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Features and impacts on the prognosis of gene mutations in patients with acute myeloid leukemia

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To explore features and impacts on the prognosis of common gene mutations in acute myeloid leukemia (AML), we assessed mutation status as well as variant allele frequency (VAF) of 24 genes in 81 AML patients by next-generation sequencing (NGS) technology. Eighty-six percentages of patients showed at least one mutation. Mutation in BCOR was associated with lower complete remission (CR) rate, whereas double mutation in CEBPA was associated with a favorable odds ratio for CR achievement. TP53 mutation was associated with inferior overall survival (OS) in univariate analysis. Multivariate analysis confirmed the negative effect of adverse cytogenetic abnormalities on survival. Mutation in RUNX1 and ZRSR2 had negative impacts on OS in patients with wild-type TP53. VAF of SRSF2 mutation was observed negatively correlated with OS. In conclusion, our study suggested that mutations in BCOR and spliceosomes might predict worse outcomes, and VAF of gene mutations may play a crucial role in outcomes of AML patients.

Key words: acute myeloid leukemia, next-generation sequencing, gene mutation, variant allele frequency

Acute myeloid leukemia (AML), characterized by the clonal expansion of myeloid precursors, is a highly heterogeneous hematological malignancy. Gene mutations have been proved to be crucial for the prognostic stratification of AML patients. The European Leukemia Net (ELN) classification classifies AML patients into three groups based on cytogenetic and molecular alterations. According to the 2017 ELN genetic-risk classification, favorable-risk group-defining mutations include double mutation in CEBPA and NPM1 mutation with no FLT3-internal tandem duplication (FLT3-ITD) or low FLT3-ITD allelic ratio (FLT3-ITDlow); the intermediate-risk group-defining mutations include NPM1 mutation and FLT3-ITD with high allelic ratio (FLT3-ITDhigh); and the adverse-risk group-defining mutations include RUNX1 mutation, ASXL1 mutation, TP53 mutation, and NPM1 wild-type with FLT3-ITD high [1]. With the wild use of next-generation sequencing (NGS) technology, the mutational spectrum in AML has been further expanded [2]. Moreover, the variant allele frequency (VAF) of genes, which has not been involved in the current stratification system, has been reported to have impacts on clinical characteristics and outcomes in AML patients [3].

In this study, to strengthen the data of features and impacts of gene mutations in AML patients, we focused on the mutational status as well as VAFs of commonly mutated genes in AML patients and assessed whether or not they could predict different prognoses of patients.

Patients and methods

Subject population. The study was approved by the Ethics Committees of the Institute of Hematology, The Zhongshan People's Hospital according to the guidelines of the declaration of Helsinki. In this retrospective review, NGS analyses were performed in samples from 81 newly diagnosed AML who were presented to our hospital between July 10, 2017, and December 28, 2019. Patients with newly diagnosed acute promyelocytic leukemia were excluded. Demographic data including age, sex, prior history of dysplasia, blood counts with differentials, percentages of blasts in bone marrow (BM), percentages of blasts in peripheral blood (PB), and karyotype were collected and recorded at diagnosis. Patients received idarubicin plus cytarabine-based or hypomethylating agent-based therapy as induction therapy. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) therapy was performed in patients in first complete remission (CR) when an agreement of patients was obtained.

Cytogenetic and molecular analysis. Germline materials for analysis were isolated from mononuclear cells obtained from BM or PB at diagnosis. Cytogenetic analyses were

performed using R-banding techniques. Gene mutational status was determined by targeted amplicon sequencing using the MiSeq platform (Illumina, San Diego, California) targeting the following 24 genes: ASXL1, BCOR, CEBPA, DNMT3A, ETV6, EZH2, FLT3, GATA2, IDH1, IDH2, KIT, KRAS, NPM1, NRAS, PHF6, PTPN11, RUNX1, SF3B1, SRSF2, TET2, TP53, U2AF1, WT1, and ZRSR2. Human genome builds 19 was used as the reference for sequence alignment. All sequence variations were determined based on information from single nucleotide polymorphism databases, including Single Nucleotide Polymorphism Database (dbSNP), and the 1000 Genomes Project. For the FLT3 gene, the identification of ITDs was performed. The VAF of genes was calculated from the results of sequence reads detected divided by the overall coverage at the specific locus.

Statistical analysis. T-tests or Mann-Whitney U tests were used to assess the differences in continuous variables between groups depending on data normality. The χ^2 test or Fisher exact test was used to analyze the categorical variables. The correlation of two continuous variables was tested by the Pearson test or Spearman test. The CR rates were evaluated after induction therapy. The overall survival (OS) was defined as the duration from the initiation of therapy to the date of death or the date of the last follow-up. The probability of OS and survival curves were estimated using the Kaplan-Meier method, with the log-rank test used to compare the differences between groups. A Cox proportional hazards regression analysis model was built for the multivariate analysis of OS after initial variable selection. SPSS (version 26; IBM SPSS Statistics, Armonk, New York) was used for statistical analysis. A two-tailed p-value <0.05 was considered to indicate statistical significance.

Results

Clinical characteristics and outcomes. The median age at diagnosis of our patient cohort was 56 years (range, 18–87) and 54% of them were men. The overall CR rate was 53% and the median OS was 4 months (range, 1–12). The clinical characteristics of patients assigned to the genetic-risk groups according to the 2017 ELN classification are summarized in Table 1. There were 21%, 35%, and 44% of patients belonging to the favorable-, intermediate- and adverse-risk groups, respectively. Clinical characteristics, including sex ratio, percentage of prior history of dysplasia, white blood cell (WBC) counts, hemoglobin (Hb) counts, platelet (Plt) counts, percentages of blasts in PB, and percentages of blasts in BM, were comparable among the three risk groups. Five prognosis-related karyotypes, including t(8;21), inv(16), t(9;11), 11q rearrangements, and complex karyotype, were observed. None of the patients were found with other abnormal karyotypes mentioned in the 2017 ELN classification, like t(9;11), t(6;9), t(9;22), inv(3)/t(3;3), monosomy 5/del 5q, monosomy 7, monosomy 17/abnormalities in 17p, and monosomal karyotype. Response after induction therapy differed among groups. CR rates were 79%, 46%, and 44%, respectively, for the favorable-, intermediate-, and

Table 1. Clinical characteristics and outcomes of patients assigned to the risk groups according to the 2017 ELN classification.

Parameter	All patients N=81	Favorable-risk, N=17	Intermediate-risk N=28	Adverse-risk N=36	p-value
Median age (range), y	56 (18–87)	53 (22–87)	53 (18–78)	59 (20–83)	0.165
Male, no. (%)	44 (54)	9 (53)	17 (61)	18 (50)	0.708
Prior history of dysplasia, no. (%)	14 (17)	1 (6)	4 (14)	9 (25)	0.219
Median WBC count (range), × 10^9/l	34.4 (0.4–309.5)	35.2 (1.4–196.0)	45.4 (1.3–309.5)	25.1 (0.4–163.4)	0.390
Median Hb count (range), g/l	77 (35–140)	80 (44–140)	79 (53–129)	73 (35–114)	0.404
Median Plt count (range), × 10^9/l	76 (3–485)	103 (3–485)	81(5–340)	57 (7–348)	0.559
Median percentage of blasts in PB (range), %	43 (3–94)	46 (7–85)	46 (3–94)	38 (4–88)	0.729
Median percentage of blasts in BM (range), %	52 (13–94)	55 (13–83)	55(13–94)	49 (13–89)	0.489
Karyotype, no. (%)					
t(8;21)	9 (11)	6 (35)	1 (4)	2 (6)	0.003
inv(16)	2 (2)	2 (12)	0 (0)	0 (0)	0.043
t(9;11)	2 (2)	0 (0)	2 (7)	0 (0)	0.160
11q23 rearrangements	2 (2)	0 (0)	0 (0)	2 (6)	0.351
Complex	8 (10)	0 (0)	0 (0)	8 (22)	0.002
CR achievement, no. (%)	31/59 (53)	11 (79)	10 (46)	10 (44)	0.090
Allo-HSCT, no. (%)	8/81 (10)	1 (6)	5 (18)	2 (6)	0.242
Median OS (range), months	4 (1–12)	7 (1–12)	6 (1–12)	2 (1–11)	0.005

Abbreviations: WBC-white blood cell; Hb-hemoglobin; Plt-platelet; PB-peripheral blood; BM-bone marrow; CR-complete remission; Allo-HSCT-allogeneic hematopoietic stem cell transplantation; OS-overall survival

adverse-risk groups (p=0.090). The median OS of favorable-risk patients was 7 months, differed from 6 months and 2 months of intermediate-risk and adverse-risk patients, respectively (p=0.005).

Mutational landscape. At least one mutation was detected in 70/81 (86%) patients, with an average of 3 mutations per patient (range, 0–6). A single mutation was observed in 16 patients (20%), double mutations were observed in 22 patients (27%), triple mutations were observed in 15 patients (19%), and quadruple mutations were observed in 12 patients (15%). Co-occurrence of mutations in different genes is summarized in Figure 1, while no significant negative correlations were found. FLT3-ITD and IDH1 mutations were associated with the presence of NPM1 mutations, respectively (p<0.001, p=0.003, respectively). Mutations in DNMT3A were positively associated with NRAS mutations (p=0.047). Double mutation in CEBPA often co-occurred with GATA2 and WT1 mutations, respectively (p=0.006 for both). SRSF2 mutations were most commonly found in patients with RUNX1, ASXL1, TET2, and STAG2 mutations, respectively (p=0.022, 0.007, 0.044, 0.006, respectively).

The frequencies of gene mutations listed according to the 2017 ELN classification are shown

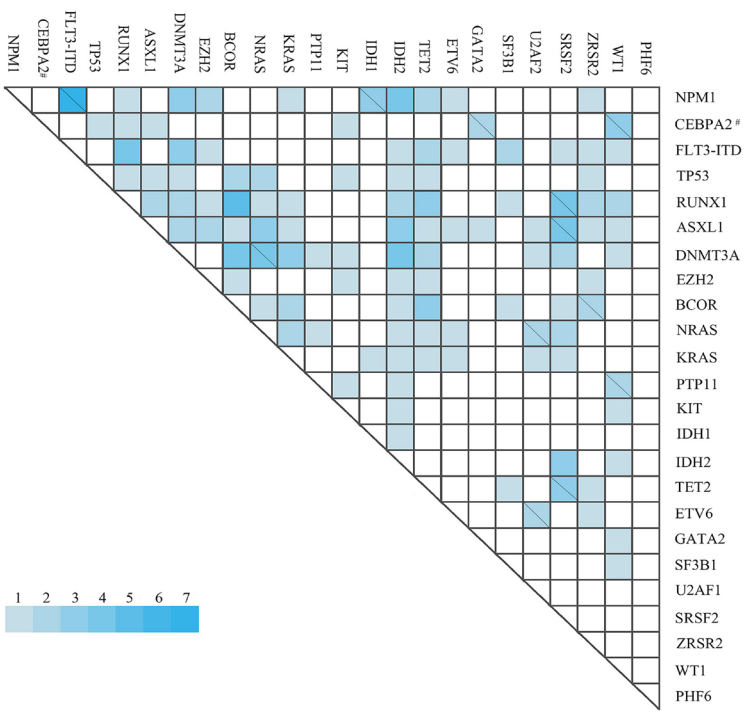

Figure 1. Co-occurrence of mutations in different genes. Differential blue intensity represents a different co-occurrence of mutations in terms of the number of patients. Patterned in diagonals indicates a significantly positive correlation. #CEBPA2 indicates the presence of a double mutation in CEBPA.

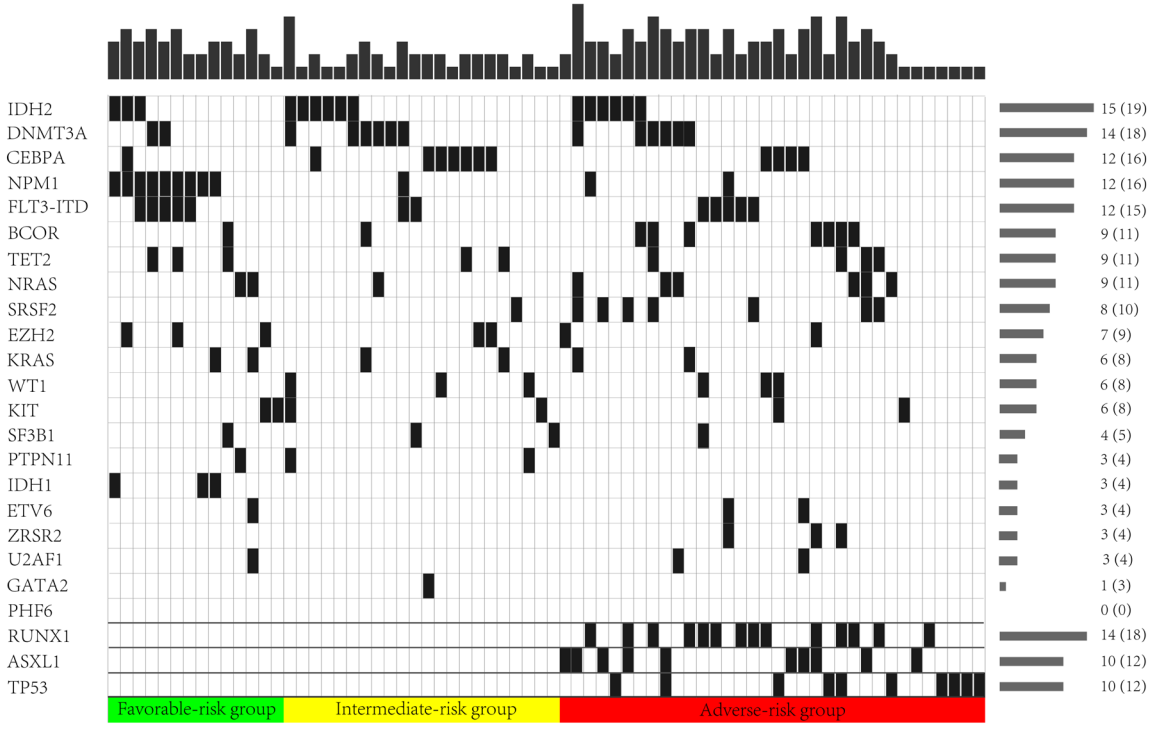

Figure 2. The landscape of mutated genes categorized into genetic-risk groups according to the 2017 ELN classification. Each black column represents an individual patient.

in Figure 2. Among risk group-defining genes, RUNX1 was the most frequently found mutated gene (18%), followed by NPM1 mutations and CEBPA mutations (both 16%). Besides these mutations, mutations in IDH2 (19%), DNMT3A (18%), BCOR (11%), and TET2 (11%) were commonly found. Comparisons of each gene mutation among risk groups are summarized in Supplementary Table S1. All IDH1 mutations were found in patients in the favorable-risk group (p=0.009). Twenty percent of patients in the adverse-risk group were found to have SRSF2 mutations, significantly differing from 4% of patients in the intermediate-risk group and none in the favorable-risk group (p=0.048). Mutations in IDH1, IDH2, EZH2, and TET2 were most frequently found in patients in the favorable-risk group (all detected in 18% of patients). In the intermediate-risk group, DNMT3A and IDH2 were the most frequently mutated genes (both detected in 22% of patients). In the adverse-risk group, in addition to risk group-defining genes (ASXL, TP53, RUNX1), mutations in BCOR and SRSF2 were the most commonly found mutations (both detected in 20% of patients), followed by mutations in DNMT3A and IDH2 (both detected in 17% of patients).

VAFs and Localization of mutations. For risk group-defining genes, the median VAFs of NPM1, ASXL1, RUNX1, and TP53 mutations were 36.10% (range, 22.10–47.30%), 33.90% (range, 11.30–44.20%), 36.40% (range, 4.70–92.50%), and 45.70% (range, 22.60–89.70%), respectively. For other commonly mutated genes in this study, the median VAFs of IDH1, IDH2, TET2, SRSF2, EZH2, BCOR, and DNMT3A mutations were 35.70% (range, 5.60–46.00%), 41.90% (range, 7.20–48.90%), 43.50% (range, 1.60–90.30%), 33.95% (range, 5.50–48.40%), 10.20% (range, 1.70–46.40%), 49.00% (range, 3.50–100%), and 42.70% (range, 2.00–95.70%), respectively.

We also demonstrated localizations of mutations in those commonly mutated genes (Figure 3). All the three IDH1 mutations were detected in exon 4 with a hotspot for missense mutations noted at P132, resulting in an arginine to glycine or histidine substitution. All IDH2 mutations were missense mutations in exon 4 with hotspots for missense mutations at R140 and R172. The majority of SRSF2 mutations were detected in exon 1, with a hotspot for missense mutations noted at P95. EZH2, TET2, and BCOR mutations were distributed throughout the exons without hotspots. DNMT3A mutations were commonly detected from exons 16 to 23, with a hotspot for missense mutations noted at R882.

Association of mutations with clinical characteristics. Relationships between clinical characteristics and the mutational status of genes were studied. Patients with RUNX1 mutations were found to be older (age ≥ 60 years, p=0.045). Patients with NPM1 mutations had higher percentages of blasts in BM (p=0.002). KIT mutations were positively associated with t(8;21) (p=0.019). ZRSR2 mutations were positively associated with a complex karyotype (p=0.011).

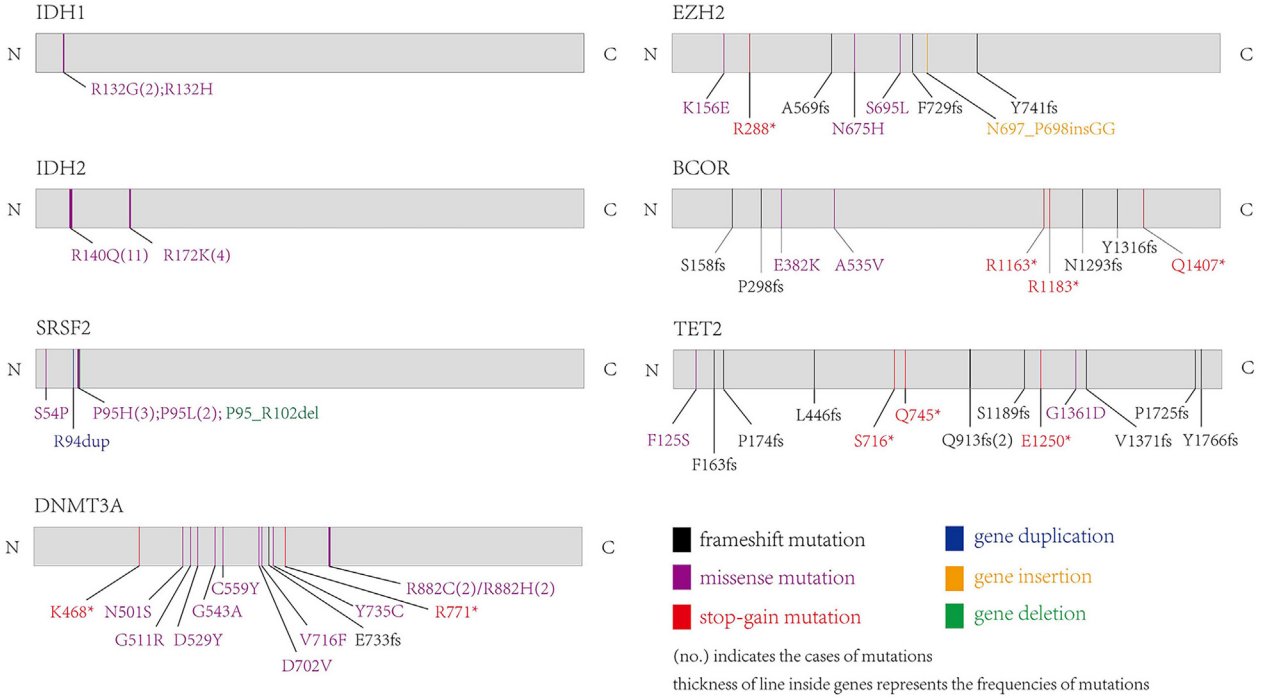

Figure 3. Schematic localization of commonly detected mutated genes other than risk group-defining genes. Hotspots were observed in IDH1, IDH2, and SRSF2 mutations.

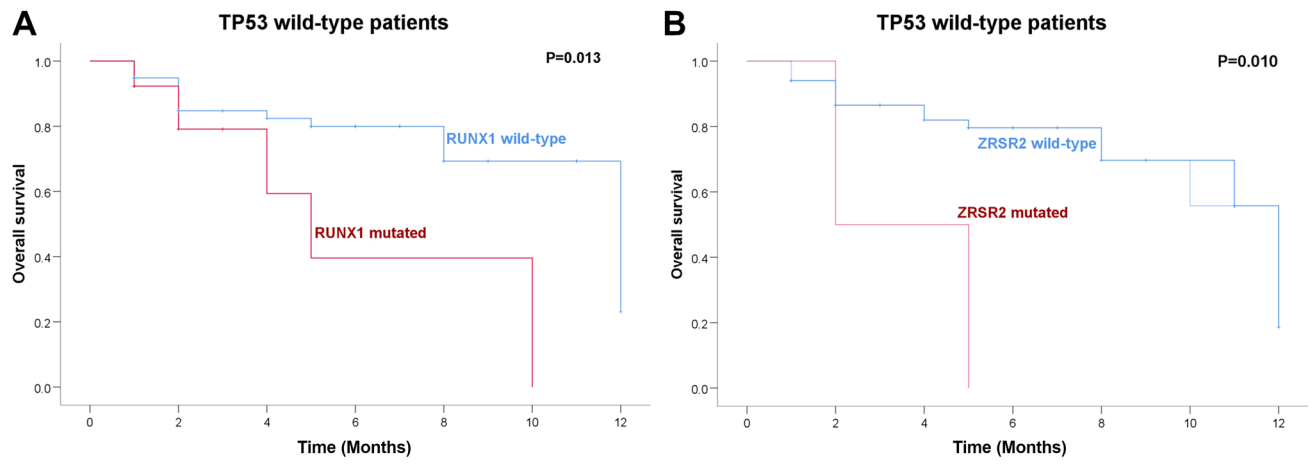

Figure 4. The negative impact of gene mutations in TP53 wild-type patients. A) RUNX1 mutation; B) ZRSR2 mutation.

We also explored the correlation between clinical characteristics and VAFs of genes. It was observed that VAFs of DNMT3A and IDH2 mutations were positively correlated with WBC counts (p=0.003, 0.044, respectively), VAFs of RUNX1 mutations were positively associated with Hb counts (p=0.017), and VAFs of SRSF2 mutations were positively associated with percentages of blasts in BM (p=0.047). In addition, the TP53 VAFs had a tendency toward a negative correlation with percentages of blasts in BM (p=0.770), whereas VAFs of other mutations had a tendency toward positive associations. Patients with adverse cytogenetics had higher TP53 VAFs than those without any adverse cytogenetic abnormalities (27.49% vs. 70.12%, p=0.032). Neither a positive nor negative correlation between VAFs and prior history of dysplasia was observed.

Impacts on CR achievement and OS. By univariate analysis (Supplementary Table S2), lower CR rates were observed in patients aged ≥60 years (p=0.034) and patients with BCOR mutations (p=0.018). The presence of double mutation of CEBPA was associated with a favorable odds ratio (OR) for CR achievement (p=0.032). Univariate analysis of OS showed that prior history of dysplasia, adverse cytogenetics, and TP53 mutation was negatively associated with survival (p=0.027, 0.004, 0.020, respectively). Variables that potentially had impacts on CR achievement and OS (p<0.08 in the univariate analysis or indicated by 2017 ELN classification) entered into the multivariable analysis (Table 2). The negative effect of adverse cytogenetic abnormalities on survival was observed (p=0.034), whereas none of the mutations was found to have significant impacts on CR achievement or survival. We also evaluated whether the VAFs of detected gene mutations were associated with survival time. Notably, negative correlation between VAFs of SRSF2 mutation and survival was observed (rs=–0.761, p=0.028).

To further evaluate the impacts of gene mutations on outcomes of patients, we performed univariate analyses for the CR achievement and OS within some subgroups. Among patients with a wild-type TP53, the presence of double mutation in CEBPA had a tendency toward better CR achievement rate (100% vs. 54.5%, respectively, p=0.070), while the presence of SRSF2 and BCOR mutation had a tendency toward resistance to induction therapy (0% vs. 59.2%, p=0.080; 16.7% vs. 60.9%, p=0.076, respectively). Besides, mutations in RUNX1 (p=0.013, HR=3.152) and ZRSR2 (p=0.010, HR=5.532) were found to be associated with an inferior OS in this subgroup (Figure 3). Among patients without adverse cytogenetics, the presence of RUNX1 mutation also had negative impacts on OS (p=0.054). Mutations in NPM1 were associated with higher odds of achieving a CR (100% vs. 48.6%, p=0.014), whereas mutations in BCOR were associated with lower odds (0% vs. 64.1%, p=0.011).

Discussion

In this study, we conducted a retrospective study of 81 newly diagnosed AML adults to evaluate the features and impacts of mutational status as well as VAF of commonly mutated genes detected by the NGS technology. We identified at least one mutation in the vast majority of patients and focused on the risk group-defining genes according to 2017 ELN classification as well as the most frequently found mutated genes in this patient cohort.

IDH2 mutation was the most frequently found mutation in this study, followed by RUNX1 mutation. Mutations in genes encoding for chromatin remodeling, transcription factors, and spliceosomes were more common in the adverse-risk group than the other two groups. The upper ranges of VAFs of NPM1, ASXL1, IDH1, IDH2, SRSF2, and EZH2, were restricted to approximately 50%, which suggested

Table 2. Multivariable analysis on CR achievement and OS.

Variable	CR achievement			OS		
	OR	95% CI	p-value	HR	95% CI	p-value
Age ≥60 years	0.394	0.096–1.619	0.196	1.964	0.779–4.954	0.153
Prior history of dysplasia	–	–	–	1.623	0.498–5.293	0.422
Adverse cytogenetics[$]	–	–	–	3.391	1.097–10.485	0.034
NPM1	9.431	0.754–117.935	0.082	–	–	–
RUNX1	0.185	0.012–2.911	0.230	3.007	0.890–10.162	0.076
TP53	–	–	–	2.649	0.600–11.690	0.198
CEBPA2[#]	7.1E8	0.000–	0.999	–	–	–
ASXL1	–	–	–	0.184	0.018–1.836	0.149
BCOR	0.246	0.021–2.878	0.246	–	–	–

Abbreviations: CR-complete remission; OS-overall survival; CI-confidence interval; OR-odds ratio; HR-hazard ratio. Notes: [$]Adverse-risk cytogenetics includes 11q23 rearrangement and complex; [#]CEBPA2 indicates double mutations in CEBPA

heterozygous mutations in genes. Higher VAFs suggested the presence of both heterozygous and homozygous mutations. Besides, consistent with the previous study, we observed high TP53 VAF with a low percentage of bone marrow blast in this study, which may suggest the presence of mutations in both leukemic and normal clones [3].

As expected, survival outcomes of patients among different risk groups assigned according to the 2017 ELN classification were significantly different, though the percentages of patients who underwent allo-HSCT were comparable among groups. Our study confirmed that double mutation in CEBPA was a favorable factor for CR achievement. TP53 and RUNX1 remain crucial genes to define patient subgroups with different prognoses. In the subset of patients with wild-type TP53, RUNX1 mutation has a negative effect on survival. Besides, RUNX1 mutations were associated with inferior survival in patients without adverse cytogenetics. However, we did not observe any effect of FLT3-ITD, NPM1, nor ASXL1 mutation on outcomes, which might be due to the small sample size.

BCOR gene is a key transcription regulatory factor, which plays an essential role in hematopoiesis [4]. We observed mutation in BCOR in 11% of patients and 20% of patients in the adverse-risk group. Despite the limited number of studies, BCOR mutations have been reported to tend toward a negative effect on OS in myelodysplastic syndrome (MDS) as well as AML patients [5–7]. In this study, BCOR mutation was observed associated with lower CR achievement rate but not inferior OS, which indicated further studies on the impacts of BCOR on outcomes might be necessary.

Spliceosome mutations are the most common mutations found in patients with MDS [8, 9]. The frequency of spliceosome mutations in our study is higher than previous studies reported in AML patients [10, 11]. We noticed that mutations of spliceosomes were more frequently found in the adverse-risk group than the other two groups, especially that the SRSF2 mutations were the most frequent mutations in the adverse-risk group. There was a significantly positive correlation between SRSF2 mutations and adverse risk genes including RUNX1 and ASXL1, which is consistent with other studies [12]. Although mechanisms remain unknown, SRSF2 mutations have been reported to have negative impacts on outcomes of MDS patients [13–16]. In AML, SRSF2 mutations were found to associate with a longer time to hematologic recovery [17]. In our study, ZRSR2 mutations were associated with worse outcomes in the subset of patients with wild-type TP53. Interestingly, we observed that higher VAF of SRSF2 mutations was associated with inferior survival, which has not been reported in previous studies to our knowledge. These results suggested that the spliceosome may be a potential biomarker in AML.

There are several limitations in this study. First, the number of patients in this study was limited.

Second, we did not evaluate the impact of a history of mutations prior to AML and whether the clearance of mutations after the induction therapy was associated with the outcomes. Third, we did not assess the features and impacts of different gene combinations.

In conclusion, in addition to 2017 ELN risk group-defining mutations, our results strengthen the data that mutations in BCOR and spliceosome might have prognostic impacts on outcomes of AML patients. Moreover, we demonstrated that VAF of gene mutations may be associated with survival in AML patients, so that the integration of VAF into prognostic classification might help to improve the identification of patients with different prognoses.

Supplementary information is available in the online version of the paper.

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Supplementary Information

Supplementary Table S1. Frequencies of gene mutations in patients with AML assigned to the genetic-risk groups according to the 2017 ELN classification.

Gene	All Patients	Favorable-risk	Intermediate-risk	Adverse-risk	p-value
NPM1, n/N tested (%)					<0.001
Mutated	12/76	9/17 (53)	1/25 (4)	2/34 (16)	
Wild-type	64/76	8/17 (47)	24/25 (96)	32/34 (84)	
Chromatin remodeling, n/N tested (%)					0.010
Mutated	23/79	4/17 (24)	3/27 (11)	16/35 (46)	
Wild-type	56/79	13/17 (76)	24/27 (89)	19/35 (54)	
ASXL1, n/N tested (%)					0.001
Mutated	10/81 (12)	0/17 (0)	0/28 (0)	10/36 (28)	
Wild-type	71/81 (88)	17/17 (100)	28/28 (100)	26/36 (72)	
EZH2, n/N tested (%)					0.359
Mutated	7/79 (9)	3/17 (18)	2/27 (7)	2/35 (6)	
Wild-type	72/79 (91)	14/17 (82)	25/27 (93)	33/35 (94)	
BCOR, n/N tested (%)					0.105
Mutated	9/79 (11)	1/17 (6)	1/27 (4)	7/35 (20)	
Wild-type	70/79 (89)	16/17 (94)	26/27 (96)	28/35 (80)	
RAS pathway, n/N tested (%)					0.813
Mutated	15/79	3/17 (3)	4/27 (5)	8/35 (7)	
Wild-type	64/79	14/17 (14)	23/27 (22)	27/35 (28)	
NRAS, n/N tested (%)					0.312
Mutated	9/79 (11)	2/17 (12)	1/27 (4)	6/35 (17)	
Wild-type	70/79 (89)	15/17 (88)	26/27 (96)	29/35 (83)	
KRAS, n/N tested (%)					0.753
Mutated	6/79 (8)	2/17 (12)	2/27 (7)	2/35 (6)	
Wild-type	73/79 (92)	15/17 (88)	25/27 (93)	33/35 (94)	
PTPN11, n/N tested (%)					0.228
Mutated	3/79 (4)	1/17 (6)	2/27 (7)	0/35 (0)	
Wild-type	76/79 (96)	16/17 (94)	25/27 (93)	35/35 (100)	
Kinase, n/N tested (%)					0.258
Present/Mutated	24/73 (33)	8/17 (50)	6/25 (24)	10/32 (31)	
Absent/Wild-type	49/73 (67)	9/17 (50)	19/25 (76)	22/32 (69)	
FLT3-ITD, n/N tested (%)					0.163
Present	12/80 (15)	5/17 (29)	2/27 (7)	5/36 (14)	
Absent	68/80 (85)	12/17 (71)	25/27 (93)	31/36 (86)	
FLT3-TKD, n/N tested (%)					0.898
Present	4/75 (5)	1/17 (6)	2/24 (8)	1/34 (3)	
Absent	71/75 (95)	16/17 (94)	22/24 (92)	33/34 (97)	
KIT, n/N tested (%)					0.753
Mutated	6/73 (8)	2/16 (13)	2/25 (8)	2/32 (6)	
Wild-type	69/73 (92)	14/16 (88)	23/25 (92)	30/32 (94)	
Methylation-related, n/N tested (%)					0.560
Mutated	34/79 (43)	9/17 (53)	12/27 (44)	13/35 (37)	
Wild-type	45/79 (57)	8/17 (47)	15/27 (56)	22/35 (63)	

Supplementary Table S1. ***Continued ...***

Gene	All Patients	Favorable-risk	Intermediate-risk	Adverse-risk	p-value
DNMT3A, n/N tested (%)					0.750
Mutated	14/79 (18)	2/17 (12)	6/27 (22)	6/35 (17)	
Wild-type	65/79 (82)	15/17 (88)	21/27 (78)	29/35 (83)	
IDH1, n/N tested (%)					0.009
Mutated	3/79 (4)	3/17 (18)	0/27 (0)	0/35 (0)	
Wild-type	76/79 (96)	14/17 (82)	27/27 (100)	35/35 (100)	
IDH2, n/N tested (%)					0.932
Mutated	15/79 (19)	3/17 (18)	6/27 (22)	6/35 (17)	
Wild-type	64/79 (81)	14/17 (82)	21/27 (78)	29/35 (83)	
TET2, n/N tested (%)					0.530
Mutated	9/79 (11)	3/17 (18)	2/27 (7)	4/35 (11)	
Wild-type	70/79 (89)	14/17 (82)	25/27 (93)	31/35 (89)	
Transcription factors, n/N tested (%)					0.013
Mutated	27/75 (37)	2/16 (13)	7/25 (28)	18/34 (53)	
Wild-type	48/75 (63)	14/16 (88)	18/25 (72)	16/34 (47)	
RUNX1, n/N tested (%)					<0.001
Mutated	14/81 (18)	0/17 (0)	0/28 (0)	14/36 (39)	
Wild-type	67/81 (82)	17/17 (100)	28/28 (100)	22/36 (61)	
CEBPA, n/N tested (%)					0.189
Mutated	12/74 (16)	1/16 (6)	7/25 (27)	4/33 (12)	
Wild-type	62/74 (84)	15/16 (94)	18/25 (72)	29/33 (88)	
CEBPA1*, n/N tested (%)					0.251
Mutated	6/74 (8)	1/16 (6)	4/25 (16)	1/33 (3)	
Wild-type	68/74 (92)	15/16 (94)	21/25 (84)	32/33 (97)	
CEBPA2#, n/N tested (%)					0.474
Mutated	6/74 (8)	0/16 (3)	3/25 (12)	3/33 (9)	
Wild-type	68/74 (92)	16/16 (100)	22/25 (88)	30/33 (91)	
ETV6, n/N tested (%)					0.438
Mutated	3/79 (4)	1/17 (6)	0/27 (0)	2/35 (6)	
Wild-type	76/79 (96)	16/17 (94)	27/27 (100)	33/35 (94)	
GATA2, n/N tested (%)					1.000
Mutated	1/79 (3)	0/17 (0)	0/27 (0)	1/35 (3)	
Wild-type	78/79 (97)	17/17 (100)	27/27 (100)	34/35 (97)	
Spliceosomes, n/N tested (%)					0.031
Mutated	18/79 (23)	2/17 (12)	3/27 (11)	13/35 (37)	
Wild-type	61/79 (77)	15/17 (88)	24/27 (89)	22/35 (63)	
SF3B1, n/N tested (%)					0.818
Mutated	4/79 (5)	1/17 (6)	2/27 (7)	1/35 (3)	
Wild-type	75/79 (95)	16/17 (94)	25/27 (93)	34/35 (97)	
U2AF2, n/N tested (%)					0.438
Mutated	3/79 (4)	1/17 (6)	0/27 (0)	2/35 (6)	
Wild-type	76/79 (96)	16/17 (94)	27/27 (100)	33/35 (94)	
SRSF2, n/N tested (%)					0.048
Mutated	8/79 (10)	0/17 (0)	1/27 (4)	7/35 (20)	
Wild-type	71/79 (90)	17/17 (100)	26/27 (96)	28/35 (80)	
ZRSR2, n/N tested (%)					0.310
Mutated	3/79 (4)	0/17 (0)	0/27 (0)	3/35 (9)	
Wild-type	76/79 (96)	17/17 (100)	27/27 (100)	32/35 (91)	
Tumor suppressors, n/N tested (%)					0.005
Mutated	15/81 (18)	0/17 (0)	3/28 (11)	12/36 (33)	
Wild-type	66/81 (82)	17/17 (100)	25/28 (89)	24/36 (67)	

Supplementary Table S1. ***Continued ...***

Gene	All Patients	Favorable-risk	Intermediate-risk	Adverse-risk	p-value
TP53, n/N tested (%)					0.001
Mutated	10/81 (12)	0/17 (0)	0/28 (0)	10/36 (28)	
Wild-type	71/81 (88)	17/17 (100)	28/28 (100)	26/36 (72)	
WT1, n/N tested (%)					0.478
Mutated	6/79 (8)	0/17 (0)	3/27 (11)	3/35 (9)	
Wild-type	73/79 (92)	17/17 (100)	24/27 (89)	32/35 (91)	
PHF6, n/N tested (%)					NA
Mutated	0/79 (0)	0/17 (0)	0/27 (0)	0/35 (0)	
Wild-type	79/79 (100)	17/17 (100)	27/27 (100)	35/35 (100)	

Note: *CEBPA1 indicates single mutation in CEBPA; #CEBPA2 indicates double mutations in CEBPA

Supplementary Table S2. Univariate analysis on CR achievement and OS.

Variable	CR achievement			OS		
	OR	95% CI	p-value	HR	95% CI	p-value
Age ≥60 years	0.292	0.095–0.895	**0.034**	2.003	0.882–4.550	**0.073**
Prior history of dysplasia	0.268	0.063–1.137	0.095	2.558	3.461–6.298	**0.027**
WBC counts ≥100×10^9/l	2.893	0.283–29.558	0.614	1.501	0.439–5.133	0.498
Adverse-risk cytogenetics[$]	1.400	0.299–6.560	0.720	3.414	1.335–8.731	**0.004**
NPM1	7.000	0.799–61.329	**0.063**	0.639	0.147–2.784	0.534
FLT3-ITD	1.185	0.241–5.839	1.000	0.791	0.234–2.677	0.694
FLT3-ITD -/low NPM1+	5.760	0.645–51.459	0.116	0.813	0.186–3.545	0.775
RUNX1	0.207	0.039–1.098	**0.071**	2.365	0.917–6.098	**0.056**
DNMT3A	0.433	0.122–1.539	0.220	1.118	0.412–3.029	0.815
IDH2	1.008	0.269–3.777	1.000	0.604	0.178–2.045	0.381
TP53	0.575	0.089–3.719	0.661	3.359	1.088–10.366	**0.020**
CEBPA	3.652	0.695–19.180	0.161	0.324	0.043–2.450	0.232
CEBPA1[*]	0.724	0.094–5.563	1.000	0.636	0.084–4.820	0.646
CEBPA2[#]	1.4E9	0.000–	**0.032**	0.044	0.000–150.99	0.233
KIT	3.111	0.323–29.942	0.389	0.043	0.000–71.806	0.184
ASXL1	0.643	0.131–3.162	0.698	0.435	0.058–3.243	0.383
IDH1	1.4E9	0.000–	1.000	2.163	0.285–16.401	0.428
TET2	0.140	0.015–1.287	0.083	1.075	0.315–3.662	0.905
SF3B1	0.000	0.000–	0.204	1.709	0.392–7.457	0.455
U2AF1	0.400	0.034–4.681	0.587	1.464	0.193–11.085	0.699
SRSF2	0.000	0.000–	0.089	0.929	0.266–3.245	0.900
ZRSR2	0.000	0.000–	0.204	3.728	0.851–16.328	**0.051**
EZH2	1.286	0.198–8.346	1.000	0.588	0.078–4.440	0.590
BCOR	0.090	0.010–0.795	**0.018**	1.166	0.342–3.972	0.799
STAG2	0.833	0.050–14.011	1.000	1.403	0.393–5.012	0.564
WT1	1.286	0.198–8.346	1.000	0.045	0.000–729.15	0.322
NRAS	1.778	0.299–10.587	0.678	1.335	0.304–5.861	0.690
KRAS	0.183	0.019–1.756	0.167	0.835	0.111–6.267	0.856
ETV6	0.833	0.050–14.011	1.000	1.464	0.193–11.085	0.699
GATA2	1.4E9	0.000–	0.495	0.048	0.000–13347	0.456
PTPN11	0.400	0.034–4.681	0.587	0.047	0.000–2062.0	0.379

Abbreviations: CR-complete remission; OS-overall survival; CI-confidence interval; OR-odds ratio; HR-hazard ratio. Notes: [$]Adverse-risk cytogenetics includes 11q23 rearrangement and complex karyotype; *CEBPA1 indicates single mutation in CEBPA; #CEBPA2 indicates double mutations in CEBPA