RESEARCH ARTICLE

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Comprehensive Mutation Profile in Acute Myeloid Leukemia Patients with *RUNX1-RUNX1T1* or *CBFB-MYH11* Fusions

RUNX1-RUNX1T1 veya *CBFB-MYH11* Füzyonları Olan Akut Myeloid Lösemili Hastalarda Detaylı Mutasyon Profili

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Abstract

Objective: This study was undertaken with the aim of better understanding the genomic landscape of core-binding factor (CBF) acute myeloid leukemia (AML).

Materials and Methods: We retrospectively analyzed 112 genes that were detected using next-generation sequencing in 134 patients with *de novo* CBF-AML. *FLT3-ITD*, *NPM1*, and *CEBPA* mutations were detected by DNA-PCR and Sanger sequencing.

Results: In the whole cohort, the most commonly mutated genes were *c-KIT* (33.6%) and *NRAS* (33.6%), followed by *FLT3* (18.7%), *KRAS* (13.4%), *RELN* (8.2%), and *NOTCH1* (8.2%). The frequencies of mutated genes associated with epigenetic modification, such as *IDH1*, *IDH2*, *DNMT3A*, and *TET2*, were low, being present in 1.5%, 0.7%, 2.2%, and 7.5% of the total number of patients, respectively. Inv(16)/t(16;16) AML patients exhibited more mutations of *NRAS* and *KRAS* (p=0.001 and 0.0001, respectively) than t(8;21) AML patients. Functionally mutated genes involved in signaling pathways were observed more frequently in the inv(16)/t(16;16) AML group (p=0.016), while the mutations involved in cohesin were found more frequently in the t(8;21) AML group (p=0.011). Significantly higher white blood cell counts were found in inv(16)/t(16;16) AML patients with *c-KIT* (*c-KIT*^{mut}) or *NRAS* (*NRAS*^{mut}) mutations compared to the corresponding t(8;21) AML/*c-KIT*^{mut} and t(8;21) AML/*NRAS*^{mut} groups (p=0.001 and 0.009, respectively).

Conclusion: The mutation profiles of t(8;21) AML patients showed evident differences from those of patients with inv(16)/t(16;16) AML. We have provided a comprehensive overview of the mutational landscape of CBF-AML.

Keywords: Core-binding factor, Acute myeloid leukemia, Mutation, Next-generation sequencing

Öz

Amaç: Bu çalışma çekirdek bağlama faktörü (ÇBF) akut myeloid löseminin (AML) genomik durumunu daha iyi anlamak amacıyla yapılmıştır.

Yöntemler: Yüz otuz dört de novo ÇBF-AML hastasında yeni nesil dizileme ile tespit edilen 112 geni geriye dönük olarak analiz ettik. *FLT3-ITD*, *NPM1* ve *CEBPA* mutasyonları DNA-PCR ve Sanger dizileme ile tespit edildi.

Bulgular: Bütün kohortta en sık mutasyonlu genler *c-KIT* (33,6%) ve *NRAS* (33,6%) ve ardından *FLT3* (%18,7), *KRAS* (%13,4), *RELN* (%8,2), *NOTCH1* (%8,2) idi. *IDH1, IDH2, DNMT3A* ve *TET2* gibi epigenetik modifikasyonla ilişkili mutasyona uğramış genlerin sıklığı düşüktü ve toplam hastaların sırasıyla %1,5, %0,7, %2,2 ve %7,5'inde mevcuttu. Inv(16)/t(16;16) AML hastalarında *NRAS* ve *KRAS* mutasyonları t(8;21) AML hastalarına göre daha fazlaydı (sırasıyla; p=0,001; 0,0001). İşlevsel olarak sinyal yolaklarında yer alan mutasyonlu genler inv(16)/t(16;16) AML grubunda daha çok gözlenirken (p=0,016), kohezin içinde yer alan mutasyonlar t(8;21) AML grubunda daha çok bulundu (p=0,011). *c-KIT (c-KIT*^{mut}) veya *NRAS* mutasyonları (*NRAS*^{mut}) olan inv(16)/t(16;16) AML hastalarında karşılığındaki t(8;21) AML/*c-KIT*^{mut} ve t(8;21) AML/*NRAS*^{mut} gruplarına göre beyaz küre sayısı daha yüksek bulundu (sırasıyla; p=0,001; 0,009,).

Sonuç: t(8;21) AML hastalarının mutasyon profilleri inv(16)/t(16;16) AML'den belirgin farklılıklar gösterdi. Bu çalışmada ÇBF-AML'nin mutasyon profili kapsamlı bir biçimde incelenmiştir.

Anahtar Sözcükler: Çekirdek bağlama faktörü, Akut myeloid lösemi, Mutasyon, Yeni nesil sekanslama

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Introduction

Cases of acute myeloid leukemia (AML) involving the core-binding factor (CBF) include AML with t(8;21) and inv(16)/t(16;16) chromosomal translocations, leading to the *RUNX-RUNX1T1* and *CBFB-MYH11* fusions genes, respectively. Such AML patients account for approximately 25% of pediatric and 15% of adult de novo AML cases [1], and CBF-AML was recognized as a unique entity in the 2016 World Health Organization classification of myeloid neoplasms and acute leukemia [2]. Accumulating evidence has revealed that the t(8;21)(q22;q22) and inv(16)/t(16;16) CBF rearrangements are associated with favorable outcomes relative to other cytogenetic subtypes and that allogeneic hematopoietic stem cell transplantation is not generally recommended during the first complete remission (CR) [3,4]. However, relapse occurs in up to 40% of these cases, indicating clinicopathological heterogeneity within this AML subset [5,6,7,8]. Further investigation is still needed to better understand leukemogenesis and disease progression.

Previous findings have demonstrated that expressions of translocation-encoded AML1 or CBF fusion proteins are insufficient by themselves to induce a full leukemic phenotype [9]. Further evidence supporting this model comes from the fact that mutations in genes activating tyrosine kinase signaling (including *KIT*, *N/KRAS*, and *FLT3*) are frequent in both CBF-AML subtypes [6,10]. Nonetheless, data regarding the prognostic significance of *KIT* and *RAS* in CBF-AML are contradictory. Duployez et al. [11] reported the presence of additional aberrations in >90% of CBF-AML cases, and mutations in epigenetic modifications or cohesin genes were associated with poor prognosis in t(8;21) AML patients with TK pathway mutations using next-generation sequencing (NGS). Ishikawa et al. [12] revealed that the c-*KIT* exon 17 mutation and the presence of extramedullary tumors in t(8;21) AML patients were poor prognostic factors for relapse-free survival, as were the loss of chromosome X or Y and *NRAS* mutation in patients with inv(16)/t(16;16). These findings highlight the multiclonality of CBF-AML and suggest that the prognostic impact may differ in the context of certain gene mutations between AML patients with t(8;21) and those with inv(16)/t(16;16).

Comprehensive genetic analysis using NGS may be helpful in refining our understanding of the prognosis of CBF-AML [11]. To the best of our knowledge, limited data are available regarding the impact of companion gene mutations in CBF-AML. To better characterize this subtype and to better understand the role of co-mutations in CBF-AML, we performed extensive mutational analysis by NGS for 134 CBF-AML patients. The clinical value of co-mutations in CBF-AML patients was also explored.

Materials and Methods

Patients

A total of 134 newly diagnosed de novo CBF-AML patients were selected from the Affiliated Changzhou Second Hospital of Nanjing Medical University, Wuxi Third People's Hospital, and First Affiliated Hospital of Soochow University from May 2016 to June 2021. The diagnosis of CBF-AML was based on the 2008 definition of the World Health Organization [2]. Eighty AML patients with t(8;21)/*RUNX1-RUNX1T1* and 54 patients with inv(16)/t(16;16)/*CBFB-MYH11* were included in the analysis. The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

Mutational Analysis by Next-Generation Sequencing

Genomic DNA was extracted from fresh bone marrow or peripheral blood samples with the QIAamp DNA Mini Kit (QIAGEN GmbH, Germany) following the manufacturer's instructions. The NGS library was prepared using at least 200 ng of genomic DNA. Massively parallel sequencing was performed with an Illumina next-generation sequencer and a variant allele frequency of >3% was used as the threshold for calling single-nucleotide variants. A high depth of coverage (1000x) was obtained for 112 genes, including whole coding regions known to be frequently mutated in hematological malignancies, such as genes involved in epigenetic regulators, signaling pathways, transcription factors, spliceosomes, cohesin complex, tumor suppressors, and chromatin modifiers (Table 1). Searches were performed in the COSMIC database for altered DNA sequences deemed to be mutations or variants with IGV software and were confirmed in the SNP database (dbSNP). Polymerase chain reaction (PCR) followed by direct Sanger sequencing was used to detect *FLT3-ITD*, *NPM1* (exon 12), and *CEBPA* to avoid possible false-negative results due to limitations of NGS, as previously described [13,14].

Other Cytogenetic and Molecular Abnormality Screening

Bone marrow (BM) cells were collected and cultured at the time of the initial diagnosis. The presence of the t(8;21) or inv(16)/t(16;16) rearrangement was determined by the conventional G/R banding method. Fluorescence in situ hybridization (FISH) of interphase nuclei and/or metaphases was performed for the chimeric genes *RUNX1-RUNX1T1* and *CBFB-MYH11*. The fusion transcripts were identified by real-time quantitative PCR (RT-qPCR) method using bone marrow or peripheral blood samples at diagnosis, as previously described [15].

Statistical Analysis

CR was defined as <5% blast cells, no Auer rods, and no clusters of blast cells by bone marrow analysis as well as no evidence

of extramedullary leukemia. All statistical analyses of the data were carried out using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY, USA). The chi-square and Fisher exact tests were used for comparisons of categorical variables between different cohorts. The Student t-test was used to analyze continuous variables with normal distribution and the Mann-Whitney U test was used for data that did not comply with normal distribution. Values of $p<0.05$ were considered statistically significant.

Results

Patient Characteristics

The 134 CBF-AML patients enrolled in this study included 65 women and 69 men with a median age of 35.5 years (range: 16.0-73.0 years). The median white blood cell (WBC) count was $15.3x10^9$/L (range: 0.9 to $156.0x10^9$/L), median hemoglobin (Hb) level was 81 g/L (range: 39.0 to 124.0 g/L), and median platelet count was $28.0x10^9$/L (range: 2.0 to $170.0x10^9$/L). Patients with inv(16) or t(16;16) AML tended to be older than t(8;21) AML patients (41 vs. 32 years, p=0.048) and also had higher WBC counts ($35.9x10^9$/L vs. $7.8x10^9$/L, p=0.0001). No significant differences were identified regarding gender, Hb level, or platelet count between patients with t(8;21) and inv(16)/t(16;16) AML.

By conventional chromosome analysis, 41.0% (55/134) of the CBF-AML patients were found to have secondary cytogenetic abnormalities. Additional chromosomal alterations were found in 45.0% (36/80) and 35.2% (19/54) of the patients with t(8;21) and inv(16)/t(16;16), respectively. Loss of the X or Y chromosome was identified as the most common secondary alteration (33/134, 24.6%), followed by trisomy 22 (13/134, 9.7%). In patients with inv(16)/t(16;16), the most frequently identified additional cytogenetic alterations were trisomy 22 and trisomy 8, which were found in 20.1% (13/54) and 9.3% (5/54) of these patients, respectively.

Table 1. The 112 genes analyzed in this study.

Number	Gene	Number	Gene	Number	Gene	Number	Gene
1	*ABL1*	29	*DNM2*	57	*KMT2A*	85	*SETBP1*
2	*ACD*	30	*DNMT3A*	58	*KRAS*	86	*SETD2*
3	*ANKRD26*	31	*DNMT3B*	59	*MAPK1*	87	*SF1*
4	*ARIDIA*	32	*ECT2L*	60	*KMT2D*	88	*SF3A1*
5	*ASXL1*	33	*EED*	61	*MPL*	89	*SF3B1*
6	*ATG2B*	34	*EP300*	62	*MYC*	90	*SH2B3*
7	*ATM*	35	*ETNK1*	63	*MYD88*	91	*SMC1A*
8	*B2M*	36	*EZH2*	64	*NF1*	92	*SMC3*
9	*BCOR*	37	*FAM46C*	65	*NOTCH1*	93	*SRP72*
10	*BCOR1*	38	*BRINP3*	66	*NOTCH2*	94	*SRSF2*
11	*BIRC3*	39	*FAT1*	67	*NPM1*	95	*STAG2*
12	*BRAF*	40	*FBXW7*	68	*NRAS*	96	*STAT3*
13	*CALR*	41	*FGFR3*	69	*PAX5*	97	*SUZ12*
14	*CBL*	42	*FLT3*	70	*PDS5B*	98	*ETV6*
15	*CCND1*	43	*GATA1*	71	*PHF6*	99	*TERC*
16	*CCND3*	44	*GATA2*	72	*PIGA*	100	*TERT*
17	*CCR4*	45	*GATA3*	73	*PLCG1*	101	*TET2*
18	*CD79B*	46	*JAK1*	74	*PRKCB*	102	*TNFAIP3*
19	*CDC25C*	47	*JAK2*	75	*PRPF40B*	103	*TP53*
20	*CDKN2A*	48	*JAK3*	76	*PRPS1*	104	*TPMT*
21	*CEBPA*	49	*HNRNPK*	77	*PTEN*	105	*TRAF3*
22	*CREBBP*	50	*ID3*	78	*PTPN11*	106	*U2AF1*
23	*CSF3R*	51	*IDH1*	79	*RAD21*	107	*U2AF2*
24	*CUX1*	52	*IDH2*	80	*RB1*	108	*WHSC1*
25	*CXCR4*	53	*IKZF1*	81	*RBBP6*	109	*WT1*
26	*DDX3X*	54	*IL7R*	82	*RELN*	110	*XPO1*
27	*DDX41*	55	*KDM6A*	83	*RHOA*	111	*ZRSR2*
28	*DIS3*	56	*KIT*	84	*RUNX1*	112	*ZMYM3*

Trisomies 8 and 22 were more frequently observed in inv(16)/t(16;16) AML patients (p=0.006 and 0.0001, respectively), while loss of the X or Y chromosome was more common in patients with t(8;21) AML (p=0.0001). Del(9q) was not noted in any inv(16) patients, while trisomies 8 and 22 were not found in any t(8;21)patients. In all cases for which FISH and/or RT-qPCR testing was performed, the results were in agreement with the results of chromosome analysis (data not shown). Both CD19 and CD56 antigen expressions were observed more frequently in the t(8;21) AML group (both p=0.0001). Clinical and biological characteristics of the patients are provided in Table 2.

Comparison of Clinical Features and Incidence of Genetic Mutations Between AML Patients with t(8;21) and inv(16)/t(16;16)

Among the participating 134 CBF-AML patients, 68 mutated genes were detected by screening the 112-gene panel. Thirty-two of those 68 genes could be classified as transcription factor, DNA methylation, signaling, spliceosome, cohesin, or tumor suppressor genes. An average of 3.19 (range: 1-10) mutations per individual were detected among these CBF-AML cases. While 22 patients had 1 alteration, 20 had 2, 32 had 3, and 60 had 4 or more. Among all genes sequenced, the most commonly mutated genes were *c-KIT* (45/134, 33.6%) and *NRAS* (45/134, 33.6%), followed by *FLT3* (25/134, 18.7%), *KRAS* (18/134, 13.4%), *RELN* (11/134, 8.2%), *NOTCH1* (11/134, 8.2%), *TET2* (10/134, 7.5%), and *WT1* (10/134, 7.5%). The other genes had mutation prevalences of <5%. The most frequently affected functional pathway was the signaling pathway, with such mutations observed in as many as 86.6% of cases. In addition, our findings suggest that the frequencies of mutations in genes associated with epigenetic modification, such as *IDH1*, *IDH2*, *DNMT3A*, and *TET2*, are low in CBF-AML, being identified in 1.5%, 0.7%, 2.2%, and 7.5% of the total number of participating patients, respectively.

Concomitant gene abnormalities were found in 100% of the patients with t(8;21) and 100% of the patients with inv(16)/t(16;16). The patients with inv(16)/t(16;16) AML were found to have more mutations in the *NRAS* and *KRAS* genes (53.7% vs. 20.0%, p=0.001 and 27.8% vs. 3.8%, p=0.0001, respectively) compared to t(8;21) AML patients. The distributions of *c-KIT*, *FLT3*, *RELN*, *TET2*, *FAT1*, and *NOTCH1* mutations within these two groups were similar (Table 3). Functionally mutated genes involved in signaling pathways were observed more frequently in the inv(16)/t(16;16) AML group

Table 2. Clinical and biological characteristics of the CBF-AML patients at diagnosis.

Variable	Total (n=134)	*RUNX1-RUNX1T1* (n=80)	*CBFB-MYH11* (n=54)	p
Gender				
Male, n (%)	69 (51.5%)	42 (52.5%)	27 (50%)	0.776
Female, n (%)	65 (48.5%)	38 (47.5%)	27 (50%)	
Age (years)				
Median (range)	35.5 (16-73)	32 (16-73)	41 (16-63)	0.048
WBC count (x10^9/L)				
Median (range)	15.3 (0.9-156.0)	7.8 (0.9-123)	35.9 (1.6-156)	0.0001
Hb (g/L)				
Median (range)	81 (39.0-124.0)	78 (39-120)	86.5 (40-124)	0.169
PLT count (x10^9/L)				
Median (range)	28.0 (2.0-170.0)	32 (2-170)	26 (3-121)	0.725
Secondary cytogenetic abnormalities				
Loss of X or Y chromosome, n (%)	33 (24.6%)	32 (40.0%)	1 (1.9%)	0.0001
del(9q), n (%)	4 (3.0%)	4 (5.0%)	0	0.095
Trisomy 8, n (%)	5 (3.7%)	0	5 (9.3%)	0.006
Trisomy 22, n (%)	13 (9.7%)	0	13 (20.1%)	0.0001
Immunophenotyping				
CD19 expression, n (n/N, %)	59 (59/116, 50.86%)	58 (58/73, 79.5%)	1 (1/43, 2.33%)	0.0001
NA, n	18	7	11	
CD56 expression, n (n/N, %)	51 (51/92, 55.43%)	50 (50/58, 86.21%)	1 (1/34, 2.94%)	0.0001
NA, n	42	22	20	

CBF: Core-binding factor; AML: acute myeloid leukemia; WBC: white blood cell; Hb: hemoglobin; PLT: platelet; NA: not available.

(p=0.016), while cohesin mutations were found more frequently in the t(8;21) AML group (11.3% vs. 0%, p=0.011). All cohesin mutations were mutually exclusive among each other (Figure 1). The mutation distribution is provided in detail in Table 3.

Relationships Between Clinical Characteristics, CR Rate, and Mutations

We analyzed the clinical characteristics of patients with mutations in *c-KIT*, *NRAS*, *KRAS*, *CSF3R*, *TET2*, and *FLT3-ITD*. As listed in Table 4, significantly higher WBC counts were found in inv(16)/t(16;16) AML patients with *c-KIT* (*c-KIT*^{mut}) or *NRAS* (*NRAS*^{mut}) mutations than in t(8;21) AML patients with *c-KIT*^{mut} and *NRAS*^{mut} (p=0.001 and 0.009, respectively).

Patients with both inv(16)/t(16;16) AML/*TET2*^{mut} and inv(16)/t(16;16) AML/*FLT3-ITD*^{mut} also had higher WBC counts than those with t(8;21) AML/*TET2*^{mut} and t(8;21) AML/*FLT3-ITD*^{mut}, but these differences did not reach statistical significance (p=0.088 and 0.067, respectively). No difference was found between other factors such as age, gender, Hb level, or platelet count.

This study also aimed to assess the impact of common gene mutations on the rate of CR after initial induction therapy. Among the 134 participating patients, relevant data were available for 128. The overall CR rate among these cases was 94.53% (121/128). No differences in CR rate were observed according to mutated genes (*KIT*, *NRAS*, *KRAS*, *TET2*, *FLT3-ITD*) between t(8;21) AML and inv(16)/t(16;16) AML patients. Clinical characteristics and CR rates of CBF-AML patients with common mutations are shown in Table 4.

Discussion

In this study, patients with inv(16)/t(16;16) AML had higher WBC counts than those with t(8;21) AML. Trisomies 8 and 22 were more frequently observed in inv(16) patients, while loss of the X or Y chromosome was more common in t(8;21) AML. Patients with t(8;21) AML also expressed CD19 and CD56 more frequently than those with inv(16) AML. These findings are consistent with the conclusions of previous reports [11,16].

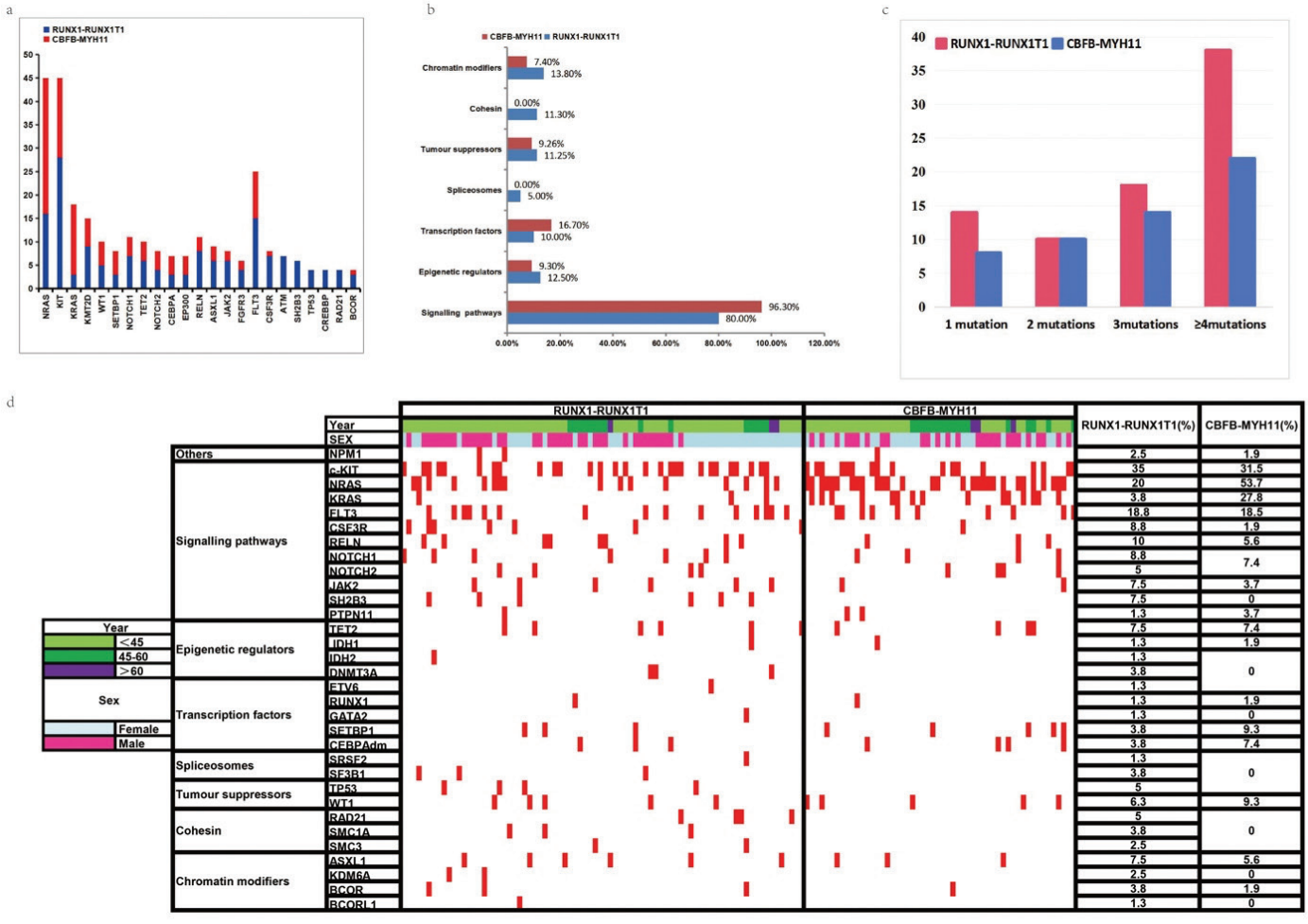

Figure 1. Comparisons of genetic mutations between AML patients with t(8;21) and inv(16)/t(16;16).

AML: Acute myeloid leukemia

Table 3. Concomitant gene abnormalities of CBF-AML at diagnosis.

Mutational genes	Total (n=134)	*RUNX1-RUNX1T1* (n=80)	*CBFB-MYH11* (n=54)	p
NPM1	3 (2.2%)	2 (2.5%)	1 (1.9%)	0.804
Signaling pathways, n (%)	116 (86.6%)	64 (80.0%)	52 (96.3%)	0.016
c-KIT, n (%)	45 (33.6%)	28 (35.0%)	17 (31.5%)	0.672
NRAS, n (%)	45 (33.6%)	16 (20.0%)	29 (53.7%)	0.0001
KRAS, n (%)	18 (13.4%)	3 (3.8%)	15 (27.8%)	0.0001
FLT3, n (%)	25 (18.7%)	15 (18.8%)	10 (18.5%)	1
CSF3R, n (%)	8 (6.0%)	7 (8.8%)	1 (1.9%)	0.143
RELN, n (%)	11 (8.2%)	8 (10.0%)	3 (5.6%)	0.524
NOTCH1, n (%)	11 (8.2%)	7 (8.8%)	4 (7.4%)	1
NOTCH2, n (%)	8 (6.0%)	4 (5.0%)	4 (7.4%)	0.714
JAK2, n (%)	8 (6.0%)	6 (7.5%)	2 (3.7%)	0.363
SH2B3, n (%)	6 (4.5%)	6 (7.5%)	0	0.081
PTPN11, n (%)	3 (2.2%)	1 (1.3%)	2 (3.7%)	0.565
Epigenetic regulators, n (%)	15 (11.2%)	10 (12.5%)	5 (9.3%)	0.781
TET2, n (%)	10 (7.5%)	6 (7.5%)	4 (7.4%)	1
IDH1, n (%)	2 (1.5%)	1 (1.3%)	1 (1.9%)	1
IDH2, n (%)	1 (0.7%)	1 (1.3%)	0	1
DNMT3A, n (%)	3 (2.2%)	3 (3.8%)	0	0.273
Transcription factors, n (%)	17 (12.7%)	8 (10.0%)	9 (16.7%)	0.296
ETV6, n (%)	1 (0.7%)	1 (1.3%)	0	1
RUNX1, n (%)	2 (1.5%)	1 (1.3%)	1 (1.9%)	1
GATA2, n (%)	1 (0.7%)	1 (1.3%)	0	1
SETBP1, n (%)	8 (6.0%)	3 (3.8%)	5 (9.3%)	0.267
CEBPA^{dm}, n (%)	7 (5.2%)	3 (3.8%)	4 (7.4%)	0.439
Spliceosomes, n (%)	4 (3.0%)	4 (5.0%)	0	0.148
SRSF2, n (%)	1 (0.7%)	1 (1.3%)	0	1
SF3B1, n (%)	3 (2.2%)	3 (3.8%)	0	0.273
Tumor suppressors, n (%)	14 (10.45%)	9 (11.25%)	5 (9.26%)	0.712
TP53, n (%)	4 (3.0%)	4 (5.0%)	0	0.148
WT1, n (%)	10 (7.5%)	5 (6.3%)	5 (9.3%)	0.516
Cohesin, n (%)	9 (6.7%)	9 (11.3%)	0	0.011
RAD21, n (%)	4 (3.0%)	4 (5.0%)	0	0.148
SMC1A, n (%)	3 (2.2%)	3 (3.8%)	0	0.273
SMC3, n (%)	2 (1.5%)	2 (2.5%)	0	0.515
Chromatin modifiers, n (%)	15 (11.2%)	11 (13.8%)	4 (7.4%)	0.403
ASXL1, n (%)	8 (6.0%)	6 (7.5%)	3 (5.6%)	0.659
KDM6A, n (%)	2 (1.5%)	2 (2.5%)	0	0.515
BCOR, n (%)	4 (3.0%)	3 (3.8%)	1 (1.9%)	0.648
BCORL1, n (%)	1 (0.7%)	1 (1.3%)	0	1
Number of mutated genes				
1	22 (16.4%)	14 (17.5%)	8 (14.8%)	0.681
2	20 (14.9%)	10 (12.5%)	10 (18.5%)	0.338
3	32 (23.9%)	18 (22.5%)	14 (25.9%)	0.648
≥4	60 (44.8%)	38 (47.5%)	22 (40.7%)	0.440
Average number (range)	3.19 (1-10)	3.17 (1-8)	3.22 (1-10)	0.873

CBF: Core-binding factor; AML: acute myeloid leukemia.

Table 4. Clinical characteristics of the common co-mutations in patients with *RUNX1-RUNX1T1* or *CBFB-MYH11* fusions.

Variables	Age, years, median (range)	Male/female, n/n	WBC count, $x10^9$/L, median (range)	Hb, g/L, median (range)	PLT count, $x10^9$/L, median (range)	CR rate, n/N (%)
***c-KIT* mutations** (n=45)						
RUNX1-RUNX1T1 (n=28)	31.5 (16-56)	13/15	10.1 (2.0-49.9)	78 (38-19)	32.5 (11-170)	50.0% (13/26) *
CBFB-MYH11 (n=17)	32 (16-57)	10/7	29 (5.7-137)	93 (63-124)	40 (15-117)	58.8% (10/17)
p	0.566	0.542	0.001	0.081	0.419	0.571
***NRAS* mutations** (n=45)						
RUNX1-RUNX1T1 (n=16)	30.5 (16-69)	9/7	18.6 (2.4-123)	80 (57-119)	28 (7-105)	87.5% (14/16)
CBFB-MYH11 (n=29)	35 (18-63)	14/15	43 (5.0-156)	87 (40-122)	25 (3-117)	92.8% (26/28) *
p	0.618	0.758	0.009	0.506	0.585	0.552
***KRAS* mutations** (n=18)						
RUNX1-RUNX1T1 (n=3)	41 (16-51)	3/0	30.9 (3.2-49.9)	79 (64-110)	11 (7-58)	100% (3/3)
CBFB-MYH11 (n=15)	36 (19-58)	9/6	32 (1.56-144)	82 (59-110)	40 (15-121)	80% (12/15)
p	0.824	0.515	0.783	0.824	0.138	1.000
***CSF3R* mutations** (n=8)						
RUNX1-RUNX1T1 (n=7)	21 (16-32)	2/5	5.1 (0.9-52.4)	63 (59-119)	28 (8-105)	57.1% (4/7)
CBFB-MYH11 (n=1)	56 (56-56)	1/0	34.9	98 (98-98)	14 (14-14)	100% (1/1)
p	0.127	0.375	0.275	0.275	0.275	1.000
***TET2* mutations** (n=10)						
RUNX1-RUNX1T1 (n=6)	36 (29-49)	2/4	4.2 (0.9-74)	89.5 (45-107)	30.5 (2-80)	66.7% (4/6)
CBFB-MYH11 (n=4)	39.5 (26-52)	1/3	28.6 (18.3-65.4)	77.5 (59-98)	22 (22-44)	100% (4/4)
p	1.00	1	0.088	0.593	0.892	0.467
***FLT3-ITD* mutations** (n=10)						
RUNX1-RUNX1T1 (n=7)	43 (24-64)	2/5	23.9 (5.6-40)	75 (57-108)	23 (6-45)	42.9% (3/7)
CBFB-MYH11 (n=3)	40 (36-42)	3/0	54 (33-137)	82 (77-85)	40 (20-57)	66.7% (2/3)
p	0.732	0.167	0.067	0.21	0.138	1.000

WBC: White blood cell; HB: hemoglobin; PLT: platelet; CR: complete remission. *: Some patients did not receive any chemotherapy or were only treated with low-dose cytosine arabinoside.

Both t(8;21) and inv(16)/t(16;16) disrupt the normal functioning of the heterodimeric transcription factor CBF complex in AML with relatively similar clinical outcomes. However, the molecular genetic abnormalities potentially explaining the differences between these two AML subtypes have not yet been explored in detail. We performed extensive mutational analysis by NGS for 134 patients with CBF-AML who ranged in age from 16 to 73 years. As expected, additional aberrations were found in 100% of these CBF-AML cases and the most commonly mutated gene was *c-KIT*, as seen in 35.0% of AML cases with t(8;21) and 31.5% of AML cases with inv(16)/t(16;16). This is in accordance with the previous research conducted by Duployez et al. [11]. Interestingly, a significantly different spectrum of gene mutations was demonstrated in AML between patients with

t(8;21) and inv(16)/t(16;16). We noticed that fewer signaling pathways were involved in cases of t(8;21) in comparison to inv(16)/t(16;16) (80.0% vs. 96.3%, p=0.016), while patients with inv(16)/t(16;16) AML exhibited more mutations in *KRAS* and *NRAS* compared to t(8;21) AML patients.

RAS genes encode a family of membrane-associated proteins that regulate signal transduction upon the ligand binding to a variety of membrane receptors, and they play important roles in physical processes including proliferation, differentiation, and apoptosis [17,18]. Activating point mutations of *RAS* genes have generally been accepted as oncogenic events in the tumorigenesis of numerous malignancies, including hematological malignancies such as AML [11,19,20]. *RAS* mutations seem to be particularly frequent in inv(16)/t(16;16) AML, with a reported incidence of up to 54% [11]. Duployez et al. [11] and Boissel et al. [21] reported that *NRAS* and *KRAS* mutations were more common among AML patients with inv(16)/t(16;16) than those with t(8;21). Further studies showed that *RAS* mutations had no effect on overall/disease-free survival, CR, or relapse rates [22,23,24]. In our cohort, both *NRAS* and *KRAS* mutations were more frequently found among inv(16)/t(16;16) AML patients than in the t(8;21) AML group, a finding previously demonstrated among other cohorts [11,21]. These data suggest that the synergic cooperation between inv(16)/t(16;16) and *RAS* mutations may influence the pathophysiology of CBF-AML.

Cohesin is a multimeric protein complex that is involved in the cohesion of sister chromatids, post-replicative DNA repair, and transcriptional regulation and it is composed of 4 core subunits: the SMC1A, SMC3, RAD21, and STAG proteins [25]. Cohesin mutations have been reported in about 6% of AML patients [26] and fewer than 2% of cases of CBF-AML [11]. Recent data revealed the identification of cohesin and chromatin modifier mutations in t(8;21) but not inv(16) patients [11,27]. In the present study, mutations in genes encoding members of the cohesin complex were present in 11.3% of the t(8;21) AML patients and none of the inv(16) AML patients, and all cohesin mutations were mutually exclusive among each other, which is consistent with previous studies [26,27]. It is interesting that cohesin gene mutations are more frequent in patients with *RUNX1*-mutated AML [28]. Indeed, Mazumdar et al. [29] demonstrated that cohesin mutations led to a state of elevated chromatin accessibility and higher levels of binding at *RUNX1* binding sites. These findings suggest links between alterations in cohesin and the RUNX1-RUNXT1 fusion oncoprotein. In addition, our study also suggests that the frequencies of mutations in genes associated with epigenetic modification (*IDH1*, *IDH2*, *DNMT3A*, and *TET2*), are low in CBF-AML, which is in accordance with the findings of Park et al. [30] and Duployez et al. [11]. These results may support the idea that mutations involved in epigenetic modification do not contribute to leukemogenesis in CBF-AML.

Yang et al. [23] showed that AML patients with *RAS* mutations had significantly higher WBC counts at diagnosis than those without mutations (p=0.001). Boissel et al. [21] demonstrated that CBF-AML patients with *c-KIT* mutations had a significantly higher median WBC count at presentation, but this difference was mainly observed among patients with inv(16), with the mutations being less common in patients with t(8;21). Additionally, no gene mutations predicted poor response to induction in comparisons with patients without mutations for particular genes (*c-KIT*, *RAS*), except for *FLT3* [21]. Jahn et al. [31] found that both *DNMT3A* and *TET2* were associated with a significantly worse prognosis in univariate analysis. However, regarding the subgroup with *c-KIT*, *NRAS*, *FLT3*, *CSF3R*, and *TET2* mutations, WBC count and CR rate were not compared between inv(16)/t(16;16) AML and t(8;21) AML patients in previous studies. Our results showed that patients with inv(16)/t(16;16) had significantly higher WBC counts than those with t(8;21) in the context of *c-KIT* or *NRAS* mutations, but no significant differences were found for CR rates.

Conclusion

This study has comprehensively analyzed the genetic mutations of 134 CBF-AML patients to characterize certain crucial genetic characteristics, compare the mutational profiles of t(8;21) AML and inv(16)/t(16;16) AML, and establish unique genetic maps. The major limitations of our study are the absence of survival data, because these patients received treatment in different medical institutions, and the fact that the prognosis of these patients was affected by diverse factors including physical status, financial situation, and the different consolidation regimens that were administered. The molecular mechanisms, exact characteristics, and clinical implications of these mutations require further study.

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Ethics

Ethics Committee Approval: The study was approved by the local ethics committee and conducted in accordance with the Declaration of Helsinki.

Authorship Contributions

Surgical and Medical Practices: H.H.; Concept: N.J.; Design: W.Q.; Data Collection or Processing: X.Ch.; Analysis or Interpretation: H.J.S., , X.Ca.; Literature Search: Z.W.; Writing: N.J.

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References

1. Schoch C, Kern W, Schnittger S, Büchner T, Hiddemann W, Haferlach T. The influence of age on prognosis of de novo acute myeloid leukemia differs according to cytogenetic subgroups. Haematologica 2004;89:1082-1090.
2. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le BMM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391-2405.
3. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, Levine RL, Lo-Coco F, Naoe T, Niederwieser D, Ossenkoppele GJ, Sanz M, Sierra J, Tallman MS, Tien HF, Wei AH, Löwenberg B, Bloomfield CD. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood 2017;129:424-447.
4. Borthakur G, Kantarjian H. Core binding factor acute myelogenous leukemia 2021 treatment algorithm. Blood Cancer J 2021;11:114.
5. Solh M, Yohe S, Weisdorf D, Ustun C. Core-binding factor acute myeloid leukemia: heterogeneity, monitoring, and therapy. Am J Hematol 2014;89:1121-1131.
6. Jourdan E, Boissel N, Chevret S, Delabesse E, Renneville A, Cornillet P, Blanchet O, Cayuela JM, Recher C, Raffoux E, Delaunay J, Pigneux A, Bulabois CE, Berthon C, Pautas C, Vey N, Lioure B, Thomas X, Luquet I, Terré C, Guardiola P, Béné MC, Preudhomme C, Ifrah N, Dombret H; French AML Intergroup. Prospective evaluation of gene mutations and minimal residual disease in patients with core binding factor acute myeloid leukemia. Blood 2013;121:2213-2223.
7. Schlenk RF, Benner A, Krauter J, Büchner T, Sauerland C, Ehninger G, Schaich M, Mohr B, Niederwieser D, Krahl R, Pasold R, Döhner K, Ganser A, Döhner H, Heil G. Individual patient data-based meta-analysis of patients aged 16 to 60 years with core binding factor acute myeloid leukemia: a survey of the German Acute Myeloid Leukemia Intergroup. J Clin Oncol 2004;22:3741-3750.
8. Marcucci G, Mrózek K, Ruppert AS, Maharry K, Kolitz JE, Moore JO, Mayer RJ, Pettenati MJ, Powell BL, Edwards CG, Sterling LJ, Vardiman JW, Schiffer CA, Carroll AJ, Larson RA, Bloomfield CD. Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): a Cancer and Leukemia Group B study. J Clin Oncol 2005;23:5705-5717.
9. Downing JR. The core-binding factor leukemias: lessons learned from murine models. Curr Opin Genet Dev 2003;13:48-54.
10. Quan X, Deng J. Core binding factor acute myeloid leukemia: advances in the heterogeneity of *KIT*, *FLT3*, and *RAS* mutations (review). Mol Clin Oncol 2020;13:95-100.
11. Duployez N, Marceau-Renaut A, Boissel N, Petit A, Bucci M, Geffroy S, Lapillonne H, Renneville A, Ragu C, Figeac M, Celli-Lebras K, Lacombe C, Micol JB, Abdel-Wahab O, Cornillet P, Ifrah N, Dombret H, Leverger G, Jourdan E, Preudhomme C. Comprehensive mutational profiling of core binding factor acute myeloid leukemia. Blood 2016;127:2451-2459.
12. Ishikawa Y, Kawashima N, Atsuta Y, Sugiura I, Sawa M, Dobashi N, Yokoyama H, Doki N, Tomita A, Kiguchi T, Koh S, Kanamori H, Iriyama N, Kohno A, Moriuchi Y, Asada N, Hirano D, Togitani K, Sakura T, Hagihara M, Tomikawa T, Yokoyama Y, Asou N, Ohtake S, Matsumura I, Miyazaki Y, Naoe T, Kiyoi H. Prospective evaluation of prognostic impact of *KIT* mutations on acute myeloid leukemia with *RUNX1-RUNX1T1* and *CBFB-MYH11*. Blood Adv 2020;4:66-75.
13. Lin LI, Chen CY, Lin DT, Tsay W, Tang JL, Yeh YC, Shen HL, Su FH, Yao M, Huang SY, Tien HF. Characterization of *CEBPA* mutations in acute myeloid leukemia: most patients with *CEBPA* mutations have biallelic mutations and show a distinct immunophenotype of the leukemic cells. Clin Cancer Res 2005;11:1372-1379.
14. Chen W, Konoplev S, Medeiros LJ, Koeppen H, Leventaki V, Vadhan-Raj S, Jones D, Kantarjian HM, Falini B, Bueso-Ramos CE. Cuplike nuclei (prominent nuclear invaginations) in acute myeloid leukemia are highly associated with *FLT3* internal tandem duplication and *NPM1* mutation. Cancer 2009;115:5481-5489.
15. Wang K, Zhou F, Cai X, Chao H, Zhang R, Chen S. Mutational landscape of patients with acute myeloid leukemia or myelodysplastic syndromes in the context of *RUNX1* mutation. Hematology 2020;25:211-218.
16. Iriyama N, Hatta Y, Takeuchi J, Ogawa Y, Ohtake S, Sakura T, Mitani K, Ishida F, Takahashi M, Maeda T, Izumi T, Sakamaki H, Miyawaki S, Honda S, Miyazaki Y, Taki T, Taniwaki M, Naoe T. CD56 expression is an independent prognostic factor for relapse in acute myeloid leukemia with t(8;21). Leuk Res 2013;37:1021-1026.
17. Beaupre DM, Kurzrock R. RAS and leukemia: from basic mechanisms to gene-directed therapy. J Clin Oncol 1999;17:1071-1079.
18. Simanshu DK, Nissley DV, McCormick F. RAS proteins and their regulators in human disease. Cell 2017;170:17-33.
19. Faber ZJ, Chen X, Gedman AL, Boggs K, Cheng J, Ma J, Radtke I, Chao JR, Walsh MP, Song G, Andersson AK, Dang J, Dong L, Liu Y, Huether R, Cai Z, Mulder H, Wu G, Edmonson M, Rusch M, Qu C, Li Y, Vadodaria B, Wang J, Hedlund E, Cao X, Yergeau D, Nakitandwe J, Pounds SB, Shurtleff S, Fulton RS, Fulton LL, Easton J, Parganas E, Pui CH, Rubnitz JE, Ding L, Mardis ER, Wilson RK, Gruber TA, Mullighan CG, Schlenk RF, Paschka P, Döhner K, Döhner H, Bullinger L, Zhang J, Klco JM, Downing JR. The genomic landscape of core-binding factor acute myeloid leukemias. Nat Genet 2016;48:1551-1556.
20. Sood R, Hansen NF, Donovan FX, Carrington B, Bucci D, Maskeri B, Young A, Trivedi NS, Kohlschmidt J, Stone RM, Caligiuri MA, Chandrasekharappa SC, Marcucci G, Mullikin JC, Bloomfield CD, Liu P. Somatic mutational landscape of AML with inv(16) or t(8;21) identifies patterns of clonal evolution in relapse leukemia. Leukemia 2016;30:501-504.
21. Boissel N, Leroy H, Brethon B, Philippe N, Botton S, Auvrignon A, Raffoux E, Leblanc T, Thomas X, Hermine O, Quesnel B, Baruchel A, Leverger G, Dombret H, Preudhomme C. Incidence and prognostic impact of *c-Kit*, *FLT3*, and *Ras* gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia 2006;20:965-970.
22. Bowen DT, Frew ME, Hills R, Gale RE, Wheatley K, Groves MJ, Langabeer SE, Kottaridis PD, Moorman AV, Burnett AK, Linch DC. *RAS* mutation in acute myeloid leukemia is associated with distinct cytogenetic subgroups but does not influence outcome in patients younger than 60 years. Blood 2005;106:2113-2119.
23. Yang X, Qian J, Sun A, Lin Jiang, Xiao G, Yin Jia, Chen S, Wu D. *RAS* mutation analysis in a large cohort of Chinese patients with acute myeloid leukemia. Clin Biochem 2013;46:579-583.
24. Bacher U, Haferlach T, Schoch C, Kern W, Schnittger S. Implications of *NRAS* mutations in AML: a study of 2502 patients. Blood 2006;107:3847-3853.
25. Kon A, Shih LY, Minamino M, Sanada M, Shiraishi Y, Nagata Y, Yoshida K, Okuno Y, Bando M, Nakato R, Ishikawa S, Sato-Otsubo A, Nagae G, Nishimoto A, Haferlach C, Nowak D, Sato Y, Alpermann T, Nagasaki M,

Shimamura T, Tanaka H, Chiba K, Yamamoto R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Nakamaki T, Ishiyama K, Nolte F, Hofmann WK, Miyawaki S, Chiba S, Mori H, Nakauchi H, Koeffler HP, Aburatani H, Haferlach T, Shirahige K, Miyano S, Ogawa S. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. Nat Genet 2013;45:1232-1237.

26. Thol F, Bollin R, Gehlhaar M, Walter C, Dugas M, Suchanek KJ, Kirchner A, Huang L, Chaturvedi A, Wichmann M, Wiehlmann L, Shahswar R, Damm F, Göhring G, Schlegelberger B, Schlenk R, Döhner K, Döhner H, Krauter J, Ganser A, Heuser M. Mutations in the cohesin complex in acute myeloid leukemia: clinical and prognostic implications. Blood 2014;123:914-920.
27. Sood R, Hansen NF, Donovan FX, Carrington B, Bucci D, Maskeri B, Young A, Trivedi NS, Kohlschmidt J, Stone RM, Caligiuri MA, Chandrasekharappa SC, Marcucci G, Mullikin JC, Bloomfield CD, Liu P. Somatic mutational landscape of AML with inv(16) or t(8;21) identifies patterns of clonal evolution in relapse leukemia. Leukemia 2016;30:501-504.
28. Thota S, Viny AD, Makishima H, Spitzer B, Radivoyevitch T, Przychodzen B, Sekeres MA, Levine RL, Maciejewski JP. Genetic alterations of the cohesin complex genes in myeloid malignancies. Blood 2014;124:1790-1798.
29. Mazumdar C, Shen Y, Xavy S, Zhao F, Reinisch A, Li R, Corces MR, Flynn RA, Buenrostro JD, Chan SM, Thomas D, Koenig JL, Hong WJ, Chang HY, Majeti R. Leukemia associated cohesin mutants dominantly enforce stem cell programs and impair human hematopoietic progenitor differentiation. Cell Stem Cell 2015;17:675-688.
30. Park SH, Lee HJ, Kim IS, Kang JE, Lee EY, Kim HJ, Kim YK, Won JH, Bang SM, Kim H, Song MK, Chung JS, Shin HJ. Incidences and prognostic impact of *c-KIT*, *WT1*, *CEBPA*, and *CBL* mutations, and mutations associated with epigenetic modification in core binding factor acute myeloid leukemia: a multicenter study in a Korean population. Ann Lab Med 2015;35:288-297.
31. Jahn N, Terzer T, Sträng E, Dolnik A, Cocciardi S, Panina E, Corbacioglu A, Herzig J, Weber D, Schrade A, Götze K, Schröder T, Lübbert M, Wellnitz D, Koller E, Schlenk RF, Gaidzik VI, Paschka P, Rücker FG, Heuser M, Thol F, Ganser A, Benner A, Döhner H, Bullinger L, Döhner K. Genomic heterogeneity in core-binding factor acute myeloid leukemia and its clinical implication. Blood Adv 2020;4:6342-6352.