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ORIGINAL ARTICLE

Genome-wide genotype-based risk model for survival in core binding factor acute myeloid leukemia patients

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Abstract

The present study attempted to build a single nucleotide polymorphism (SNP)-based risk model for predicting overall survival (OS) and event-free survival (EFS) in patients with core binding factor acute myeloid leukemia (CBF-AML). Adopting genome-wide SNP array using Affymetrix SNP array 6.0, we analyzed 868,157 SNPs with respect to OS and EFS in 104 patients with CBF-AML. Significant SNPs were identified from single SNP analysis. The risk model was constructed with incorporation of six SNPs and three clinical factors (age, c-kit exon 17 mutation, and LDH) for OS and six SNPs and three clinical factors (age, WBC, and LDH) for EFS. The model was further defined into low- and high-risk groups based on risk scores. The median age was 39 years, and the subgroup of t(8;21) and inv(16) or t(16;16) was assessed in 68 (65.4%) and 36 patients (34.6%). Finally, six SNPs per each OS (rs4353685, rs4908185, rs7709207, rs12034, rs1554844, and rs17241868) and EFS (rs13385610, rs11210617, rs11169282, rs7709207, rs4438401, and rs16894846) were incorporated into the risk model. OS was significantly different in favor of the low risk group (80.4 ± 8.4%) compared to the high-risk group (22.0 ± 7.3% at 3 years; $p = 8.75 \times 10^{-13}$; HR 8.67). For EFS, there was also a significant difference between the low- (75.0 ± 5.8%) versus high-risk group (17.1 ± 6.3% at 3 years; $p = 5.95 \times 10^{-13}$; HR 7.67). A genome-wide SNP-based risk model can stratify CBF-AML patients according to their OS and EFS in 104 patients.

Keywords Acute myeloid leukemia · Core binding factor · Genome-wide single nucleotide polymorphism array

Introduction

Acute myeloid leukemia (AML) is a heterogeneous group of disease that is characterized by a high degree of heterogeneity with respect to chromosomal abnormalities and gene mutations, which is translated to marked difference in treatment response and survival [1]. Approximately 50 to 60% of AML patients exhibit cytogenetic abnormalities at the time

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of diagnosis [2], and majorities are associated with nonrandom chromosomal translocations but other types of abnormalities including monosomies, deletions or trisomy are also commonly encountered [3, 4]. Cytogenetics is one of the most important disease-related prognostic factors for predicting clinical outcomes following AML treatment.

Core binding factor (CBF)-positive acute myeloid leukemia is a genetically distinct group of acute myeloid leukemia, which is associated with chromosomal changes of t(8;21) and inv(16)(p13q22) or t(16;16)(p13;q22). These arrangements are responsible for the production of fusion gene RUNX1/RUNX1T1 and CBFB/MYH11. Because RUNX1 and CBFB are the genes that encode α and β subunit of CBF, a heterodimeric transcription factor which is essential in normal hematopoiesis, chimeric proteins derived from aforementioned fusion genes disrupt the CBF complex and serve as pathogenic attributes of this specific type of AML [5]. CBF-AML has several distinctive characteristics compare to other forms of AML, as such that it often begins in young adults although AML is generally a disease of older adults and it often presents favorable prognosis [6, 7].

Although CBF-AML is generally believed to comprise better risk group of AML, the prognosis varies from patients and only 50% of CMF-AML patients achieve long-term remissions [8, 9]. Recently, the presence of kit mutation has known to be important for prognostication in CBF-AML patients [10]. However, there have been several controversies whether the prognostic significance is different according to the mutation location—exon 8 mutation vs exon 17 mutation—or the type of CBF—t(8;21) type vs inv(16)/t(16;16) type [11–13]. Besides, recent analysis from the German-Austrian AML study group reported that secondary chromosomal and genetic abnormalities were found in 39 and 84% of CBF-AML patients, which has prognostic impact on CBF-AML patients [14]. Taken together, CBF-AML, although characterized by distinctive chromosomal and genetic rearrangement, is also a heterogeneous group of disease, which may have diverse cytogenetic abnormalities differ by individual. Thus, information on series of mutations or additional chromosome abnormality alone is not full enough to understand disease heterogeneity with respect to different remission rate, relapse rate, and survival after CBF-AML treatment.

Single nucleotide polymorphism (SNP) is a variation at a single position in a deoxyribonucleic acid (DNA) sequence among individuals that could explain inter-individual differences of the response or toxicity to chemotherapy. In this study, we performed SNP-based approach to CBF-AML and attempted to build a SNP-based risk model for predicting overall survival (OS) and event-free survival (EFS).

Material and methods

Study population

In the current study, a total of 104 patients were included from eight hospitals in the Republic of Korea diagnosed between 1995 and 2008. The diagnosis of CBF-AML had been confirmed by the presence of AML blasts over 20% in the marrow with the presence of t(8;21), inv(16), or t(16;16) in metaphase cytogenetics. FISH findings were adopted adjunctively to make a diagnosis of CBF-AML.

A total of 104 patients had received standard induction chemotherapy consisted of 3 days of idarubicin and 7 days of either cytarabine ($n = 35$) or enocitbine (behenoyl cytarabine, BHAC; $n = 68$) [15]. For all patients achieving a CR, consolidation therapy was given subsequently [16]. Three or four cycles of high-dose cytarabine or BHAC had been given as consolidation therapy. Alternatively, autologous stem cell transplantation following 1 or 2 cycles of consolidation had proceeded. If indicated (e.g., in CR2 or other high risk feature of disease at the time of diagnosis), allogeneic stem cell transplantation using matched sibling donor or matched unrelated donor was performed. Baseline characteristics of patients are provided in Table 1. This study was approved by the Institutional Research Board of the Samsung Medical Center, Seoul, Korea. The study was conducted in accordance with the tenets of the Declaration of Helsinki.

Statistical analysis

The results were analyzed according to the information available as of December 2011. The primary end point of this study was OS and EFS. The OS was calculated from the beginning of induction therapy to the date of the last follow-up or death from any cause. EFS was defined as the time from the evaluation of the induction to the date of death due to any cause, relapse, or not achieving complete remission. Complete remission (CR) was defined as follows [17]: (1) Normal values for neutrophil ($> 1.0 \times 10^9$/L) and platelet count ($> 100 \times 10^9$/L), and independence from red cell transfusion; (2) blast cells less than 5%, no clusters or collections of blasts, absence of Auer rods on bone marrow examination; (3) absence of extramedullary leukemia. For clinical risk factors, we evaluated four clinical variables for OS and EFS including age (< 55 vs ≥ 55 years), white blood cell (WBC) counts at diagnosis (< 10.0 vs ≥ 10.0×10^9/L), lactate dehydrogenase (LDH) level at diagnosis (< 1000: ≥ 1000 mg/dL) and C-kit exon 17 mutation (positive vs negative). Among them, age, c-kit exon 17 mutation, and LDH were statistically significant for OS, thus being incorporated into the risk model. In the case of EFS, age, WBC, and LDH were significant and were incorporated into the risk model.

Table 1 Patient and disease characteristics and summary of treatment outcome ($n = 104$)

Variables	Total, $n = 104$
Age, median (range), years	39 (15–75)
Gender (male: female), *n*	63 (60.6%): 41 (39.4%)
Subtype [t(8;21): inv.(16) or t(16;16)], *n*	68 (65.4%): 36 (34.6%)
WBC, median (range), ($\times 10^9$/L)	14.8 (1.1–367.1)
LDH, median (range), mg/dl	1040 (324–8385)
C-kit mutation exon 17 mutation	38 (36.5%) 12/38
Complete remission, *n* (%)	91/104 (87.5%)
Overall survival rate at 2 years	65.1 ± 4.9%
Event-free survival rate at 2 years	54.7 ± 5.2%

WBC white blood cell, *LDH* lactate dehydrogenase

Genome-wide SNP array procedures

Genotyping methods

DNA was extracted from peripheral blood collected at the time of diagnosis using the QIAGEN DNA purification kit according to the manufacturer's instructions. Genome-Wide Human SNP Arrays 6.0 (Affymetrix, CA, USA) was used for genome-wide SNP array analysis per the manufacturer's instructions. The samples which met the quality control (QC) thresholds were included in the analyses. The QC call rate of all the samples was at least 95%.

Quality control of genome-wide SNP array genotyping data (Table 5)

The genotyping call rate in 104 cases was a median of 99.7796% (range, 97.4758–99.9294%). All the samples were successfully genotyped with more than 95.0% of call rate. The genotype data filtration criteria were as follows: (1) genotypes with minor allele frequency less than 0.05 were removed; (2) those with call rate less than 95% were eliminated; (3) the cluster QC p value less than 10^{-4} were also removed from the analysis. Finally, after the genotype data filtration, a total of 538,357 and 541,328 autosomal SNPs were remained from 868,157 SNPs into the final analysis for OS and EFS (Table 5).

Single SNP analysis for OS and EFS (Table 5) (Fig. S1)

Summary of the study flow is presented in Table 5. Three genetic models including additive, dominant, and recessive models were used for the analysis. Among these three genetic models, a model with the lowest p values was selected per each SNP with the criteria of (1) p value cutoff of less than 10^{-4} and (2) the presence of at least two significant SNPs within 100 kb in order to reduce the risk of false positive result. A total of 578 SNPs for OS and 579 SNPs for EFS were selected for further step of analysis, respectively. Full detailed data of single SNP analysis for OS and EFS are presented as Manhattan plots in Supplemental Fig. 1..

Risk model generation for OS and EFS

Selection of the best SNP risk score (Table 5, Fig. 1)

Next, SNP reduction procedure was performed remaining in 578 and 579 SNPs for OS and EFS respectively. To move on to next step of model construction, the following statistical criteria was used: (i) p value < 0.000025 from Cox proportional hazard regression model (adjusted for clinical factors: age, c-kit exon 17 mutation, and LDH for OS; age, WBC, and LDH for EFS) and (ii) high linkage disequilibrium $r^2 < 0.8$. Consequently, a total of nine SNPs for each OS and EFS were selected. Next, we summed the number of deleterious genotypes for all the SNPs (adverse 1, reference 0) in each particular combination [18], which was then grouped by number of adverse SNPs and ranked by likelihood. In addition, we ran 1000 bootstrap stepwise selection Cox models using these SNPs and used scree plot to graphically determine the optimal number of SNPs to retain (Fig. 1a, scree plot for OS; Fig. 1c, scree plot for EFS). Finally, six highest ranked SNPs for each OS and EFS were selected and incorporated into risk model generation.

Risk model generation: SNP risk models, SNP risk score + clinical risk score (Fig. 1b, d)

Based on above result, a multiple SNP-based risk model was determined with incorporation of six SNPs and three clinical factors (age, c-kit exon 17 mutation, and LDH) for OS and six SNPs and three clinical factors (age, WBC, and LDH) for EFS. To assess the relevance of the incorporating of SNPs and clinical factors, the final model was assessed using time-dependent receiver-operator characteristics (ROC) curves and compared among the models generated using SNP score only versus clinical factors only versus risk score generated based on SNP score plus clinical factors, as shown in Fig. 1 (Fig. 1b, ROC curve for OS; Fig. 1d, ROC curve for EFS).

Risk group definition by risk score (Fig. S4)

The model was grouped into two groups according to the number of deleterious variables: risk score 0–3 as a low risk ($n = 71$) and 4–8 as a high risk ($n = 33$). For EFS, the same procedure was repeated. The model was grouped into two groups according to the number of deleterious variables: risk score 0–5 as a

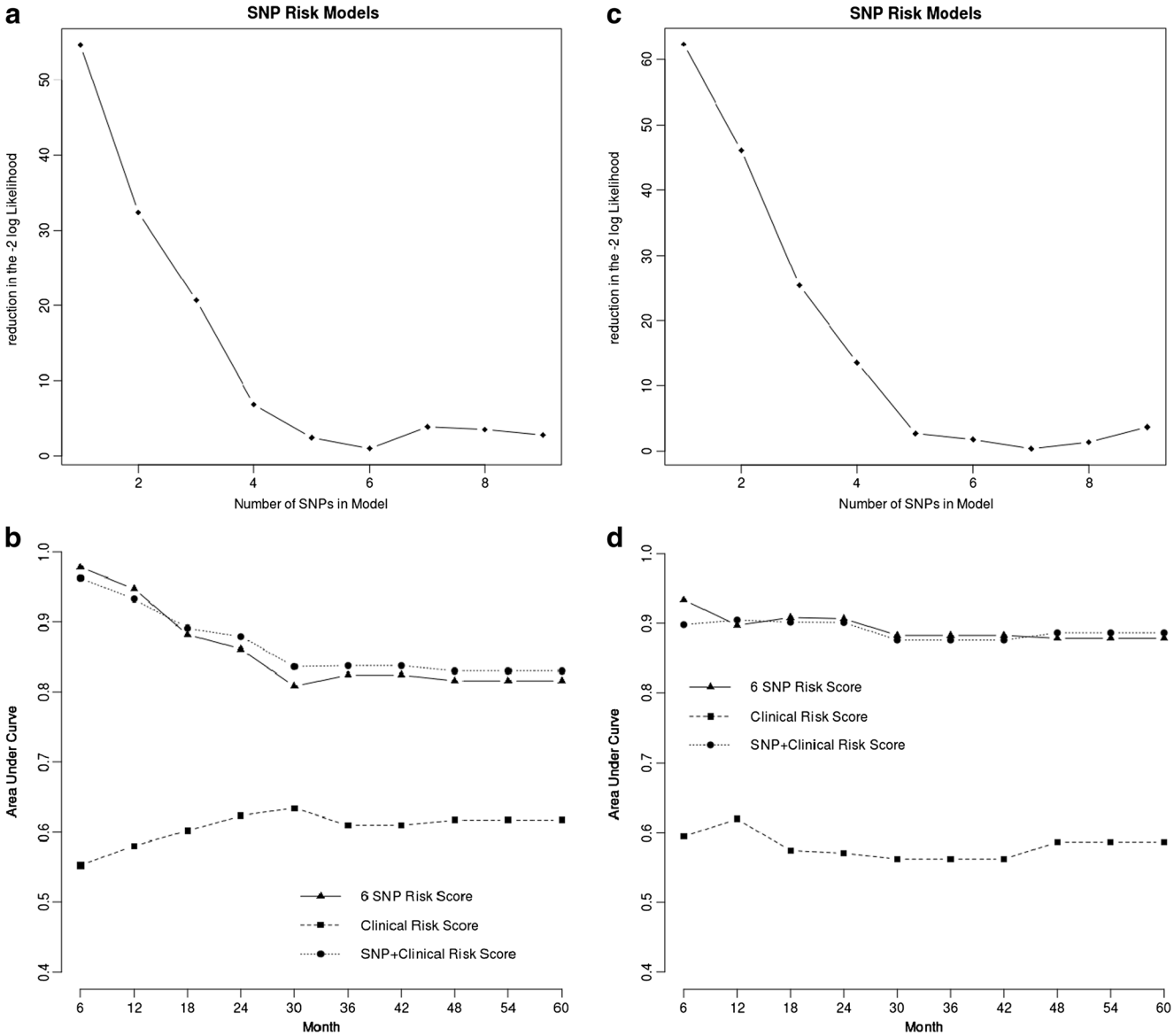

Fig. 1 SNP selection and predictive risk model generation. **a** SNP risk models for overall survival: reduction procedure of SNP numbers incorporated into the risk model. **b** Time-dependent ROC analysis for overall survival. **c** SNP risk models for event-free survival: reduction procedure of SNP numbers incorporated into the risk model. **d** Time-dependent ROC analysis for event-free survival

low risk ($n = 65$) and 6–9 as a high risk ($n = 39$). The cutoff points for classifying two groups were based on the reversal of events/no event, according to the risk score (Fig. S2).

Results

Patients' characteristics and treatment outcomes (Table 1)

Patients' characteristics and treatment outcomes are presented in Table 1. Median age of the overall population ($n = 104$) was 39 years (range 15–75) and male to female ratio was 63 (60.6%):41 (39.4%). The median follow-up duration was 36.2 months. The subgroup of t(8;21) was 68 patients (65.4%) and inv(16) or t(16;16) was 36 patients (34.6%) among the assessed patients. The CR has been achieved in 91 patients with probability of achieving CR of 87.5% (95% C.I. 81.1–93.9%) after one cycle of induction ($n = 86$) or two courses ($n = 5$). The 2 years' OS and EFS rates were 65.1 ± 4.9 and $54.7 \pm 5.2\%$, respectively. No differences of CR, OS, and EFS were noted between the group with t(8;21) vs with inv(16)/t(16;16) ($p = 0.743$ for CR, $p = 0.609$ for OS, $p = 0.759$ for EFS) and between the patients receiving cytarabine vs BHAC ($p = 0.173$ for CR, $p = 0.603$ for OS, $p = 0.188$ for EFS).

Table 2 Candidate SNPs for OS and EFS

SNP (rs number)	Chromosome loci	Position	Major allele	Minor allele	MAF	Genotype frequency	p-HWE	Call rate	Involved gene
Candidate SNPs for OS									
rs4353685	2p14	68676231	A	C	0.4471154	34/47/23	0.3808685	1.0000000	NA
rs4908185	1p21	101817983	C	T	0.3605769	46/41/17	0.1390456	1.0000000	OLFM3 (intron)
rs7709207	5q22	112636498	C	G	0.4656863	32/45/25	0.2517916	0.9807692	NA
rs12034	21q21	17569905	A	G	0.4807692	26/56/22	0.4232851	1.0000000	CXADR
rs1554844	12q13.1	50120582	C	T	0.1346154	77/26/1	0.4565022	1.0000000	COX14
rs17241868	3q22	133548850	A	G	0.1009615	83/21/0	0.2521121	1.0000000	ACPP(intron)
Candidate SNPs for EFS									
rs13385610	2q37.1	241123935	A	G	0.4903846	24/58/22	0.2376405	1.0000000	PASK(intron)
rs11210617	1q34.2	42536775	A	G	0.3942308	42/42/20	0.1151856	1.0000000	FOXJ3(intron)
rs11169282	12q13.1	50136188	C	T	0.1372549	75/26/1	0.4409564	0.9807692	CERS5(intron)
rs7709207	5q22	112636498	C	G	0.4656863	32/45/25	0.2517916	0.9807692	NA
rs4438401	18q21.2	52061362	A	G	0.1831683	69/27/5	0.2838739	0.9711538	NA
rs16894846	6q21.3	34101146	A	G	0.2067308	65/35/4	0.7903079	1.0000000	GRM4(intron)

SNP single nucleotide polymorphism, *MAF* minor allele frequency, *p-HWE p* value for Hardy-Weinberg equilibrium, *NA* not applicable, *OLFM3* olfactomedin III, *CXADR* coxsackie and adenovirus receptor, *COX14* cytochrome c oxidase 14, *ACPP* acid phosphatase, prostate, *PASK* Per-Arnt-Sim domain-containing kinase, *FOXJ3* Forkhead box J3, *CERS5* ceramide synthases 5, *GRAM4* glutamate receptor metabotropic 4

Candidate SNPs for OS and EFS (Table 2)

Table 2 demonstrates final SNPs selected for risk model prediction. The risk model was constructed with six SNPs for OS: rs4353685 on chromosome 2p14, rs4908185 on chromosome 1p21, rs7709207 on chromosome 5q22, rs12034 on chromosome 21q21, rs1554844 on chromosome 12q13.1, and rs17241868 on chromosome 3q22. For EFS, risk model was constructed with other six SNPs: rs13385610 on chromosome 2p37.1, rs11210617 on chromosome 1q34.2, rs11169282 on chromosome 12q13.1, rs7709207 on chromosome 5q22, rs4438401 on chromosome 18q21.2, and rs16894846 on chromosome 6q21.3. The details of each SNP are listed in Table 2, in which relevance with specific genes are demonstrated. Chromosome loci of 5q22 (rs7709207) and 12q13.1 (rs1554844, rs11169282) are associated with both OS and EFS.

Overall survival and event free survival by each SNP (Fig. S3)

Overall survival by SNPs

Table 3 summarizes the influence of SNP genotypes on overall survival. The CC/CA genotype group with rs4353685 demonstrated better OS than the AA genotype group ($p = 1.76 \times 10^{-5}$ after adjustment for age/c-kit exon17/LDH). The CC/CT genotype compared to the TT genotype with rs4908185 ($p = 1.02 \times 10^{-6}$), the CC/CG genotype compared to GG genotype with rs7709207 ($p = 1.22 \times 10^{-5}$), the AA/AG genotype compared to the GG genotype with rs12034 ($p = 1.30 \times 10^{-5}$), the CC genotype compared to the GT genotype with rs1554844 ($p = 1.46 \times 10^{-5}$), and the AA genotype compared to the GG/GA genotype with rs17241868 ($p = 9.61 \times 10^{-6}$) showed better OS.

The rs4908185 is located in the intronic region of olfactomedin III (OLFM3) and the rs12034 is located on coxsackie and adenovirus receptor (CXADR) as demonstrated in Table 2. Both genes are known to be associated with resistance of "anoikis," a term indicating detachment-induced apoptosis.

Event-free survival by SNPs

As shown in Table 3, the AA/AG genotype group with rs13385610 demonstrated better EFS than the GG genotype group ($p = 1.33 \times 10^{-5}$ after adjustment for age/c-kit exon17/LDH). The GG/GA genotype compared to the AA genotype with rs11210617 ($p = 1.22 \times 10^{-5}$), the CC genotype compared to CT genotype with rs11169282 ($p = 1.29 \times 10^{-5}$), the CC/CG genotype compared to the GG genotype with rs7709207 ($p = 2.49 \times 10^{-5}$), the AA genotype compared to the AG genotype with rs4438401 ($p = 1.51 \times 10^{-5}$), and the AA genotype compared to the GG/GA genotype with rs16894846 ($p = 1.85 \times 10^{-5}$) showed better EFS.

The rs11169282 is located in the intronic regions of ceramide synthases 5(CerS5), which is one of the isoforms of ceramide synthases (CerSs) family gene. Ceramide signaling is of great interest as a therapeutic target based on its relevance to apoptosis pathway and antitumor activity. The rs16894846 is located in the intronic regions of glutamate receptor metabotropic 4 (GRAM4), which is involved in inhibition of the cyclic adenosine 3′,5′-monophosphate (cAMP) cascade.

Results of risk model generation

Figure 2 and Table 4 demonstrate OS and EFS according to risk groups, which were defined by low- and high-risk groups based on risk scores from the number of deleterious SNPs and clinical factor variables. OS was significantly different in favor of the low- risk group compared to the high-risk group ($p = 8.75 \times 10^{-13}$, HR 8.67 with 95% CI 4.30–17.5). For EFS, there was also a significant difference between the low- and high-risk groups ($p = 5.95 \times 10^{-13}$, HR 7.67 with 95% CI 4.03–14.6).

Time-dependent receiver-operating curve characteristics analysis

Time-dependent ROC was performed in order to confirm SNP-based risk score model is independent from clinical factor derived risk model. As shown in Fig. 1, the models generated using SNP score only or generated based on SNP score plus clinical factors showed significantly higher AUC over time compared to that generated with clinical factors only, thus demonstrating that SNP-based risk model can improve prognostication power in CBF (+) AML patients (Fig. 1b, ROC curve for OS; Fig. 1d, ROC curve for EFS).

Discussion

The current results, analyzed from a total of 104 patients, enabled the identification of genetic variants relevant to OS and EFS in uniformly treated CBF-AML patients. We constructed the risk model with incorporation of six SNPs and three clinical factors for predicting OS and EFS, which is further divided into low- and high-risk group based on the number of deleterious SNPs and clinical factors. Survival analysis based on this SNP-based risk model showed

Table 3 Effects of SNP genotypes on overall survival and event-free survival

Candidate SNPs	Groups	Patients (*n*)	3-year OS rate	*p* value[a]	HR	95% CI
rs4353685	CC/CA	70	70.6 ± 6.1%	1.76×10^{-5}	4.75	[2.33–9.66]
	AA	34	41.2 ± 8.7%			
rs4908185	CC/CT	87	67.8 ± 5.5%	1.02×10^{-6}	6.09	[2.95–12.6]
	TT	17	25.3 ± 10.9%			
rs7709207	CC/CG	77	71.6 ± 5.6%	1.22×10^{-5}	4.57	[2.31–9.04]
	GG	27	27.3 ± 9.4%			
rs12034	AA/AG	82	71.3 ± 5.5%	1.30×10^{-5}	4.68	[2.34–9.37]
	GG	22	25.5 ± 9.8%			
rs1554844	CC	77	72.3 ± 5.6%	1.46×10^{-5}	4.36	[2.24–8.48]
	GC	26	27.4 ± 9.7%			
	TT	1	–			
rs17241868	AA	83	68.7 ± 5.5%	9.61×10^{-6}	5.06	[2.47–10.4]
	GG/GA	21	28.3 ± 11.1%			
Candidate SNPs	Groups	Patients (*n*)	3-year EFS rate	*p* value[b]	HR	95% CI
rs13385610	AA/AG	82	62.9 ± 5.7%	1.33×10^{-5}	4.08	[2.17–7.67]
	GG	22	19.3 ± 8.7%			
rs11210617	GG/GA	62	66.7 ± 6.3%	1.22×10^{-5}	4.00	[2.15–7.45]
	AA	42	31.2 ± 7.8%			
rs11169282	CC	77	62.4 ± 6.0%	1.29×10^{-5}	3.98	[2.14–7.40]
	CT	26	26.9 ± 8.7%			
	TT	1	–			
rs7709207	CC/CG	77	63.0 ± 5.8%	2.49×10^{-5}	3.90	[2.07–7.33]
	GG	27	22.8 ± 8.9%			
rs4438401	AA	72	65.1 ± 6.1%	1.51×10^{-5}	3.96	[2.12–7.40]
	AG	27	18.5 ± 7.8%			
	GG	5	–			
rs16894846	AA	65	67.4 ± 6.2%	1.85×10^{-5}	3.90	[2.09–7.29]
	GG/GA	39	28.9 ± 7.8%			

OS overall survival, *EFS* event-free survival, *HR* hazard ratio, *95% CI* 95% confidence interval, *LDH* lactate dehydrogenase, *WBC* white blood cell

[a] Adjusted for age/c-kit/LDH

[b] Adjusted for age/WBC/LDH

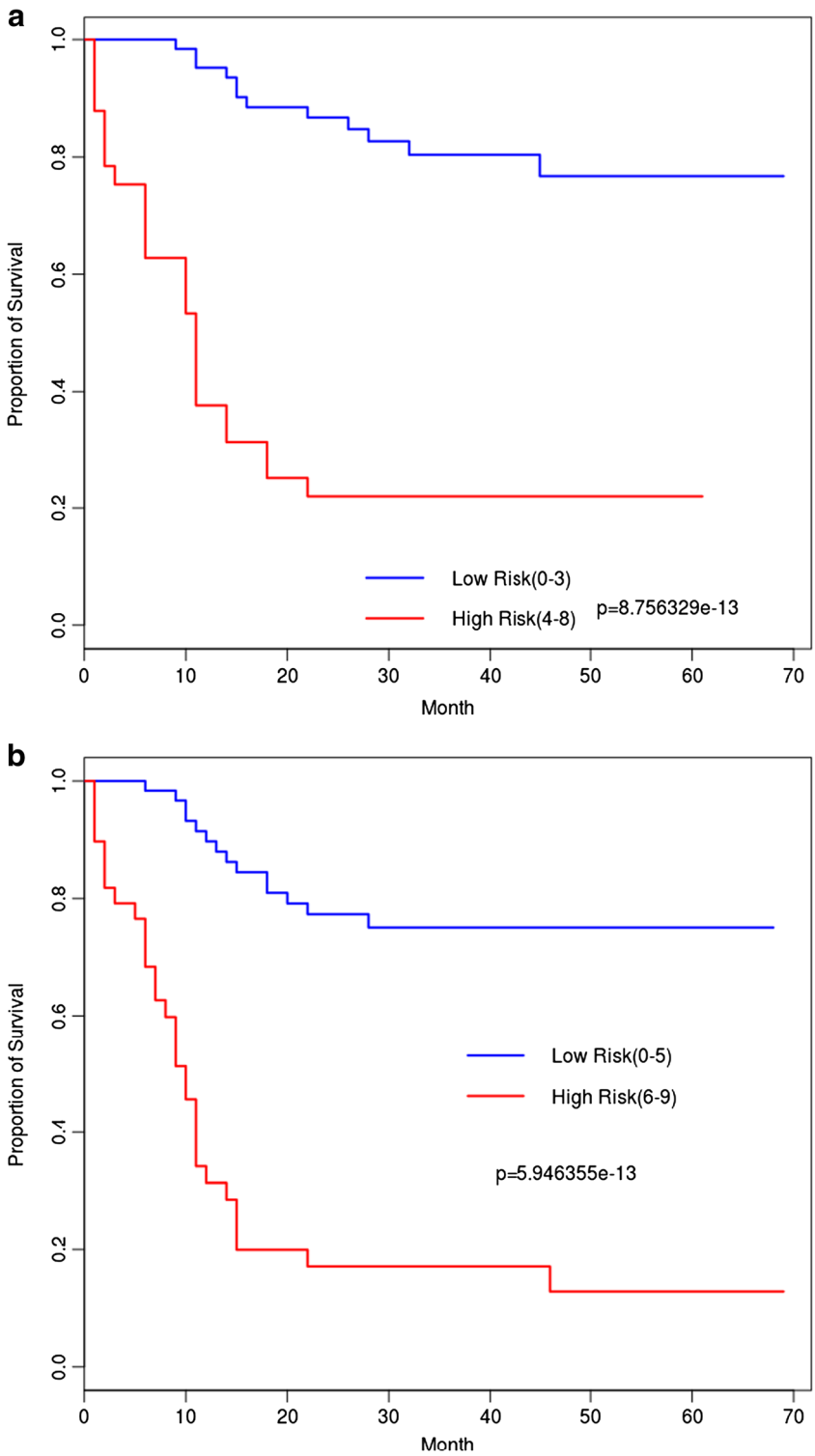

Fig. 2 Overall survival and event-free survival by risk model composed of SNPs and clinical risk factors. **a** Overall survival. **b** Event-free survival

significant difference in OS and EFS between the low- and high-risk groups, which enabled to identify a group of patients with poor OS and EFS.

In a previous study, we presented the relevance of genetic variants based on genome-wide SNP analysis on OS in normal karyotpe AML (AML-NK) patients [19]. Although there had been several previous studies demonstrating the association of genomic polymorphism and treatment outcome in AML patients [20–24], they were limited by the number of SNPs evaluated during the studies. With adopting genome-wide SNP array using Affymetrix SNP array 6.0, we could comprehensively analyze more than 500,000 SNPs at the same time with

Table 4 Overall survival and event-free survival according to risk model

Group	Patients (*n*)	Events	3-year OS rate	*p* value	HR	95% CI
Low risk (score 0–3)	71	12	80.4 ± 8.4%	8.75×10^{-13}	1.00	–
High risk (score 4–8)	33	25	22.0 ± 7.3%		8.67	[4.30–17.5]
Group	Patients (*n*)	Events	3-year EFS rate	*p* value	HR	95% CI
Low risk (score 0–5)	65	14	75.0 ± 5.8%	5.95×10^{-13}	1.00	–
High risk (score 6–9)	39	31	17.1 ± 6.3%		7.67	[4.03–14.6]

OS overall survival, *EFS* event-free survival, *HR* hazard ratio, *95% CI* 95% confidence interval

respect to OS and EFS in a homogeneous group of CBF-AML. Using a same algorithm as described in an earlier study [19], we reduced the number of SNPs incorporated into the risk model for OS and EFS. The number of SNPs was reduced from 538,357 to 578 after the first step of single SNP analysis, then to nine SNPs after processing of SNP selection procedure, and finally to six SNPs (Table 5) incorporated into the risk model for OS (rs4353685, rs4908185, rs7709207, rs12034, rs1554844, and rs17241868). After applying same SNP reduction procedure, the number of SNPs was reduced from 538,357 and finally to six SNPs for building a risk model for EFS (rs13385610, rs11210617, rs11169282, rs7709207, rs4438401, and rs16894846). The identified candidate SNPs were then evaluated to determine involved genes. We particularly paid attention to several genes including OLFM3 located in chromosome 1p21 (rs4908185), CXADR in chromosome 21q21 (rs12034), CerS5 in chromosome 12q13.1 (rs11169282), and GRM4 in chromosome 6q21.3 (rs16894846), which are demonstrated in detail in the following paragraphs.

Olfactomedin III (OLFM3) expression possibly has a role in anoikis resistance in number of human cancer cell lines [25]. Anoikis is a form of normal cell death resulting from a loss of contact with the appropriate extracellular matrix. Therefore, dysregulation of this process termed "anoikis resistance" may contribute to development and metastases of tumor [26]. Coxsackie and adenovirus receptor (CXADR), which serves as a primary receptor for adenoviral infection [27], has been also reported to have essential roles in a variety of cellular process involving cell survival, apoptosis, adhesion, and migration [28]. Although biologic function of CXADR in malignancies of different origin remains under debate, recent Japanese study revealed that CXADR signaling substantially has an impact on growth and survival of oral squamous carcinoma cells (SCC) via inhibition of anoikis [28]. Although anoikis resistance has paid attention in solid cancers as a vital step during cancer progression and metastatic colonization [29], it has rarely been described in hematologic malignancies. However, interaction between the cells in the bone marrow microenvironment and the hematopoietic

Table 5 Study process of SNPs selection for overall survival (OS) and event-free survival (EFS)

Affymetrix SNP array 6.0 genotyping ↓	Autosomal SNPs (*n* = 868,157 for OS)	Autosomal SNPs (*n* = 868,157 for EFS)
Quality control and filtration → Call rate > 0.95 each event Y and event N → 623,270 → Hardy-Weinberg Equilibrium(HWE) > 1e−07 → 643,229 → Minor allele frequency (MAF) > 0.05 each event Y and event N → 555,471 ↓	Autosomal SNPs (*n* = 538,357 for OS)	Autosomal SNPs (*n* = 541,328 for EFS)
MAX3 test < 0.001 → MAX3: additive, dominate, recessive model (a model with the lowest *p* values was selected per each SNP) → Minimal genotype frequency for each event Y and N in recessive model (OS or EFS: event Yes > 4 and event No > 2) ↓	Autosomal SNPs (*n* = 578 for OS)	Autosomal SNPs (*n* = 579 for EFS)
Cox *p* value < 0.000025 SNP and high linkage disequilibrium $r^2 < 0.8$ SNP selection ↓	Autosomal SNPs (*n* = 9 for OS)	Autosomal SNPs (*n* = 9 for EFS)
Risk score model and bootstrapping models ↓		
Final model	Autosomal SNPs (*n* = 6 for OS)	Autosomal SNPs (*n* = 6 for EFS)

SNP single nucleotide polymorphism, *MAF* minor allele frequency, *QC* quality control, *HWE* Hardy-Weinberg equilibrium, *OS* overall survival, *EFS* event-free survival

cells is known to be critical to initiation of hematologic malignancy and its maintenance [30]. Anti-apoptotic myeloid leukemia cell sequence-1 (MCL-1), suggested as an essential protein for development and survival of AML cells [31], was shown to render anoikis resistance in several types of solid cancer [32–35]. Moreover, MCL1 downregulation via targetable compound has shown sensitizes cancer cells to anoikis [36]. Our finding along with previous findings, potentially suggested that dysregulation of anoikis may contribute to pathogenesis of AML.

Ceramide, an established second messenger in apoptotic signaling pathways, is generated from the cells in response to stimuli of diverse cellular and environmental stresses [37]. Ceramide can be produced either by the hydrolysis of sphingomyelin (SM) through sphingomyelinase (SMase) or by a family of genes known as ceramide synthases (CerSs), which consists of six members of CerS1 to CerS6 [38, 39]. Alternate isoforms of this family may exert opposing roles in the same cell, for example, the pro-apoptotic role of CerS1 and the anti-apoptotic role of CerS6 has been demonstrated in head and neck cancer [40]. CerS5, a member of this family, has consistently been reported as having a pro-apoptotic role. It was observed that overexpression of CerS5 increased apoptosis in human cervical carcinoma cells [37] and mRNA level of CerS5 was higher in endometrial and colon cancer cell lines and decreased following the induction of apoptosis [41]. Based on the observations that disturbances in ceramide signaling may lead altered apoptotic signaling, and potentially leads to cancer development, manipulating CerS proteins and ceramide levels are gaining increasing attention as therapeutic target. Accordingly, ceramide generation was also shown to be relevant to apoptosis and antitumor activity in human leukemia cell lines [42], and novel approaches to enhance the efficacy of ceramide are currently investigated for the treatment of AML [43, 44]. Our finding appears to be in line with previous findings with respect to a potential association of ceramide regulation on treatment outcomes of AML patients.

Glutamate receptor metabotropic 4 (GRAM4) together with GRM6, GRF7, and GRM8, belongs to group III metabotropic glutamate receptor family and is linked to the inhibition of the cyclic AMP cascade. Because this receptor family is related to glutamatergic neurotransmission, GRM4 gene variants have been proposed to be associated with many neuropathologic conditions [45, 46]. Recently, the important role of glutamate in intercellular communication has been extended to non-neural systems and Chinese researchers reported that the GRM4 gene polymorphism was associated with the susceptibility and metastasis of osteosarcoma in a Chinese Han population [47]. Before this result, the role of cAMP pathway in osteosarcoma has been demonstrated in mice, in which tumor growth was suppressed by cAMP-dependent protein kinase [48, 49]. Although little is known about GRM4 in acute leukemia, extensive evidences have suggested that cAMP response element binding protein (CREB) is proto-oncogene in AML and its overexpression in AML cell lines results in increased cell proliferation and growth in the absence of cytokines [50]. Because CREB-dependent signaling has a role in leukemogenesis, small-molecule inhibitors of CREB are currently under development [51, 52].

To the best of our knowledge, this is the first genome-wide SNP analysis focusing on CBF-AML patients. With this non-candidate driven method, both identified and unidentified pathways in AML biology were comprehensively considered and taken into account in our analysis. However, there were also several limitations in the present study. One of the major weaknesses is the absence of validation cohort to confirm the universal validity of this risk model. For consolidating this risk model, further replication of the results in a different group of patients is needed. In addition, we did not perform functional study of each SNP. Therefore, we cannot confirm biologic role of each SNP on AML cells, and further functional study needs to be proceeded.

In summary, the present study suggests that the genome-wide SNP-based risk model obtained from 104 patients with CBF-AML was able to identify a group of patients with poor overall survival and event-free survival.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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