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Serial next-generation sequencing for detecting germline predisposition in acute myeloid leukemia

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Disclosure of competing interests

The authors declare no competing financial interests.

Contribution

JA and JHP contributed to the conceptualization and design of the study, data collection, analysis and interpretation, and manuscript writing. HK and HK contributed to the study's conceptualization and design, data interpretation, and manuscript revision. IS, MYK, SKS, HY, YP, IK, HS, SP, SK, JC, HSL, HL, SHB, YC, HL, YRD, JJH, MKK, and SP contributed to essential data. CL contributed to data analysis. All authors approved the final submitted version.

Clinical Trial Registration

This study was registered with the Clinical Research Information Service (CRIS) under the registration number **KCT0004825**.

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Data Sharing Statement

Sequencing data generated and analyzed in this study have been deposited in the European Nucleotide Archive (ENA) under the accession number **PRJEB81954**.

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Recent advances in whole-genome and next-generation sequencing (NGS) have significantly deepened our understanding of the genomic landscape of acute myeloid leukemia (AML), uncovering not only somatic mutations but also inherited germline predispositions.[1, 2] While somatic NGS panels are routinely employed for diagnostic risk stratification and therapeutic decision-making, the incidental detection of germline variants is increasingly recognized.[1-5] Persistently high variant allele frequencies (VAFs)—particularly those observed during complete remission (CR) in the absence of residual disease—may suggest a germline origin. In the context of allogeneic hematopoietic cell transplantation (HCT), confirming the germline nature of these variants is essential for donor selection, genetic counseling, and evaluation of conditioning-related toxicities. This study aimed to investigate the prevalence, gene distribution, and clinical implications of germline variants in AML through the use of serial targeted NGS.

We conducted a prospective, multicenter study enrolling 343 patients with AML from 17 academic institutions across Korea between February 2017 and June 2021. All participants provided written informed consent, and the study was conducted in accordance with the Declaration of Helsinki and registered with the Clinical Research Information Service (KCT0004825). The study protocol was approved by the institutional review boards of all participating centers. Patients included in the analysis had paired bone marrow samples collected at diagnosis and during CR. Targeted NGS was performed using the Illumina HiSeq 2500 platform (mean coverage: 1,387×), encompassing the coding regions of 83 AML-associated genes (Table S1).[6] Sequencing data were deposited in the European Nucleotide Archive (Accession number: PRJEB81954). Variants with a VAF ≥2.0% at diagnosis were included in the analysis. We screened for germline variants in 15 genes (*CBL, CEBPA, DDX41, DNAH5, ETV6, GATA2, JAK2, KRAS, MPL, NF1, NRAS, PTPN11, RUNX1, SUZ12*, and *TP53*) associated with germline predisposition to myeloid neoplasms, as defined by the 2022 European LeukemiaNet (ELN) guidelines and prior studies. [1, 7] Variants exhibiting a VAF of 40–60% in CR

samples were classified as putative germline. Variant classification followed the criteria of the American College of Medical Genetics and Genomics (ACMG); those categorized as pathogenic or likely pathogenic were considered causative.[8] In addition, variants of uncertain significance (VUS) in *CEBPA* or *DDX41* were deemed causative when accompanied by somatic mutations.[9, 10]

Among the 343 enrolled patients, 322 received intensive chemotherapy and 21 received low-intensity therapy. Germline variants were identified in 19 patients (5.5%), most frequently in *DDX41* (58%, n=11), followed by *DNAH5* (16%, n=3), *CEBPA* and *TP53* (11% each, n=2), and *MPL* and *GATA2* (5% each, n=1). Two patients initially harboring VUS were reclassified as germline cases based on the presence of coexisting somatic mutations (Table 1).

The most common *DDX41* variants were p.A500fs (n=5) and p.A550fs (n=2), both truncating mutations. Patients with germline *DDX41* mutations had a median age of 61 years (range, 50–77), and only 3 of the 11 (27.3%) had concurrent somatic *DDX41* mutations. Two additional sub-threshold variants (VAF <2%) were detected but were excluded from the somatic mutation count per protocol criteria. *CEBPA* germline mutations were biallelic and located in the C-terminal domain, whereas somatic counterparts were detected in both N- and C-terminal regions. One case involved a familial p.N356K variant shared with a first-degree relative, both diagnosed with AML and treated with allogeneic HCT, without subsequent relapse or clonal evolution (Table 2). Additional germline variants included *TP53* (p.R248Q, p.G44S), *DNAH5* (p.R1883*, p.S914*), *MPL* (p.R357*), and *GATA2* (p.G200fs). Aside from the one familial *CEBPA* case, no other patients were classified as familial at diagnosis, and a retrospective review of medical records did not identify any additional families with multiple affected individuals harboring the same germline variant. All patients demonstrated a marked reduction of somatic VAF during CR, supporting a germline origin of persistent variant (Figure S1). Compared with patients lacking germline variants, those with germline mutations had

significantly lower bone marrow cellularity (median: 50% vs. 80%, *P*<0.001) and lower blast percentages (34% vs. 65%, *P*=0.004). Germline variants were absent among patients with favorable cytogenetic risk and were predominantly identified in those with intermediate or adverse risk (*P*<0.001) (Table 3).

Overall survival showed a trend toward worse prognosis in patients with germline mutations (*P*=0.034); however, this difference did not reach statistical significance in multivariate analysis that included age, 2022 ELN risk classification, and allogeneic HCT status (HR 1.00; 95% CI:0.53–1.86; *P*=0.989) (Figure S2). Among the 19 patients with germline mutations, 12 underwent allogeneic HCT. Of these, 4 received unrelated donor grafts (only one as a primary option), and 8 received grafts from matched sibling or haploidentical donors. Only one haploidentical donor was screened for *DDX41* germline variants. Notably, germline status did not significantly influence donor type or post-transplant outcomes (Figure S2). The median follow-up duration was 30.4 months (range, 8.6–54.1), and no cases of donor-derived leukemia were observed.

In this nationwide prospective study of 343 AML patients with available CR samples, serial targeted NGS identified germline mutations in 5.5% of cases, underscoring the utility of paired-sample NGS in detecting rare yet clinically significant germline variants. Our findings support incorporating germline testing in cases with persistently high VAFs during CR in the absence of measurable residual disease. Early identification of germline predisposition could inform more individualized treatment strategies, including donor selection, therapy intensity, and the need for genetic counseling. Recognition of germline predisposition during the study has prompted changes in clinical practice, including assessment of mutation clearance for suspected *CEBPA* variants and targeted germline testing for *DDX41* when related donors are considered.

The observed prevalence differs from previous reports, which may reflect differences in sequencing panels, study design, and ethnic background. Notably, whole-exome sequencing studies have reported higher detection rates ranging from 7.2% to 23.4%.[7, 11] Our targeted panel focused on 15 genes associated with myeloid neoplasms, highlighting the impact of panel composition on germline mutation detection. *DDX41* emerged as the most frequently mutated gene (3.2%), with a predominance of truncating variants, reaffirming its established role in familial AML. Concurrent *DDX41* somatic mutations were less frequent, possibly due to the sample size and predefined VAF thresholds.[12, 13] Additionally, population-specific genetic factors may contribute to this discrepancy and warrant further investigation. These germline mutations were often identified in older patients with low somatic burden and hypocellular bone marrow, consistent with prior observations.[9] Germline mutations in *CEBPA*, *TP53*, *DNAH5*, *MPL*, and *GATA2*, though individually rare, underscore the genetic heterogeneity of AML. Familial *CEBPA* mutations further support the value of germline testing in uncovering hereditary leukemia syndromes. In this study, biallelic *CEBPA* germline mutations were confined to the C-terminal domain and co-occurred with somatic variants in both N- and C-terminal regions. While *CEBPA* germline mutations are typically associated with N-terminal frameshift variants and high penetrance (~90%), emerging data suggest that C-terminal (bZIP) or central domain mutations may be associated with incomplete penetrance and later disease onset.[14] These findings, together with our observations, suggest possible population-specific mutational patterns in Korean AML patients, which merit further investigation. *TP53* germline carriers demonstrated high-risk cytogenetic profiles, suggesting that pathogenic germline variants may exacerbate adverse somatic features.

Patients with germline mutations exhibited distinct clinical characteristics, including significantly lower marrow cellularity and blast percentages, and were more frequently classified into intermediate or adverse cytogenetic risk groups. Despite these unfavorable baseline characteristics,

germline status did not negatively impact outcomes following allogeneic HCT. Of the 12 transplanted patients, only one donor was screened for *DDX41*, reflecting the absence of standardized protocols. Importantly, no cases of donor-derived AML were observed over the median follow-up of 30.4 months. Our findings suggest that rare germline variants with approximately 50% VAF may be underrecognized in clinical settings due to the difficulty in distinguishing them from somatic mutations without confirmatory testing. Furthermore, existing bioinformatic pipelines—primarily optimized for somatic variant detection—may inadvertently filter out bona fide germline events, thereby contributing to their underdetection.

This study has several limitations, including the restricted scope of the targeted NGS panel, potential selection bias due to the inclusion of only patients who achieved CR, and the absence of functional validation or comprehensive family genetic assessments. Notably, the restriction to patients with available CR samples led to the exclusion of a substantial proportion of enrolled cases (260 out of 603 AML patients, 43%), potentially introducing selection bias. To address this limitation in future research, the use of alternative germline DNA sources—such as skin biopsies or buccal swabs—should be considered to enable broader patient inclusion and enhance the generalizability of findings. Despite these constraints, serial NGS monitoring proved effective in identifying germline mutations and distinguishing them from somatic variants. Importantly, our findings underscore the utility of paired CR sample analysis as a reliable method for detecting germline mutations and elucidating their prevalence and clinical implications in AML, particularly in the context of allogeneic HCT.

In conclusion, this study reinforces the clinical utility of germline mutation analysis using paired CR samples in AML. Such integration enhances diagnostic accuracy, informs therapeutic decision-making, and supports optimized donor selection for transplantation. As clinical awareness increases,

the implementation of standardized germline testing and donor screening protocols will be critical to advancing personalized management strategies for AML.

References

1. Dohner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. Blood. 2022;140(12):1345-1377.
2. Song GY, Kim HJ, Kim T, et al. Validation of the 2022 European LeukemiaNet risk stratification for acute myeloid leukemia. Sci Rep. 2024;14(1):8517.
3. Cho YU. The role of next-generation sequencing in hematologic malignancies. Blood Res. 2024;59(1):11.
4. Speight B, Hanson H, Turnbull C, et al. Germline predisposition to haematological malignancies: Best practice consensus guidelines from the UK Cancer Genetics Group (UKCGG), CanGene-CanVar and the NHS England Haematological Oncology Working Group. Br J Haematol. 2023;201(1):25-34.
5. Hwang SM. Genomic testing for germline predisposition to hematologic malignancies. Blood Res. 2024;59(1):12.
6. Ahn JS, Kim HJ, Kim YK, et al. Assessment of a new genomic classification system in acute myeloid leukemia with a normal karyotype. Oncotarget. 2018;9(4):4961-4968.
7. Yang F, Long N, Anekpuritanang T, et al. Identification and prioritization of myeloid malignancy germline variants in a large cohort of adult patients with AML. Blood. 2022;139(8):1208-1221.
8. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424.
9. Choi EJ, Cho YU, Hur EH, et al. Unique ethnic features of DDX41 mutations in patients with idiopathic cytopenia of undetermined significance, myelodysplastic syndrome, or acute myeloid leukemia. Haematologica. 2022;107(2):510-518.

10. Pabst T, Eyholzer M, Haefliger S, Schardt J, Mueller BU. Somatic CEBPA mutations are a frequent second event in families with germline CEBPA mutations and familial acute myeloid leukemia. J Clin Oncol. 2008;26(31):5088-5093.
11. Kim B, Yun W, Lee ST, et al. Prevalence and clinical implications of germline predisposition gene mutations in patients with acute myeloid leukemia. Sci Rep. 2020;10(1):14297.
12. Makishima H, Saiki R, Nannya Y, et al. Germ line DDX41 mutations define a unique subtype of myeloid neoplasms. Blood. 2023;141(5):534-549.
13. Duployez N, Largeaud L, Duchmann M, et al. Prognostic impact of DDX41 germline mutations in intensively treated acute myeloid leukemia patients: an ALFA-FILO study. Blood. 2022;140(7):756-768.

Table 1. Genetic features of germline variants identified by serial targeted next-generation sequencing

Patient number	Variant	KOVA_AF	gnomAD_AC	Type	NT.Change	Transcript accession	AA.Change	ACMG	Interpretation	VAF at diagnosis (%)	VAF at CR (%)
11-130	*DDX41*	9.48E-04	2	ins	c.1496dupC	NM_016222.3	p.A500fs	PVS1, PS4, PM2	P	48.64	48.74
13-34	*DDX41*	9.48E-04	2	ins	c.1496dupC	NM_016222.3	p.A500fs	PVS1, PS4, PM2	P	50.18	49.23
16-10	*DDX41*	9.96E-05	.	snv	c.19G>T	NM_016222.3	p.E7*	PVS1, PM2	LP	47.39	47.84
16-19	*DDX41*	1.47E-04	.	ins	c.1647dupA	NM_016222.3	p.A550fs	PVS1, PM2	LP	47.94	50.36
16-20	*DDX41*	1.47E-04	.	ins	c.1647dupA	NM_016222.3	p.A550fs	PVS1, PM2	LP	48.94	49.96
16-21	*DDX41*	.	.	del	c.21delA	NM_016222.3	p.E7fs	PVS1, PM2	LP	44.26	43.56
19-13	*DDX41*	9.48E-04	2	ins	c.1496dupC	NM_016222.3	p.A500fs	PVS1, PS4, PM2	P	49.58	51.19
20-1	*DDX41*	9.48E-04	2	ins	c.1496dupC	NM_016222.3	p.A500fs	PVS1, PS4, PM2	P	49.17	49.52
24-6	*DDX41*	2.94E-04	2	snv	c.455T>G	NM_016222.3	p.V152G	PM2, (PP2)	VUS	47.62	50.90
27-4	*DDX41*	.	.	ins	c.1591_1594dupATCG	NM_016222.3	p.A532fs	PVS1, PM2	LP	56.0	51.31
29-10	*DDX41*	9.48E-04	2	ins	c.1496dupC	NM_016222.3	p.A500fs	PVS1, PS4, PM2	P	50.69	48.95
13-38	*MPL*	7.57E-04	4	snv	c.1069C>T	NM_016222.3	p.R357*	PVS1, PS4, PM2	P	48.89	48.52
32-34	*DNAH5*			snv	c.2741C>G	NM_001369.2	p.S914*	PVS1, PM2, PS4	LP	47.92	45.91
18-8	*DNAH5*		7	snv	c.5647C>T	NM_001369.2	p.R1883*	PVS1, PM2, PM3	P	51.17	49.07
29-32	*DNAH5*		7	snv	c.5647C>T	NM_001369.2	p.R1883*	PVS1, PM2, PM3	P	48.7	47.26
	TP53	2.76E-04	3	snv	c.743G>A	NM_000546.5	p.R248Q	PS3, PS4, PM1, PM2, PM5, PP1, PP3	P	64.0†	47.31
11-051	*CEBPA*			del	c.872_876delTGGGC	NM_001287424.1	p.L291fs	PVS1, PM2	LP	50.86	49.03
29-106	*CEBPA*			snv	c.1068T>A	NM_001287424.1	p.N356K	PM1, PM2	VUS	47.98	49.43
32-70	*TP53*			snv	c.730G>A	NM_000546.5	p.G244S	PS3, PS4, PM1, PM2, PM5, PP1, PP3	P	45.32	34.6†
32-33	*GATA2*			del	c.599delG	NM_001145661.1	p.G200fs	PVS1, PS4, PM2	P	46.91	46.97

KOVA_AF, Korean Variant Archive allele frequency; gnomAD_AC, Genome Aggregation Database Allele Count; NT, nucleotide; AA, amino-acid; ACMG, American College of Medical Genetics and Genomics; PVS, pathogenic very strong; PS, pathogenic strong; PM, pathogenic moderate; P, pathogenic; LP, likely pathogenic; VUS, variant of uncertain significance; VAF, variant allele frequency; CR, complete remission.

† Variants marginally outside the VAF screening range (40–60%) were reviewed individually and considered germline if supported by consistent VAFs in CR samples and classified as pathogenic according to ACMG guidelines.

Table 2. Clinical Characteristics of patients with germline mutations identified by serial targeted next-generation sequencing.

Patient number	Age (years)/ sex	Variant	Cytogenetics	Accompanying somatic mutations	Sampling at CR status	Received allogeneic HCT	Last follow-up status	Follow-up duration (months)
11-130	65/M	*DDX41*	46,XY [20]	*DDX41*	After 1st decitabine and venetoclax	Yes	Dead with AML	23.2
13-34	57/M	*DDX41*	46,XY [20]	*DDX41, TP53*	After 1st induction (idarubicin + cytarabine)	Yes	Dead	12.4
16-10	67/M	*DDX41*	45,X,-Y[4]/46,XY[16]	Not detected	After 1st induction (idarubicin + cytarabine)	No	Dead with AML	22.4
16-19	73/M	*DDX41*	45,X,-Y[5]/46,XY[35]	Not detected	After 1st induction (idarubicin + cytarabine)	No	Dead with AML	36.7
16-20	77/F	*DDX41*	46,XX[50]	Not detected	After 2nd decitabine	No	Dead with AML	20.6
16-21	61/F	*DDX41*	46,XY[40]	*ASXL1*	After 1st induction (daunorubicin + cytarabine)	Yes	Dead	8.1
19-13	72/M	*DDX41*	46,XY, del(20)(q11.2)[2]/46, XY[23]	Not detected	After 4th decitabine	No	Dead with AML	25.3
20-1	59/F	*DDX41*	Unknown	Not detected	After 1st induction (idarubicin + cytarabine)	No	Alive	43.1
24-6	50/F	*DDX41*	46,XX[20]	*CEBPA, DDX41, NPM1, NRAS*	After 1st induction (idarubicin + cytarabine)	Yes	Alive	58.2
27-4	60/M	*DDX41*	46,XY[20]	Not detected	After 1st induction (idarubicin + cytarabine)	No	Dead	30.8
29-10	50/M	*DDX41*	46,XY,t(11;19)(q23;p 13.1)[8], 46,XY[12]	*NOTCH3, NRAS*	After 1st induction (daunorubicin + cytarabine)	No	Dead	4.0
13-38	52/F	*MPL*	46,XX[20]	*DNMT3A, IDH1, NPM1, FLT3*-ITD	After 1st induction (idarubicin + cytarabine)	Yes	Dead	9.1
32-34	48/M	*DNAH5*	46,XY[20]	*TET2, NPM1, FLT3*-ITD, *CEBPA*	After 1st induction (idarubicin + cytarabine)	Yes	Alive	49.0
18-8	34/M	*DNAH5*	46XY,t(7;11),inv(9)[20]	*TET2, GATA2*	After 1st induction (daunorubicin + cytarabine)	Yes	Alive	28.0
29-32	52/F	*DNAH5* / *TP53*	44~46,XX,+add(1)(q21),-5,-14,-15,-15,-21,+3~4mar[cp16] 46,XX[4]	*BCOR*	After 1st induction (daunorubicin + cytarabine)	Yes	Dead	8.2
11-051	62/F	*CEBPA*	46,XX[20]	*CEBPA, RAD21, SMC1A, WT1*	After 1st induction (idarubicin + cytarabine)	Yes	Alive	54.1
29-106	47/F	*CEBPA*	48,XX, del(9)(q?),+10, +21[7] 47,XX, del(9),+10[5] 47,X,-X,+10,+21[6] 47,XX,+21[2]	*CEBPA*	After 1st induction (daunorubicin + cytarabine)	Yes	Dead	33.4
32-70	73/M	*TP53*	45,X,-Y[20]	*DNMT3A, FLT3*-TKD, *NPM1, SMC3*	After 1st decitabine and venetoclax	Yes	Alive	31.3
32-33	21/M	*GATA2*	45,XY,-7[30]	*BRAF, CSF3R, NRAS, RUNX1*	After 1st induction (idarubicin + cytarabine)	Yes	Alive	48.1

AA, amino-acid; CR, complete remission; HCT, hematopoietic cell transplantation; AML, acute myeloid leukemia.

Table 3. Clinical characteristics of 343 patients.

	Total	Germline mutation group	Non-germline mutation group	*P* value
	N = 343	N = 19	N = 324	
Age in years, median (range)	58 (19–80)	59 (21–77)	54 (19–80)	0.081
Sex, male (%)	192 (56.0)	11 (57.9)	180 (55.6)	1.000
WBC, x 10^9/l, median (range)	10.19 (0.13–335.4)	2.91 (0.42–79.48)	12.9(0.13–335.4)	0.064
Marrow blast, % (range)	64 (0–100)	34 (3–94)	65 (0–100)	0.004
Marrow cellularity, no (%)	311 80 (5–100)	16 50 (5–95)	295 80 (10–100)	<0.001
Cytogenetic risk, total				0.016
Favorable (%)	74 (21.6)	0 (0)	74 (22.8)	
Intermediate (%)	212 (61.8)	14 (73.7)	198 (61.1)	
Adverse (%)	53 (15.5)	4 (21.0)	49 (15.1)	
Not available	4 (1.2)	1 (5.3)	3 (0.9)	
Disease risk group by 2022 ELN recommendations				<0.001
Favorable (%)	153 (44.6)	1 (5.3)	152(46.9)	
Intermediate (%)	91 (26.5)	11 (57.9)	80 (24.7)	
Adverse (%)	95 (27.7)	6 (31.6)	89 (27.5)	
Not available	4 (1.2)	1 (5.3)	3 (0.9)	
Received intensive induction therapy	322 (93.9)	15 (78.9)	307 (94.7)	0.022
No. of patients who received allogeneic HCT (%)	229 (66.8)	12 (63.2)	217 (67.0)	0.804
Donor type				0.492
Matched related (%)	91 (39.7)	3 (25.0)	88 (40.6)	
Unrelated (%)	74 (32.3)	4 (33.3)	70 (32.3)	
Haploidentical (%)	64 (27.9)	5 (41.7)	59 (27.2)	
Median number of mutations (range)	3 (0–11)	3 (1–6)	3 (0–11)	0.485

WBC, white blood cell; ELN, European LeukemiaNet; HCT, hematopoietic cell transplantation.

Serial next-generation sequencing for detecting germline predisposition in acute myeloid leukemia

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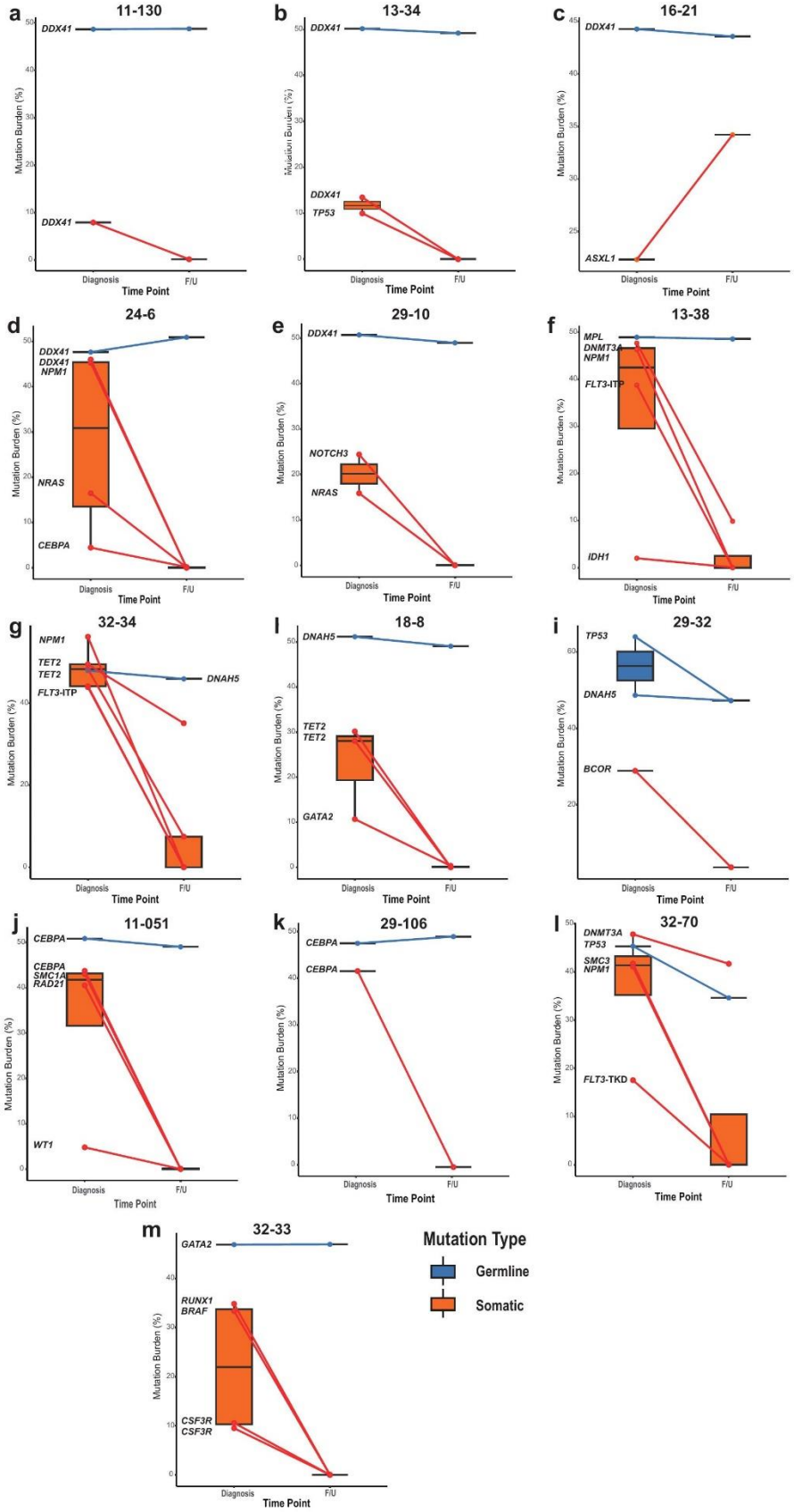

Figure S1. The dynamics of germline and somatic variant allele frequencies (VAFs) of mutations at diagnosis and at complete remission (CR) in each patient. F/U, follow-up.

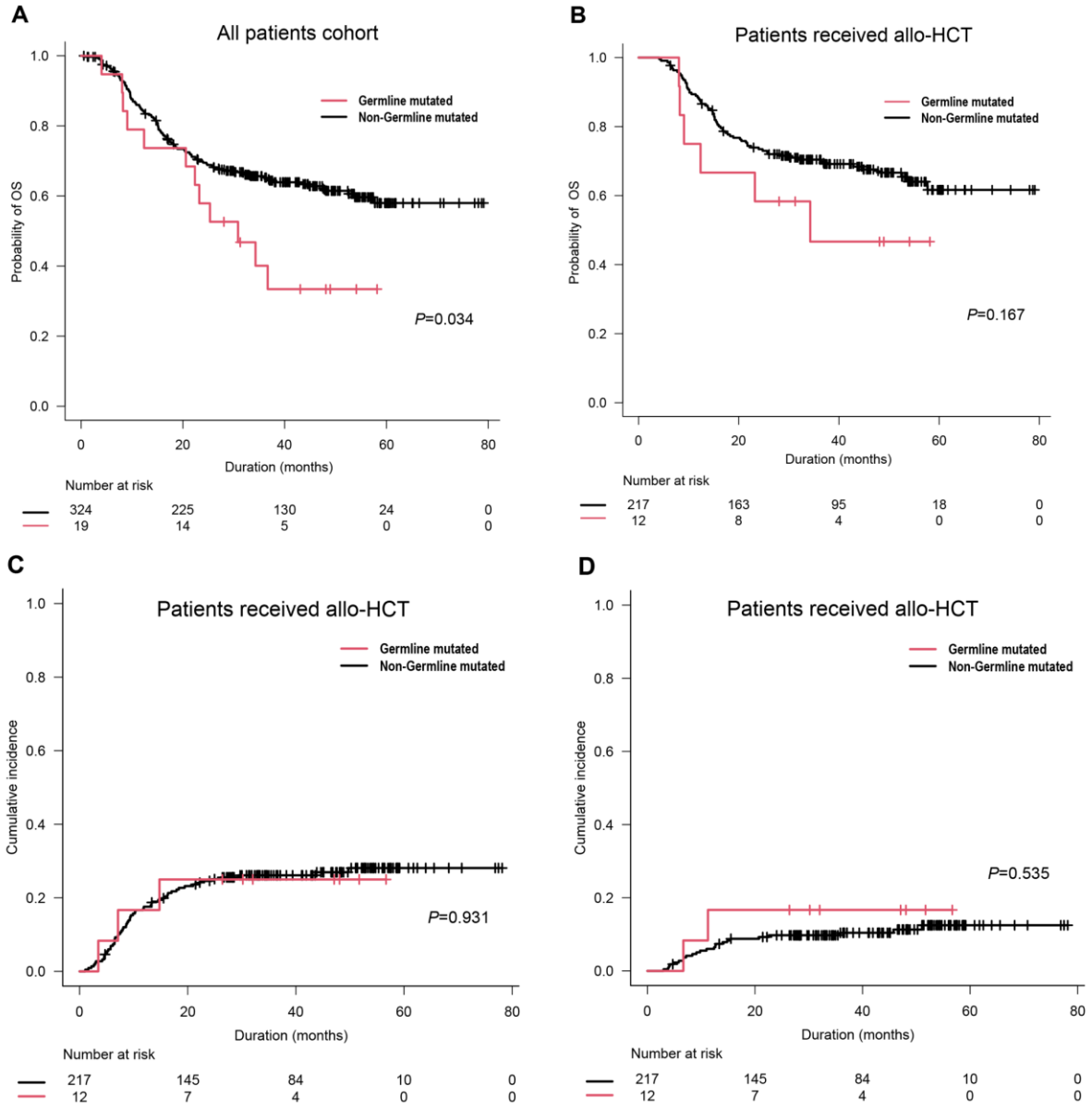

Figure S2. Prognostic significance according to presence of germline mutations. (A) Overall survival (OS) of entire patient cohort based on germline mutation status. (B) OS, (C) cumulative incidence of relapse, and (D) cumulative incidence of non-relapse mortality of patients who underwent allogeneic hematopoietic cell transplantation (HCT) stratified by germline mutation status.

Table S1. The list of 83 targeted genes

List of 83 targeted genes
ABL1
ARID2
ASXL1
ASXL2
ATRX
BCOR
BCORL1
BRAF
CALR
CBL
CBLB
CBLC
CCND2
CDKN2A
CEBPA
CREBBP
CSF3R
CUX1
DDX41
DDX5
DDX6
DNAH11
DNAH5
DNAI1
DNMT3A
ETV6
EZH2
FBXW7
FLT3
FOXP1
GATA1
GATA2
GNAS
GNB1
HMGCLL1
HRAS
IDH1
IDH2
IKZF1
JAK1
JAK2
JAK3
KDM6A

KIT
KMT2A
KMT2C
KRAS
MECOM
MGA
MN1
MPL
MYD88
NDC80
NF1
NOTCH1
NOTCH3
NPM1
NRAS
PDGFRA
PDGFRB
PHF6
PIGA
PPM1D
PTEN
PTPN11
RAD21
RB1
RUNX1
SETBP1
SF3A1
SF3B1
SF3B2
SMC1A
SMC3
SRSF2
STAG2
SUZ12
TET2
TP53
TP63
U2AF1
WT1
ZRSR2