540T>A; p.(C180*)	Het	AD	NS	VUS (BP6)	VUS/LB/B	27763634 29517769	ICUS	+	+
PV1856	24/F	***MECOM***	c.948T>G ; p.(F316L)	Het	AD	RUSAT	VUS (PM2 supporting, PP3)	-	-	ICUS	-	-
PV2660	65/M	***MECOM***	c.1103T>C; p.(F368S)	Het	AD	RUSAT	VUS (PM2, PP3)	-	-	AA	-	-
PV1946	69/M	***MECOM***	c.1133A>G; p.(H378R)	Het	AD	RUSAT	VUS (PP3, BS1)	VUS	-	ICUS	+	-
PV1146	53/F	***MPL***	c.1367G>C; p.(R456P)	Het	AR	CAMT	VUS (PM2, PP3)	VUS	-	MDS-EB-1	-	+
PV2598	44/F	***MPL***	c.1399G>A; p.(G467S)	Het	AR	CAMT	VUS (PM2, PP3)	-	-	ICUS	-	+
PV1961	61/F	***MPL***	c.1841G>T; p.(G614V)	Het	AR	CAMT	VUS (PM2, PP3)	-	17666371	ICUS	+	-
PV1843	58/F	***NF1***	c.3734C>G; p.(T1245S)	Het	AD	NF	VUS (PM2 supporting, PP3, BP1)	VUS	-	CCUS	-	-
PV2281	70/F	***NF1***	c.3734C>G; p.(T1245S)	Het	AD	NF	VUS (PM2 supporting, PP3, BP1)	VUS	-	MDS-5q	+	-
PV629	64/M	***PARN***	c.449G>A; p.(R150H)	Het	AD/AR	TBD	VUS (PM2, PM5, PP3)	VUS	-	MDS-SLD	-	-
PV1316	48/M	***PARN***	c.482A>G; p.(Y161C)	Het	AD/AR	TBD	VUS (PM2, PP3)	VUS	-	AA	-	-
PV1846	29/F	***PARN***	c.1590C>G; p.(S530R)	Het	AD/AR	TBD	VUS (PM1, PM2, BP4)	-	-	ICUS	-	+
PV1866	44/F	***PARN***	c.1613G>C; p.(R538P)	Het	AD/AR	TBD	VUS (PM2, PP3)	VUS	30523342 31268371	ICUS	+	+
PV2536	35/M	***PARN***	c.1612C>T; p.(R538W)	Het	AD/AR	TBD	VUS (PP3)	VUS	-	ICUS	-	-
PV2226	52/F	***PARN***	c.1774_1775GA>TC; p.(E592S)	Het	AD/AR	TBD	VUS (PM2, PP3)	-	-	AA	+	-
PV674	67/M	***PTPN11***	c.556C>T; p.(R186W)	Het	AD	NS	VUS (PP2, PP3, BP6)	VUS/LB	23624134 29037749 35626289	MDS-MLD	-	-
PV2476	81/F	***PTPN11***	c.1671G>C; p.(Q557H)	Het	AD	NS	VUS (PP2, PP3, BP4)	VUS	-	CCUS	+	-
PV2186	58/M	***RAF1***	c.1323G>C; p.(Q441H)	Het	AD	NS	VUS (PM2, PP2, PP3)	VUS	-	MDS-MLD	-	-
PV1956	73/M	***RAF1***	c.1721A>G; p.(Y574C)	Het	AD	NS	VUS (PP2, PP3, BP6)	VUS/LB/B	26580448	ICUS	+	+
PV652	29/M	***RPL11***	c.296_298delTCT; p.(F99del)†	Het	AD	DBA	VUS (PM2, PM4)	VUS	26220995	MDS-EB-2	-	-
PV1790	65/M	***RTEL1***	c.334G>A; p.(A112T)	Het	AD/AR	TBD	VUS (PP3, BS1)	VUS	31268371	ICUS	+	-
PV2674	19/F	***RTEL1***	c.818C>G; p.(A273G)	Het	AD/AR	TBD	VUS (PM2, PP3)	-	-	AA	-	-
PV1959	59/F	***RTEL1***	c.1189C>G; p.(Q397E)	Het	AD/AR	TBD	VUS (PP3, BS1)	VUS	29344583	ICUS	+	-
PV2307	49/M	***RTEL1***	c.1189C>G; p.(Q397E)	Het	AD/AR	TBD	VUS (PP3, BS1)	VUS	29344583	ICUS	+	-
PV2498	36/F	***RTEL1***	c.1940C>T; p.(P647L)	Het	AD/AR	TBD	VUS (PS3 moderate, PM2, PP3, BS1)	VUS	25848748	ICUS	-	-
PV2186	58/M	***RTEL1***	c.2707G>A; p.(V903M)	Het	AD/AR	TBD	VUS (PM2, PP3, BS1)	VUS	-	MDS-MLD	-	-

Supplemental Table 4. (Continued)

Patient ID	Age/Sex	Gene	Germline Variant	Zigosity	Inheritance	Congenital syndrome/disorder	ACMG/AMP classification (criteria)	ClinVar classification	PMID reference	Diagnosis	Extra-hematologic phenotype	Family history
PV2186	58/M	***RTEL1***	c.2878C>G; p.(H960D)	Het	AD/AR	TBD	VUS (PM2, PP3)	-	-	MDS-MLD	-	-
PV1866	44/F	***RTEL1***	c.3440G>A; p.(G1147E)	Het	AD/AR	TBD	VUS (PM2, PP3, BS1)	VUS	-	ICUS	+	+
PV10089	25/M	***SAMD9***	c.578C>T; p.(P193L)	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PP3, BS1)	VUS	-	ICUS	-	+
PV1210	46/F	***SAMD9***	c.1487G>A; p.(G496D)	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PM2, PP3)	-	-	ICUS	-	-
PV2370	48/F	***SAMD9***	c.2584C>G; p.(Q862E)‡	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PM2, BP4)	-	-	MDS-MLD	-	-
PV2370	48/F	***SAMD9***	c.2596G>C; p.(E866Q)‡	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PM2, PP3, BP4)	-	-	MDS-MLD	-	-
PV1338	48/M	***SAMD9L***	c.190C>G; p.(P64A)	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PM2, PP3)	-	-	AML	+	-
PV2666	42/M	***SAMD9L***	c.2069G>A; p.(G690D)	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PP3, PP4, BS1)	VUS	34621053	AA	+	+
PV1346	45/M	***SAMD9L***	c.2494dupA; p.(T832fs)	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PVS1 moderate, PM2)	VUS	-	MDS-EB-2	+	-
PV2671	50/F	***SAMD9L***	c.2494dupA; p.(T832fs)	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PVS1 moderate, PM2)	VUS	-	AA	+	+
PV30047	63/F	***SAMD9L***	c.3368T>C; p.(L1123P)	Het	AD	*SAMD9/9L*-associated predisposition	VUS (PM2, PP3)	-	-	MDS/MPN	+	+
PV2463	57/M	***SOS1***	c.985G>A; p.(E329K)	Het	AD	NS	VUS (PP2, PP3, BP4)	VUS	-	CCUS	+	-
PV1714	72/F	***SOS1***	c.1627T>C; p.(S543P)†	Het	AD	NS	VUS (PP2, PP3)	VUS	-	AA	+	-
PV1362	59/M	***TERC***	n.29T>A	Het	AD	TBD	VUS (PM2, BP4)	VUS	-	MDS-EB-1	+	+
PV1726	27/M	***TERC***	n.40_43delTTTT†	Het	AD	TBD	VUS (PM2, PP4)	-	-	ICUS	+	+
PV2353	52/M	***TERC***	n.80T>C†	Het	AD	TBD	VUS (PM2, PP4)	-	26581148	ICUS	+	+
PV2143	47/F	***TERC***	n.192C>T	Het	AD	TBD	VUS (PM2 supporting, PP3, BS1)	VUS	-	ICUS	-	-
PV2370	47/F	***TERC***	n.397G>A	Het	AD	TBD	VUS (PP3, BS1)	VUS	-	MDS-MLD	-	-
PV1987	53/M	***TERT***	c.403G>T; p.(G135W)†	Het	AD/AR	TBD	VUS (PM2, PP2, PP3, PP4)	-	-	AA	+	-
PV2216	59/M	***TERT***	c.554G>A; p.(R185Q)	Het	AD/AR	TBD	VUS (PM2, PP2, BP4)	VUS	-	MDS-RS-MLD	-	-
PV2589	21/M	***TERT***	c.2377G>A; p.(E793K)†	Het	AD/AR	TBD	VUS (PM2, PP2, PP3, PP4)	VUS	-	CCUS	+	-
PV1616	69/F	***TP53***	c.509C>T; p.(T170M)	Het	AD	LFS	VUS (PP3, BP6)	VUS/LB	31081129	MDS-MLD	-	-
PV1923	81/M	***WAS***	c.946C>A; p.(P316T)	Hem	XLR	SCN	VUS (PM2, PP3)	VUS	-	MDS-RS-MLD	-	-

F, female; M, male; Het, heterozygous; Hom, homozygous; Hem, hemizygous; AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive; NS, Noonan syndrome; SCN, severe congenital neutropenia; TBD, telomere biology disorder; SDS, Shwachman-Diamond syndrome; IBMFS, inherited bone marrow failure syndrome; FA, Fanconi anemia; DBA, Diamond-Blackfan anemia; RUSAT, radioulnar synostosis with amegakaryocytic thrombocytopenia; CAMT, congenital amegakaryocytic thrombocytopenia; NF, neurofibromatosis; LFS, Li-Fraumeni syndrome; VUS, variant of unknown significance; P, pathogenic; LP, likely pathogenic; ICUS, idiopathic cytopenia of undetermined significance; MDS-EB, myelodysplastic syndrome with excess blasts; MDS-MLD, myelodysplastic syndrome with multilineage dysplasia; AA, aplastic anemia; MDS-SLD, myelodysplastic syndrome with single lineage dysplasia; MDS/MPN, myelodysplastic syndrome/myeloproliferative neoplasm; MDS/MPN-U,

myelodysplastic syndrome/myeloproliferative neoplasm unclassifiable; MDS-5q, myelodysplastic syndrome with deletion of chromosome 5q; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome.

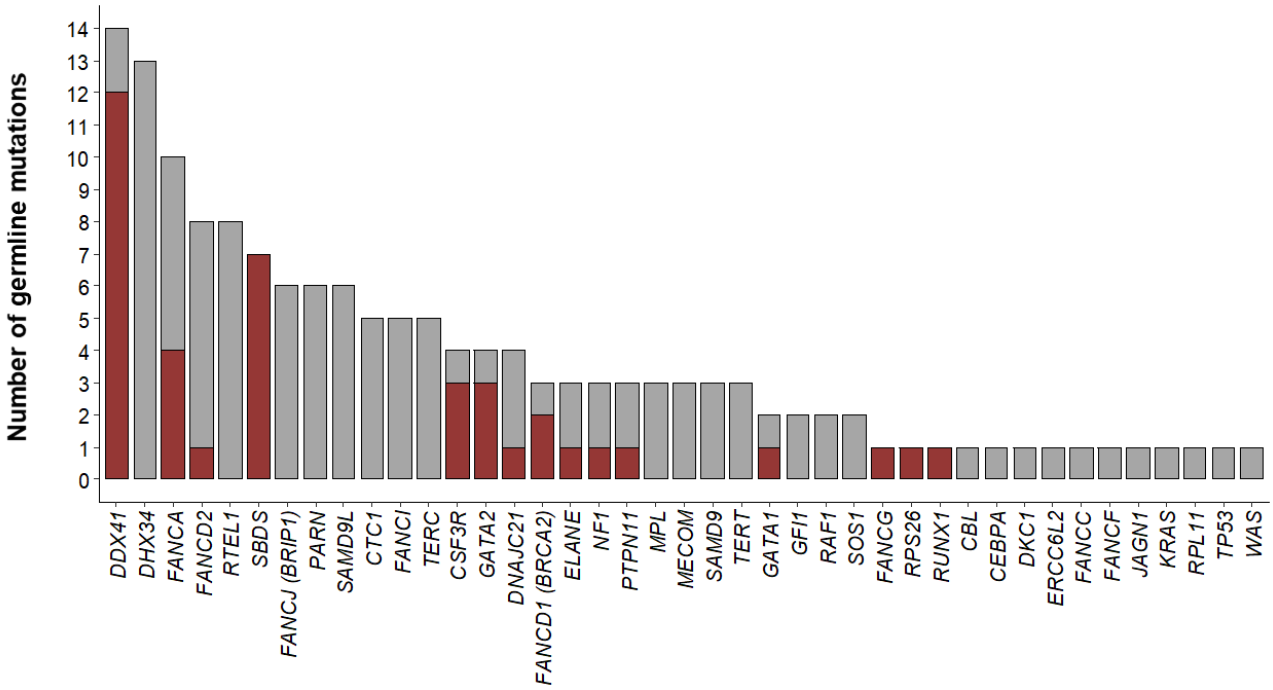
All variants showed a variant allele frequency (VAF) of 0.4 or above in both myeloid (peripheral blood granulocytes) and control germline tissue (T-lymphocytes or buccal epithelial cells). The germline origin of *SAMD9/SAMD9L* variants was confirmed in genomic DNA samples from buccal cells in mouthwash.

For *DDX41* germline variants classification the pathogenic moderate criterion PM3 was used in a modified manner to account for the known mechanism of *DDX41* somatic second hit in affected individuals. This criterion was applied to the germline variant when a second somatic pathogenic variant was also present in an affected patient in our study.[20,21]

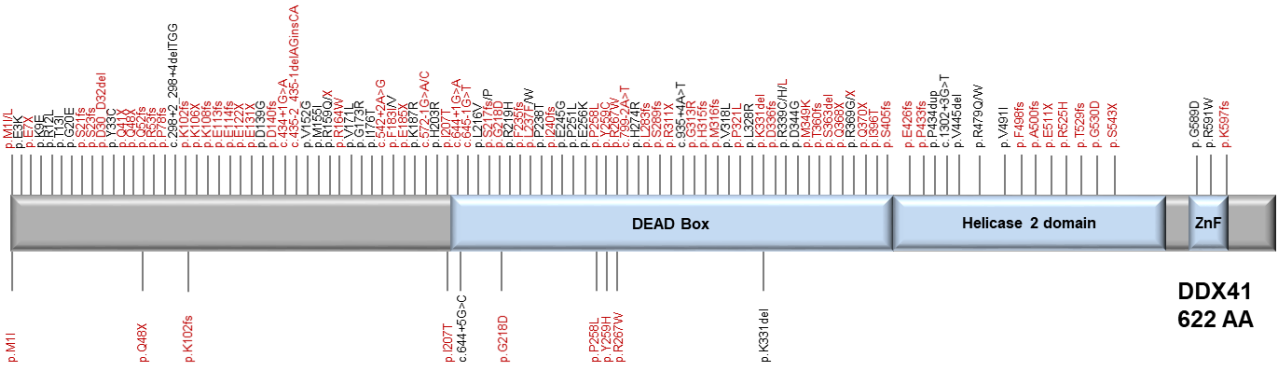
†VUSs affected genes associated with autosomal dominant or compound heterozygous predisposition to myeloid neoplasms presenting several evidences suggestive of a pathogenic effect: 1) total population variant frequency < 0.001 in gnomAD (v2.1.1), since a minor allele frequency cut-off of 0.001 is recommended for AD Mendelian disease variant discovery;[24] 2) a greater number of damaging compared to benign in silico functional predictions among the SIFT, PolyPhen-2, MutationTaster, LRT, Provean, DANN and PaPI functional prediction algorithms, or damaging dbscSNV prediction for splicing variants; 3) absence of ACMG/AMP benign criteria 4) suggestive patient's hematologic/extra-hematologic phenotype and/or suggestive family history of hematologic disorder/solid cancer and/or evidences collected from scientific literature.

‡*SAMD9* p.(Q862E) and p.(E866Q) variants were observed in *cis* in patient PV2370.

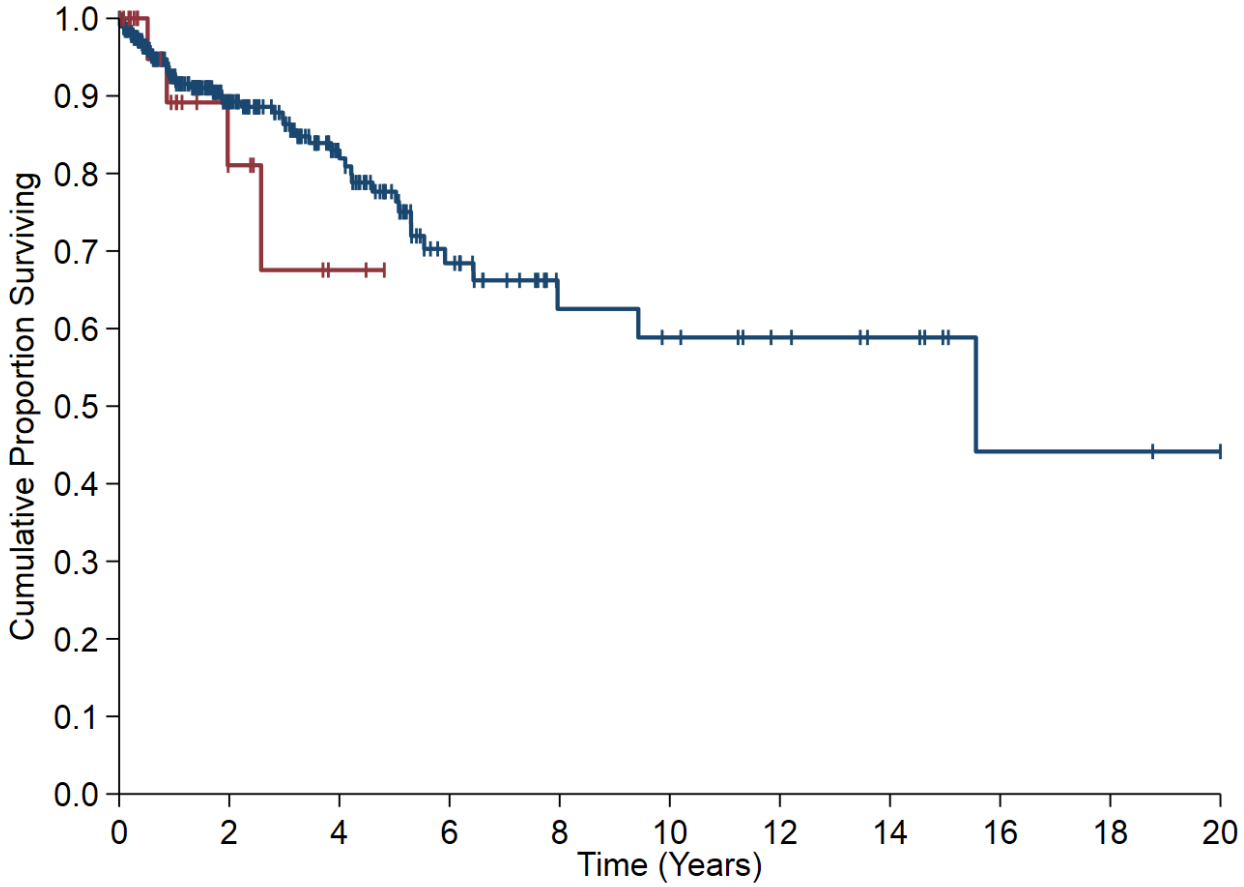
Supplemental Figure 1. Germline mutation landscape of the cohort included in the study. Red bars represent pathogenic and likely pathogenic variants (P/LP), while gray bars denote variant of unknown significance (VUS).



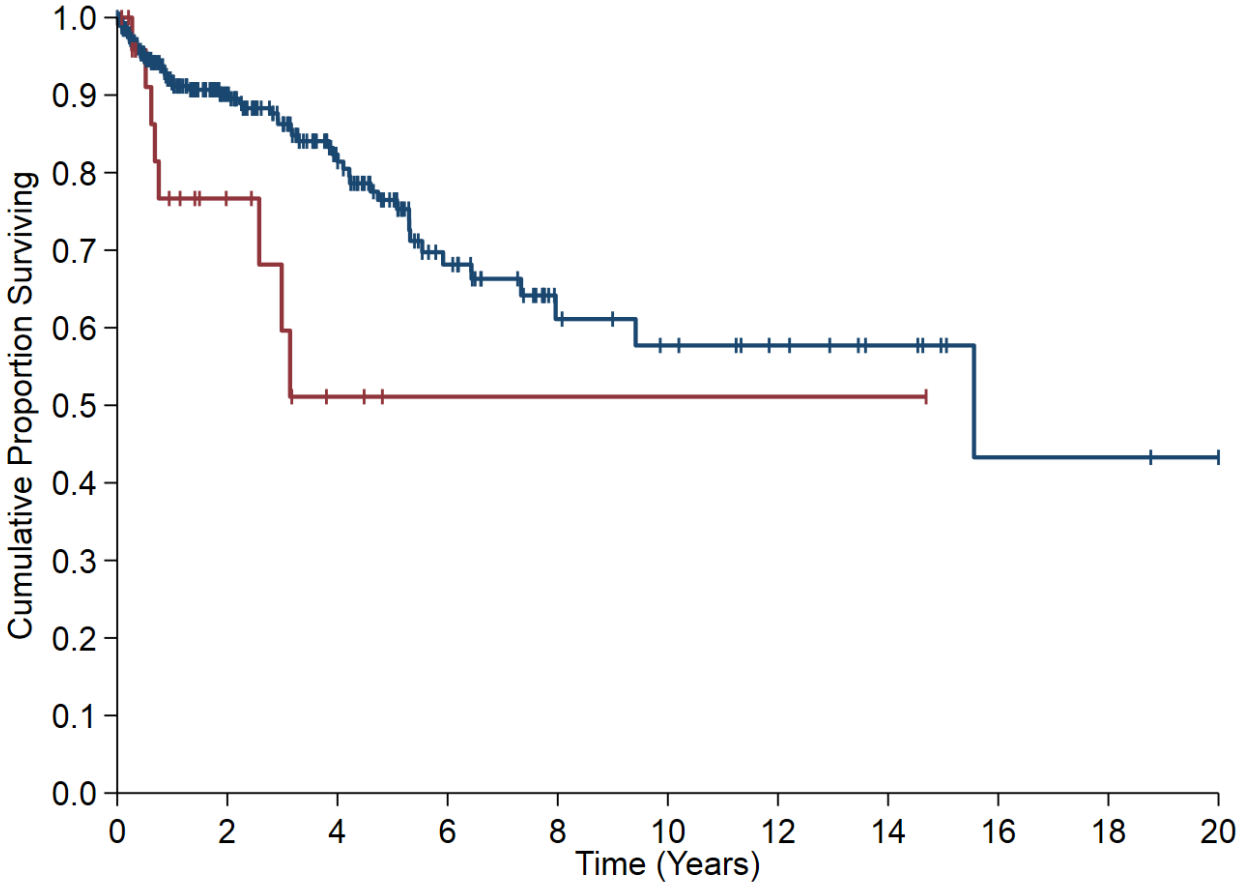
Supplemental Figure 2. Schematic domain structure of DDX41 (NM_016222.4) and distribution of germline variants. Mutations reported in the literature are shown above schematic protein representation, while mutations detected in the present study are shown below protein representation (Pathogenic/Likely Pathogenic [P/LP] variants [red], Variants of Unknown Significance [VUS] [black]).



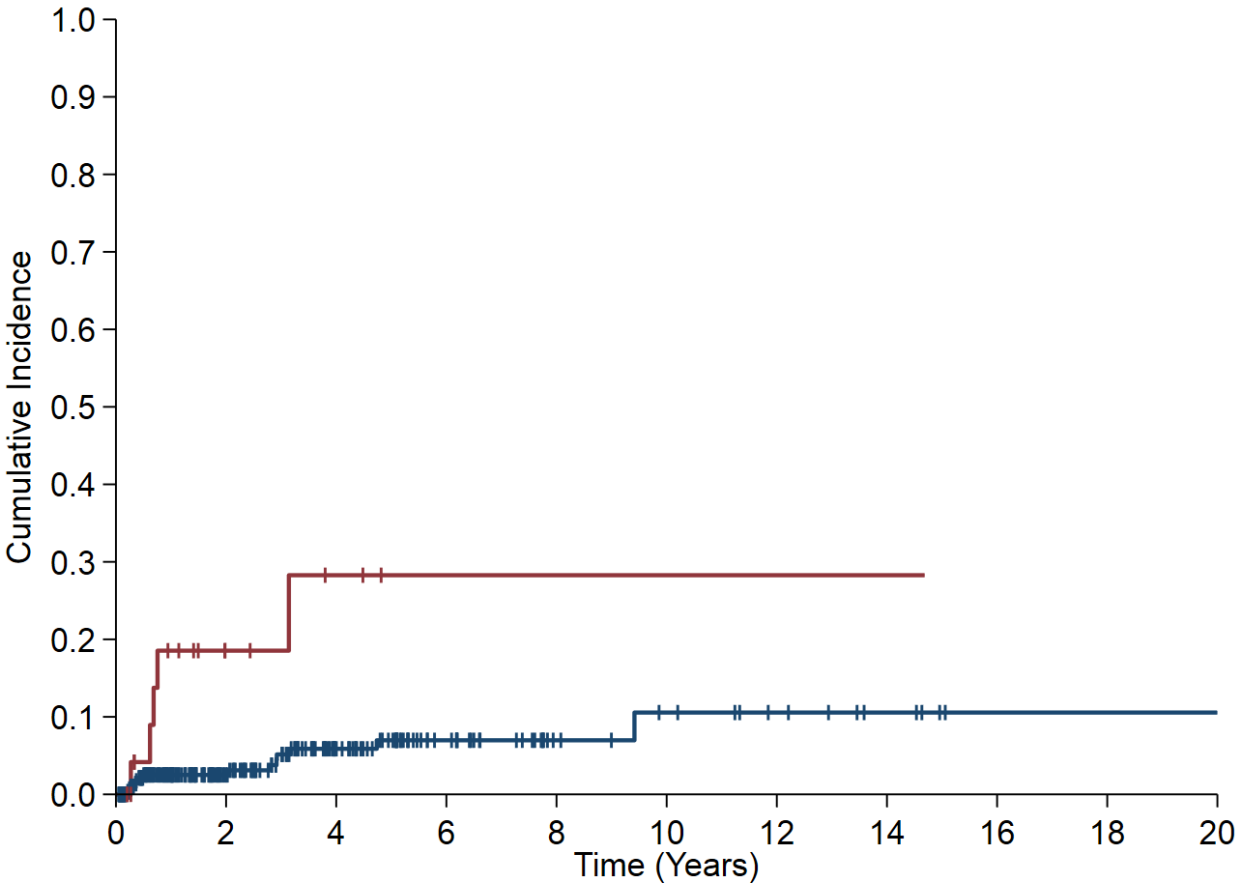
Supplemental Figure 3. Overall survival of adult patients with hypocellular bone marrow according to the presence or absence of germline mutations identified as causative of a congenital syndrome or disorder (HR=1.32, P=.59) (navy line: absence of germline predisposition; maroon line: presence of germline predisposition).



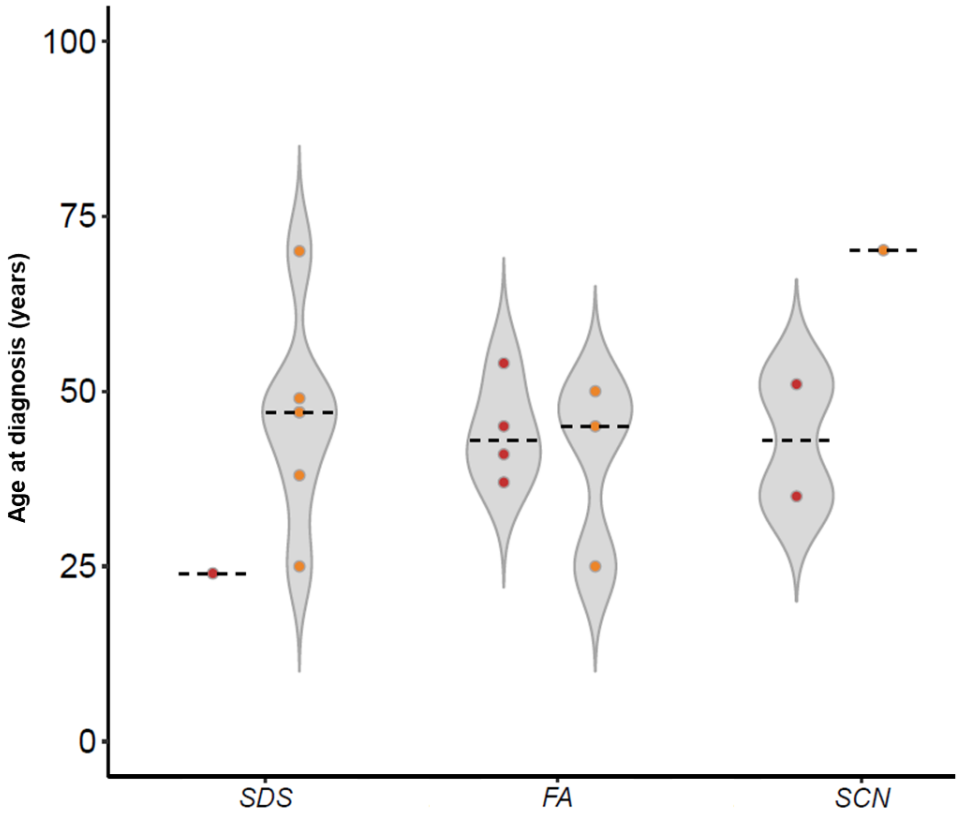
Supplemental Figure 4. Event-free survival of adult patients with hypocellular bone marrow according to the presence or absence of germline mutations identified as causative of a congenital syndrome or disorder (HR=2.18, P=.041) (navy line: absence of germline predisposition; maroon line: presence of germline predisposition).



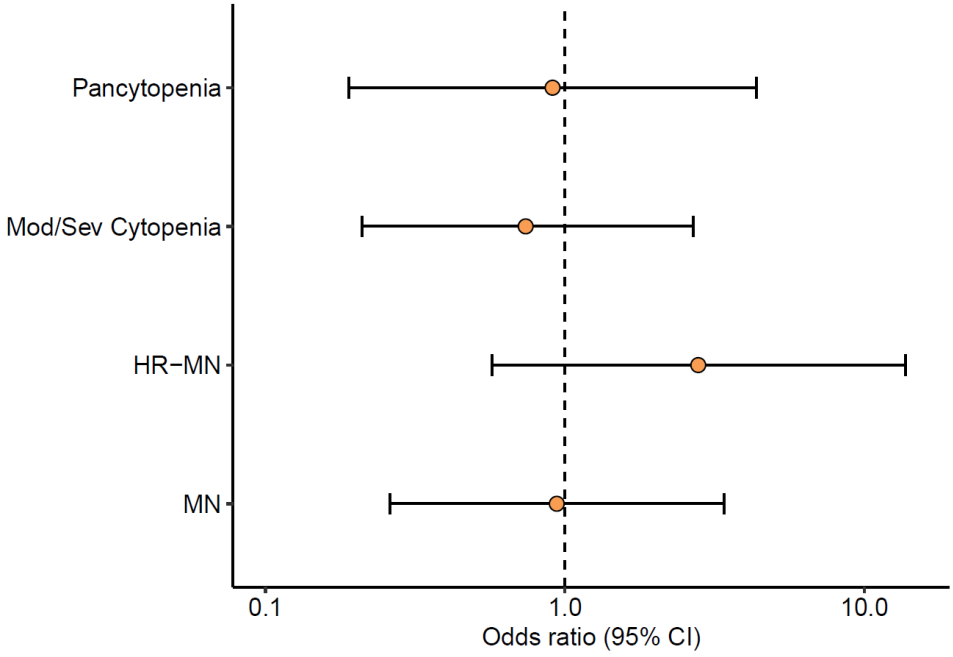
Supplemental Figure 5. Cumulative incidence of progression into AML estimated with a competing risk approach in patients with a diagnosis of myeloid neoplasm according to the presence or absence of germline mutations identified as causative of a congenital syndrome or disorder (HR=3.92, P=.008) (navy line: absence of germline predisposition; maroon line: presence of germline predisposition).



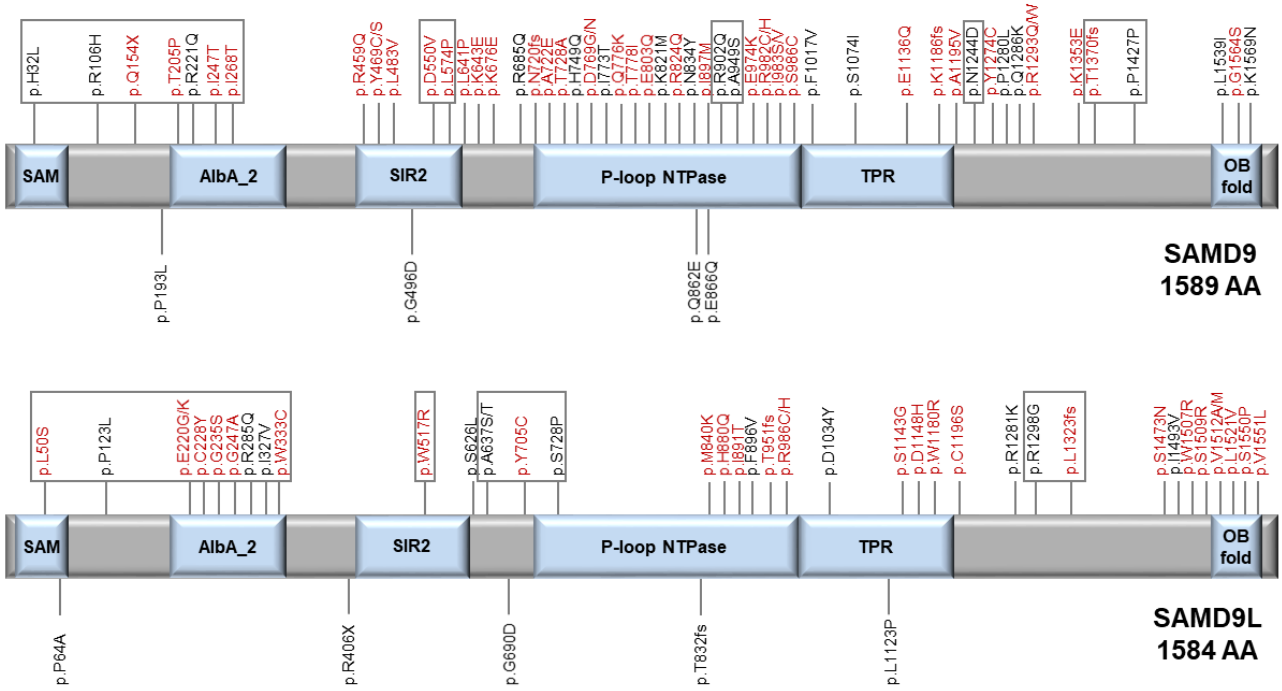
Supplemental Figure 6 Age distribution according to the underlying germline mutation in patients carriers of a unique heterozygous mutation in genes associated with autosomal recessive disorders (orange dots) and in those with congenital syndrome or disorder resulting from combined heterozygosity/homozygosity (red dots) (median age is indicated with a dashed line) (SDS, Shwachman-Diamond Syndrome; FA, Fanconi Anemia; SCN, Severe Congenital Neutropenia).



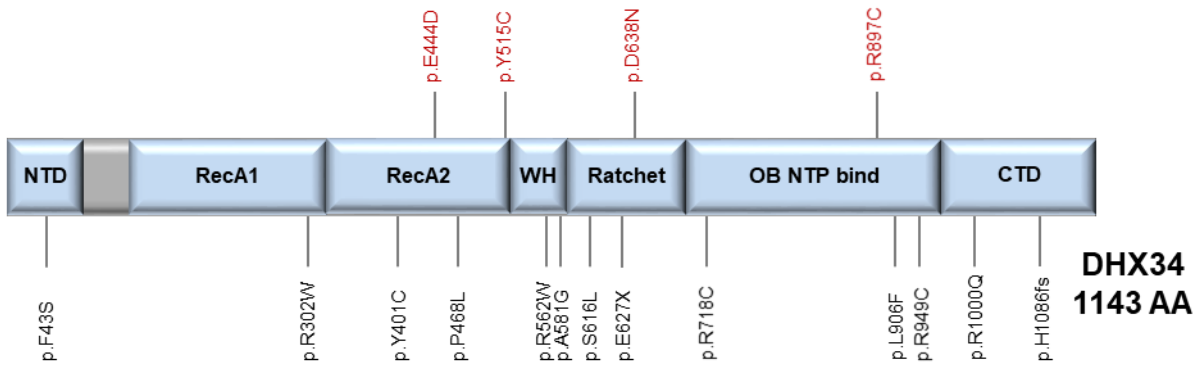
Supplemental Figure 7. Odds ratios for clinical phenotype (moderate to severe cytopenia and pancytopenia, myeloid neoplasm [MN] and high risk [HR]-MN) of the status of carrier of germline heterozygous mutation in genes associated with autosomal recessive disorders predisposing to myeloid neoplasm.



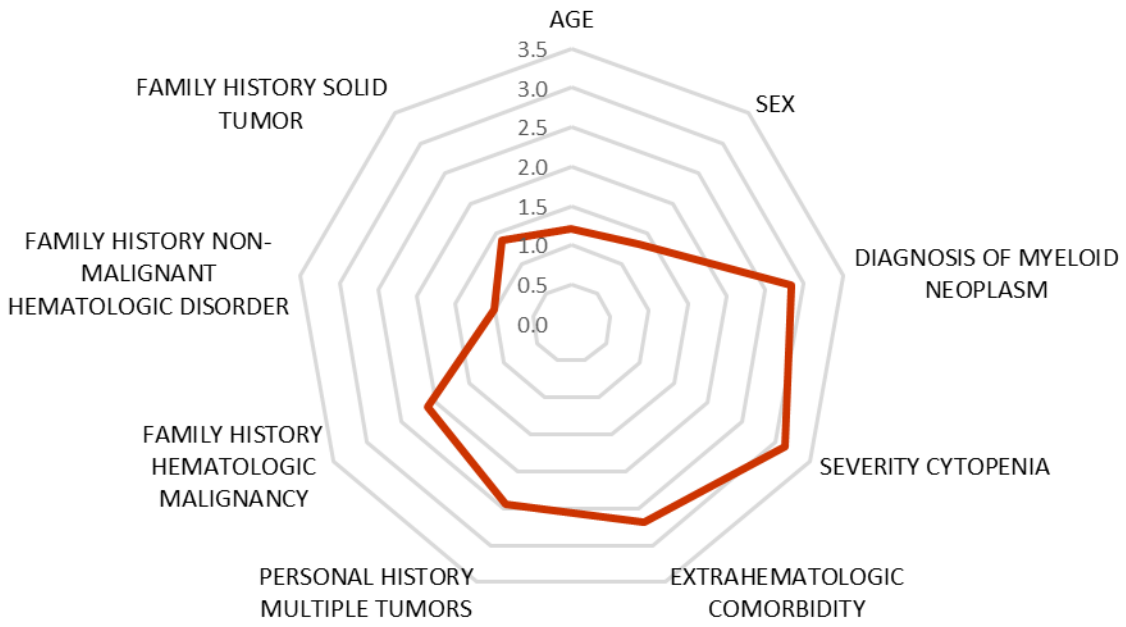
Supplemental Figure 8. Schematic domains structure of SAMD9 (NM_017654.4) and SAMD9L (NM_152703.5), and distribution of germline variants. Mutations reported in the literature are shown above schematic protein representation; boxed variants have been described in adult patients with MDS/BMF, predominantly clustering at N-terminal regions. Mutations detected in the present study are shown below protein representation and almost all localize at N-terminal and central regions of both genes (Pathogenic/Likely Pathogenic [P/LP] variants [red], Variants of Unknown Significance [VUS] [black]).



Supplemental Figure 9. Schematic domain structure of DHX34 (NM_014681.6) and distribution of germline variants. Mutations reported in the literature are shown above schematic protein representation. Mutations detected in the present study are shown below protein representation. (Pathogenic/Likely Pathogenic [P/LP] variants [red], Variants of Unknown Significance [VUS] [black]).



Supplemental Figure 10. Odds ratios from multinomial regression models of demographic, clinical and family history variables for germline predisposition in adult patients with hypocellular bone marrow.



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