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Gene polymorphisms of molecules of the cGAS-STING signalling pathway are associated with AML in Chinese patients

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Abstract

Purpose The aim of this study was to explore the relationships between single-nucleotide polymorphisms (SNPs) of crucial molecules in the cGAS-STING signalling pathway and the susceptibility to, induction chemotherapy response of, and prognosis of acute myeloid leukaemia (AML) in Chinese patients.

Methods Thirteen SNPs of crucial molecules in the cGAS-STING signalling pathway were genotyped in 262 AML patients using the Sequenom MassARRAY system. The associations of SNPs with susceptibility, and induction chemotherapy response were analysed using the chi-square test or Fisher's exact test and univariate binary logistic regression, the connection of SNPs with prognosis of AML was analysed using the log-rank test, and Kaplan–Meier curves were applied for survival estimation.

Results In our study, gene polymorphisms of cGAS-STING signalling pathway molecules could be vitally associated with AML. In the recessive model, the cGAS rs311678 gene polymorphism could be closely related to AML susceptibility (CC vs. TT + TC, odds ratio (OR) = 0.480, 95% confidence interval (CI) = 0.260–0.889, $p = 0.020$). Moreover, IKKA rs3808917 might be associated with the WBC count, cGAS rs311678 could be associated with the bone marrow (BM) blast percentage, and NF-κB rs1056890 under codominant and recessive models could be connected with the HGB level. Patients who were STING rs7380272 TT/CT carriers was likely to have higher insensitivity to induction chemotherapy than CC carriers (TT + CT vs. CC, OR = 2.917, 95% CI = 1.073–7.929, $p = 0.036$). Survival analysis indicated that the IKKB rs3747811 TT genotype might be associated with decreased overall survival (OS) ($p < 0.05$).

Conclusions SNPs of molecules in the cGAS-STING signalling pathway could be significantly associated with AML. The cGAS rs311678 gene polymorphism could be associated with AML susceptibility, the STING rs7380272 variant might be related to induction chemotherapy response, and IKKB rs3747811 tended to be associated with AML overall survival. Moreover, IKKA rs3808917 could be associated with the WBC count, cGAS rs311678 could be associated with the BM blast percentage, and NF-κB rs1056890 might be related to the HGB level.

Keywords Acute myeloid leukaemia, cGAS-STING signalling pathway, Single-nucleotide polymorphisms, Prognosis

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1 Introduction

Acute myeloid leukaemia (AML) is a type of leukaemia caused by the abnormal proliferation of naïve or immature granulocytes or monocytes in the bone marrow [1], which can rapidly progress and spread to other tissues and organs, such as the blood, lymph nodes, spleen, liver, and other organs. Although with the increasing knowledge of the biological mechanisms of AML, the prognosis of AML remains dismal due to the high heterogeneity of AML. Consequently, it is still urgent to further study the pathological mechanisms of AML in order to find new therapeutic targets to improve the prognosis.

The cGAS-STING pathway is an important intracellular signalling pathway that plays a vital role in regulating immune and inflammatory responses. The key molecules in this signalling pathway include: cyclic GMP-AMP synthase (cGAS), stimulator of interferon response cGAMP interactor (STING), interferon regulatory factor 3 (IRF3), nuclear factor kappa B (NF-κB), TANK binding kinase 1 (TBK1), inhibitor of nuclear factor kappa B kinase subunit A (IKKA), and inhibitor of nuclear factor kappa B kinase subunit B (IKKB) [2, 3]. Among them, cGAS act as a DNA sensing enzyme that recognizes and binds DNA in cells when they are infected by viruses or bacteria, and then catalyzes the production of cyclic GMP-AMP (cGAMP) [4], which further activates STING protein in cells [5–7]. Activated STING proteins can regulate immune and inflammatory responses by activating NF-κB and IRF3 signalling pathways [8, 9], which leads to the transcription of related genes such as interferon and inflammatory factors, and participates in immune and inflammatory response. Moreover, activated STING recruits and activates the TBK1 and IKKA/B complexes, which can phosphorylate and activate the transcription factors IRF3 and NF-κB, transfer them from the cytoplasm to the nucleus, and activate their transcriptional activity, thus promoting the development of immune and inflammatory responses [8, 10–12]. Recent studies have confirmed that the cGAS-STING pathway is related to many diseases, including inflammatory diseases, autoimmune and degenerative diseases [13]. Specifically, the cGAS-STING pathway can promote immune recognition and the killing of tumour cells. Tumour cells can avoid being recognized by the immune system and evade attack by reducing their exposure to their own DNA. However, when the cGAS-STING pathway is activated, the DNA in the tumour cells will be increasingly recognized and attacked by the immune system, thereby improving antitumour immunity. Like in breast cancer, cGAS-STING pathway activation cooperates with anti-PD-1 immunotherapy to improve the effect of immunotherapy [14]. Moreover, the function of cGAS-STING pathway is different in various types of tumors, and the abnormal activation of this pathway is also associated with the occurrence and progression of certain tumors [15, 16]. For example, some studies have shown that in tumours such as pancreatic [17], colorectal [18], and non-small cell lung cancer [19], abnormal activation of the cGAS-STING pathway can enhance the proliferation and metastasis of tumour cells, thereby accelerating tumour development. In summary, the cGAS-STING pathway plays a critical role in tumour progression, invasion, immunotherapy and prognosis, suggesting that it is a significant target for cancer treatment.

Gene polymorphisms refer to the multiple different sequences or expression forms of the same gene in different individuals. Single-nucleotide polymorphisms (SNPs), as the most prevalent type of gene polymorphism in humans, are single-nucleotide variations at a specific genomic position that lead to variations in gene activity, and they have significant impacts on different diseases. Numerous studies have demonstrated the connection of SNPs with AML. Brwa Ali Hussein et al. found that gene polymorphisms of NKG2A were related to enhanced NK cell effector function and improved outcomes of IL-2-based immunotherapy [20], and a gene variant of NKG2D affected the patient outcomes after haematopoietic stem cell therapy (HSCT) [21]. SNPs of Ara-C-pathway genes were associated with the chemotherapy response and clinical outcomes of AML patients [22]. Chen Hu et al. reported that a CDK9 SNP (L156F) was associated with drug resistance in AML cells, and the inhibition of the mutation could overcome the resistance [23]. Moreover, recent studies have shown that SNPs of cGAS, STING and IKKB have established links with the risk of colorectal cancer [24, 25], and gene polymorphisms of TBK1 [26] and STING [27] are associated with clinical outcomes in colorectal cancer patients. IKKA variants may affect blood pressure and lipid levels in ischaemic stroke patients [28]. NF-κB2 genetic variations participate in NSCLC susceptibility, therapeutic effects and prognosis [29], which are also related to prognosis and disease development of colorectal cancer. Zongxin Zhang et al. revealed the association of the IRF-3 SNP with the susceptibility and clinical outcomes of chronic lymphocytic leukaemia (CLL) [30], and SNPs of IRF-3 are associated with systemic lupus erythematosus [31] and autoimmune thyroid diseases [32]. Gene polymorphisms of STING 293Q are protective factors in cardiovascular disease in obese individuals. However, there are few data about the gene polymorphisms of molecules of the cGAS-STING signalling pathway and AML.

In this study, 13 SNPs of crucial molecules of the cGAS-STING pathway were selected and detected in 262 AML patients and 304 healthy controls. Genotyping was performed using the Sequenom MassARRAY system.

By analysing the associations of SNPs in molecules of the cGAS-STING signalling pathway with AML susceptibility, chemotherapy response and prognosis, we may provide important genomic information for the individualized treatment and prognosis assessment of AML and a theoretical basis for the development of new treatment strategies and precision medicine.

2 Methods

2.1 Clinical samples

A total of 262 AML patients from Qilu Hospital of Shandong University were ultimately enrolled in the study. The diagnosis and classification of AML patients are based on the French-American-British (FAB) classification. The patient's initial diagnosis was made from October 6, 2010 to December 15, 2021. Patients meeting the following criteria were excluded from the study: 1) patients with the M3 subtype owing to the specific clinical features, therapy and favourable prognosis of this subtype; and 2) patients for whom the subtype of AML was unknown. Accordingly, 304 healthy donors were enroled in the study. The enrolment criteria for healthy donors were as follows: 1) healthy people older than 18 years of age; 2) no blood system-related diseases; 3) no other tumours or cancers; and 4) a white blood cell (WBC) count of $(3.5–9.5)\times10^9$/L, a red blood cell (RBC) count of $(3.8–5.1)\times10^{12}$/L, a platelet (PLT) count of $(125–350)\times10^9$/L, and a haemoglobin (HGB) level of (115–150) g/L. To evaluate drug response, complete remission (CR) was defined as an absence of clinical signs of leukaemia, no evidence that extramedullary disease existed and so on, in accordance with international recommendations. Overall survival (OS) was applied to assess treatment outcomes. According to the principle of informed consent, all study participants were notified of the research purpose. Informed consent forms were signed by the participants or their legal guardians. The study was approved by the Medical Ethics Committee of Qilu Hospital of Shandong University.

2.2 Specimen collection, DNA isolation and genotyping

Bone marrow was collected from AML patients during bone puncture after admission, and cubital venous blood was collected from the control group on the day of physical examination. All specimens were collected in an anticoagulant tube and stored in a -80 °C refrigerator. DNA was isolated from the bone marrow monocytes of AML patients and peripheral blood white cells of healthy controls with a commercial genomic DNA isolation kit (Tiangen, Beijing, China). The extracted DNA concentration was measured with a spectrophotometer (DeNovix, United States), the A260/280 ratio was 1.8–1.9, and the final concentration was greater than 25 ng/μl. The fragment containing the SNP site was amplified by PCR, and the primer was extended with a single base. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) was used to test the changes in the selected SNPs, and genotyping results were obtained by TYPER4.0 software.

2.3 RNA extraction and real-time quantitative PCR (qPCR)

RNA was isolated from bone marrow mononuclear cells with AG RNAex Pro Reagent (AG21102; Accurate Biology, Changsha, China). gDNA wiper mix was used to remove genomic DNA, and RNA was reverse transcribed into cDNA with HIScript II Q RT SuperMIX. ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China) and a Roche 480II Light Cycler System were applied for the determination of relative mRNA expression. GAPDH was used as a reference control, and the delta Ct (ΔCt) was calculated for the comparison of amplification efficiencies. The primers for GAPDH, cGAS and IKKB were as follows:

cGAS rs311678 forwards: CCAGGATTTAGGGTG ACTCTAGT,
cGAS rs311678 reverse: GCCTCAGGACAGAAA AACTTG,
IKKB rs3747811 forwards: ACCAGCCTCTCAATG TGTTCTAG,
IKKB rs3747811 reverse: CCCACACTTTACGCA GCTGTATA.
GAPDH forwards: GGAGTCCACTGGCGTCTTCA,
GAPDH reverse: GTCATGAGTCCTTCCACG ATACC.

2.4 Statistical analysis

Hardy–Weinberg equilibrium (HWE) and minor allele frequency (MAF) were applied for the preliminary screening of 13 candidate SNPs. SNPs with a p value less than 0.05 in the HWE test or an MAF less than 5% in the general population were excluded from further analysis. Three genetic models were used to analyse the genotype data, including codominant, recessive, and dominant models. Associations between the genotype of SNPs and AML susceptibility, WBC count, BM blast percentages, HGB level, PLT count and treatment outcome were analysed. The chi-square test or Fisher's exact test was applied for initial selection; univariate binary logistic regression was used to estimate odds ratios (ORs) with a corresponding 95% confidence interval (CI), adjusted for age and sex. Univariate survival analysis was performed using the log-rank test. Kaplan–Meier curves were applied for survival estimation. $p<0.05$ was

considered statistically significant. SPSS version 25.0 software (SPSS Inc.) and GraphPad Prism were used for statistical analyses.

3 Results

3.1 SNP selection and study populations

The selected SNPs are shown in Table 1. Thirteen SNPs of key molecules of the cGAS-STING signalling pathway were selected, and 11 SNPs were further analysed after passing the HWE deviation and MAF > 0.05 criteria; TBK1 rs61933195 and cGAS rs72960018 were excluded from subsequent analyses because their MAFs were less than 5%. The clinical features of AML patients and healthy donors are shown in Table 2. There were 141 males and 121 females among the AML patients, and 106 males and 198 females among the healthy donors. The median age of AML patients was 48.5 (13–87) years, and that of healthy donors was 40.5 (20–88) years. There were no differences in the age or sex distributions between the AML patients and healthy controls. In AML patients, the median WBC count was 18.00 (0.77–452.43) $\times 10^9$/L, the median of PLT count was 39 (2–235) $\times 10^9$/L and the median HGB level was 77 (21–138) g/L. Based on the National Comprehensive Cancer Network (NCCN) clinical practice guidelines and risk stratification of prognosis, there were 58 patients in the favourable prognosis group, 135 patients in the intermediate group, and 68 patients in the adverse group. A total of 138 patients exhibited a CR and 22 patients did not exhibit CR after induction chemotherapy.

Table 2 Characteristics of AML patients and controls

Variable	control n (%)	case n (%)
Gender		
Male	106(34.9)	141(53.8)
Female	198(65.1)	121(46.2)
Age(years, median range)	40.5(20–88)	48.5(13–87)
< 60	281(92.4)	196(74.8)
≥ 60	23(7.6)	66(25.2)
WBC		
Median($\times 10^9$/L)	n.a	18.00(0.77–452.43)
< 100×10^9/L	n.a	212(80.9)
≥ 100×10^9/L	n.a	50(19.1)
PLT		
Median($\times 10^9$/L)	n.a	39(2–235)
> 50×10^9/L	n.a	107(40.8)
≤ 50×10^9/L	n.a	155(59.2)
HGB		
Median(g/L)	n.a	77(21–138)
≥ 60 g/L	n.a	219(83.6)
< 60 g/L	n.a	43(16.4)
Bone marrow blast		
Median(%)	n.a	77.5(21–98)
< 42%	n.a	31
≥ 42%	n.a	231
Risk stratification		
Favourable	n.a	58
Intermediate	n.a	135
Adverse	n.a	68
Response		
CR	n.a	138
no CR	n.a	22

n.a Not appliable

Table 1 Selected genes and SNPs

Gene	SNP	Variant	Variant allele	MAF	HWE(*p*-value)
cGAS	rs311678	73425293 T > C	C	32.5503%	0.2375
cGAS	rs72960018[a]	73452449G > A	A	1.8456%	0.9487
STING	rs7380272	139481019C > T	T	30.7047%	0.7011
TBK1	rs61933195[a]	64457057C > A	A	3.8591%	0.0522
TBK1	rs7486100	64482007 T > A	A	34.8684%	0.8846
IRF3	rs11880923	49618821 T > C	C	23.3221%	0.2408
IRF3	rs2304206	49665614G > A	A	18.1208%	0.5536
IKKA	rs2230804	100218126C > T	T	47.9866%	0.1509
IKKA	rs3808917	100230958C > A	A	24.3289%	0.9123
IKKB	rs2272736	42319645G > A	A	10.5705%	0.7175
IKKB	rs3747811	42271987 T > A	A	39.9329%	0.9921
NF-κB2	rs1056890	102403013G > A	A	23.1544%	0.9460
NF-κB2	rs12769316	102392994G > A	A	14.8026%	0.5680

[a] cGAS rs72960018 and TBK1 rs61933195 were excluded from subsequent analyses as MAFs < 5%

3.2 Association between cGAS-STING signaling pathway and AML susceptibility

Among 304 healthy controls, the genotypes of cGAS rs311678 were not detected in 6 healthy controls. The association of cGAS-STING signalling pathway molecule SNPs and susceptibility to AML was analysed using three genetic models, including the codominant model, recessive model, and dominant model. The chi-square test showed that cGAS rs311678 under the codominant and recessive model ($p < 0.05$) may be associated with susceptibility. As shown in Table 3, after adjusting for age and sex, cGAS rs311678 under the recessive model could be vitally associated with AML susceptibility, and the CC genotype of cGAS rs311678 tended to be a protective factor compared to the TT and TC genotypes (OR = 0.480, 95% CI = 0.260–0.889, $p = 0.020$).

3.3 Association between the WBC count, BM blast cells, the HGB level and SNPs

The association of SNPs in molecules of the cGAS-STING signalling pathway with the WBC count was analysed. A WBC count greater than 100×10^9/L was considered a high WBC count and a WBC count less than 100×10^9/L was considered a low WBC count. As shown in Table 4, the chi-square test showed that IKKA rs3808917 under the dominant model ($p < 0.05$) may be related to the WBC count. After adjusting for age and sex, IKKA rs3808917 under the dominant model could be significantly associated with the WBC count, and

Table 3 Association between selected SNPs and AML susceptibility

gene	SNP	model	genotype	control	case	χ2 test p-value	OR (95% CI)	Adjusted *p*-value
cGAS	rs311678	codominant	TT	142	123	0.044		
			TC	118	121		1.332(0.920–1.929)	0.129
			CC	38	18		0.549(0.290–1.043)	0.067
		recessive	TT + TC	260	244	0.021		
			CC	38	18		0.480(0.260–0.889)	0.020

Table 4 Association between WBC count, the percentage of BM blast cells, HGB level and SNPs in AML

gene	SNP	model	genotype	WBC < 100 $\times 10^9$ /L	WBC ≥ 100 $\times 10^9$ /L	*χ2 test p-value*	OR (95% CI)	Adjusted *p*-value
IKKA	rs3808917	dominant	CC	104	33	0.031		
			AA + CA	108	17		0.499 (0.261–0.954)	0.036
			genotype	BM blast cell < 42%	BM blast cell ≥ 42%	*χ2 test p-value*	OR (95% CI)	Adjusted *p*-value
cGAS	rs311678	codominant	TT	10	113	0.043		
			TC	16	105		0.578 (0.251–1.333)	0.199
			CC	5	13		0.236 (0.069–0.802)	0.021
			genotype	HGB ≥ 60 g/L	HGB < 60 g/L	*χ2 test p-value*	OR (95% CI)	Adjusted *p*-value
NF-κB2	rs1056890	codominant	GG	136	31	0.006		
			AG	76	7		0.406 (0.170–0.966)	0.042
			AA	7	5		3.099 (0.915–10.499)	0.069
		recessive	GG + AG	212	38	0.031		
			AA	7	5		3.939 (0.179–13.165)	0.026

compared to the CC genotype, the AA and CA genotypes tended to be a protective factor in those with a high WBC count (OR = 0.499, 95% CI = 0.261–0.954, $p = 0.036$). The relationship between SNPs and the percentage of BM blast cells was also analysed. A percentage of BM blast cells greater than 42% was considered a high BM blast level, and a percentage less than 42% was considered a low BM blast level. Under the codominant model, cGAS rs311678 may be associated with the BM blast cell percentage groups ($p < 0.05$). As shown in Table 4, after adjusting for age and sex, the CC genotype tended to be a protective factor in patients with a high BM blast cell percentage (OR = 0.236, 95% CI = 0.069–0.802, $p = 0.021$), and the TC genotype was not associated with the BM blast cell percentage compared with the TT genotype (OR = 0.578, 95% CI = 0.251–1.333, $p = 0.199$). The association between the HGB level and SNPs was analysed in the study. The high HGB group was defined as patients with a HGB level of ≥ 60 g/L, and the low HGB group was defined as patients with an HGB level of < 60 g/L. The results showed that NF-κB2 rs1056890 under codominant and recessive models may be related to the HGB level ($p < 0.05$). After adjusting for age and sex, compared with the GG genotype, the AG genotype of rs1056890 could be associated with the HGB level under the codominant model (OR = 0.406, 95% CI = 0.170–0.966, $p = 0.042$), the AA genotype of NF-κB2 rs1056890 may be associated with HGB level compared with the GG and AG genotypes under the recessive model (OR = 3.939, 95% CI = 0.179–13.165, $p = 0.026$).

3.4 Association between the induction chemotherapy response, risk stratification and cGAS-STING pathway molecule SNPs in AML

Among 262 enrolled AML patients, the efficacy was evaluated in 160 patients after induction chemotherapy in our hospital. In this study, the association between SNPs and the chemotherapy response was analysed. Our data showed a statistically significant relationship between the SNPs and induction chemotherapy responses. As shown in Table 5, under the dominant model, statistically significant differences in STING rs7380272 were observed between the CR and no CR groups ($p < 0.05$). After adjusting for age and sex, the TT/CT genotypes tended to be a risk factor for insensitivity to chemotherapy compared with the CC genotype (OR = 2.917, 95%

Table 5 Association between the sensitivity of induction chemotherapy, risk stratification and SNPs in AML

gene	SNP	model	genotype	CR/no CR	*χ2 test p-value*	OR (95% CI)	Adjusted *p*-value
STING	rs7380272	dominant	CC	72/6	0.030		
			TT + CT	66/16		2.917 (1.073–7.929)	0.036
				risk stratification			*χ2 test*
				Favourable	Intermediate	Adverse	*p*-value
STING	rs7380272	dominant	CC	28	73	23	0.025
			TT + CT	30	62	45	
				OR (95% CI)	1.266 (0.680–2.357)	0.559 (0.271–1.154)	
				Adjusted p-value	0.458	0.116	
TBK1	rs7486100	codominant	TT	31	63	25	0.001
			TA	20	64	28	
			AA	7	8	15	
				OR (95% CI)	2.08 (0.674–6.419)	0.388 (0.134–1.124)	
					3.157 (0.993–10.039)	0.639 (0.215–1.899)	
				Adjusted p-value	0.203	0.081	
					0.051	0.421	
		recessive	TT + TA	51	127	53	0.007
			AA	7	8	15	
				OR (95% CI)	2.504 (0.841–7.453)	0.487 (0.179–1.326)	
				Adjusted p-value	0.099	0.159	

CI = 1.073–7.929, $p = 0.036$). The association of SNPs with risk stratification was analysed. The chi-square test showed that STING rs7380272 under the dominant model, and TBK1 rs7486100 under the codominant and recessive models could be associated with risk stratification ($p < 0.05$), and logistic regression analysis after adjusting for sex and age showed that no SNPs were associated with risk stratification, we speculate that this may be attributed to the limited sample size and the high heterogeneity of patients.

3.5 SNPs and survival in AML

Preliminary screening by Kaplan–Meier analysis and the log-rank test revealed that IKKB rs3747811 under the codominant and dominant models may be related to overall survival in AML patients. As shown in Fig. 1, patients with the TA and AA genotypes of IKKB rs3747811 showed an increased OS (17 months and 20 months) compared with patients with the TT genotype (8 months) under the codominant model ($p = 0.004$) and dominant model ($p = 0.001$). Moreover, as Fig. 2 shows, IKKB was associated with AML OS in GEPIA2 (https://gepia2.cancer-pku.cn/#survival), and the group with low expression of IKKB showed an increased OS.

3.6 mRNA expression of cGAS and IKKB in AML

To assess the effect of the cGAS rs311678 and IKKB rs3747811 polymorphisms on mRNA expression, we quantified mRNA expression in AML patients with different genotypes. cGAS rs311678 showed significantly increased mRNA expression in patients carrying the CT and TT genotypes compared to those carrying the CC genotype (Fig. 3a, $p < 0.05$), and IKKB rs3747811 also showed increased mRNA expression in patients carrying the TT genotype compared with those carrying the AA and TA genotypes (Fig. 3b, $p < 0.05$). In summary, the results showed that the IKKB rs3747811 mutation may affect the expression level of IKKB mRNA and was related to prognosis of AML.

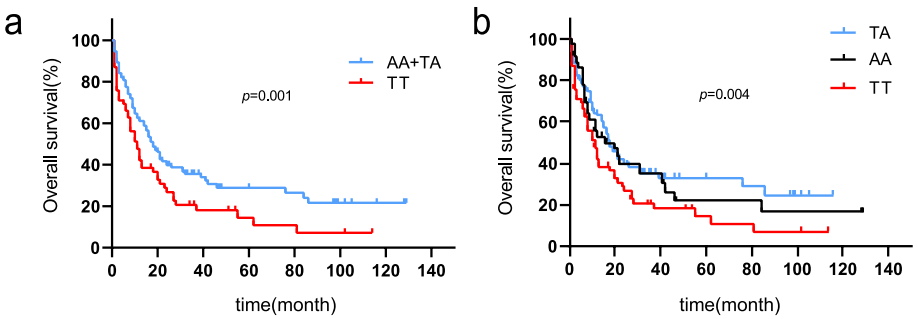

Fig. 1 The overall survival of AML patients with TT, AA, and TA genotypes in IKKB rs3747811 under different models. (**a**) dominant model, (**b**) codominant model

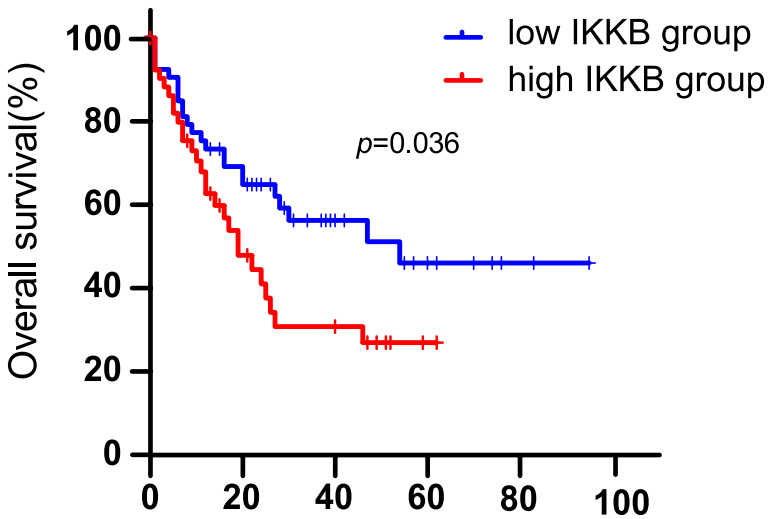

Fig. 2 The OS of AML patients with IKKB in GEPIA. The low IKKB expression group showed an increased survival time compared to high IKKB expression group

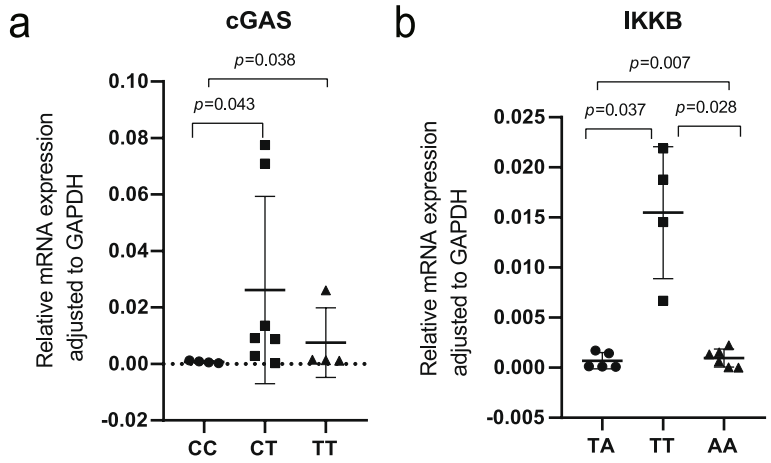

Fig. 3 cGAS and IKKB mRNA expression in AML patients. **a** Expression of cGAS mRNA in AML patients with the CC, CT, and TT genotypes of rs311678. *n* = 4, 7, and 4, respectively. **b** Expression of IKKB mRNA in AML patients with the TA, TT, and AA genotypes of rs3747811. *n* = 5, 4, and 6, respectively

4 Discussion

Thirteen SNPs of molecules from the cGAS-STING signalling pathway were selected, and the MassARRAY System was used to analyse the genotypes in 262 patients and 304 healthy donors. SNPs of cGAS-STING signalling pathway molecules may be associated with disease susceptibility, induction chemotherapy response, and AML prognosis. In our study, c-GAS rs311678 could be associated with susceptibility, and the CC genotype of cGAS rs311678 tended to be a protective factor in AML susceptibility compared with the CT and TT genotypes in the recessive model. STING rs7380272 might be significantly associated with induction chemotherapy response, the TT and CT genotypes tended to be adverse factors in drug response, IKKB rs3747811 could be meaningfully related to the outcome of AML, and the AA/TA genotypes tended to be favourable factors for AML prognosis. Moreover, IKKA rs3808917 might be associated with the WBC count, and the AA/CA genotype under the dominant model tended to be a protective factor for the high WBC count; cGAS rs311678 could be associated with the BM blast percentage, and the CC genotype was a protective factor for the high BM blast percentage; NF-κB rs1056890 under the codominant model and recessive model might be related to the HGB level, the AG genotype under the codominant model was likely to be a protective factor for a low HGB level, and the AA genotype under recessive model tended to be a risk factor for a high HGB level.

Although there are some factors that may affect the prognosis of AML, including age, disease subtype, molecular genetic abnormalities, treatment options, and response to treatment, the prognosis of AML remains dismal, and the mechanisms affecting tumorigenesis, and chemotherapy response and additional factors related to prognosis still need to be explored. The cGAS-STING pathway plays a comprehensive role in tumours, not only in improving antitumor immunity but also in inducing tumorigenesis and promoting tumour development and metastasis [33, 34]. In our study, the CC genotype of cGAS rs311678 tended to be a protective factor against AML susceptibility, which showed that gene polymorphisms of cGAS could be associated with AML susceptibility, and this was demonstrated in previous studies on colorectal cancer risk and cervical precancerous lesions [24, 35]. For the analysis of sensitivity to induction chemotherapy and SNPs, patients with the CC genotypes of STING rs7380272 tended to exhibit CR after induction chemotherapy. However, the allele frequency STING rs7380272 was not significantly different between a systemic lupus erythematosus (SLE) patient group and a healthy control group [36]. The difference in study findings may be attributed to the different diseases evaluated. Regarding the prognosis of AML, patients with the AA/TA genotype of IKKB rs3747811 showed an increased OS, and this genotype had an favourable impact on prognosis, which suggests that the mutation of IKKB rs3747811 could be a favourable mutation. In previous studies, it was found that IKKB rs3747811 was linked with a decreased risk of colon cancer [25] and an increased risk of developing wheezing [37]. However, SNPs in some genes, such as IRF3 and TBK1, are not found to be associated with AML. Previous studies reported that SNPs in these genes are associated with other diseases, such as autoimmune thyroid diseases, colorectal cancer, ischaemic stroke and schizophrenia

[24, 28, 32, 38], and the different results may be attributable to disease heterogeneity. In summary, SNPs in molecules of the cGAS-STING signalling pathway are associated with susceptibility, response to induction chemotherapy, and AML prognosis, and the cGAS-STING signalling pathway appears to play a crucial role in AML development.

Although we found that some SNPs of cGAS-STING pathway molecules could be related to AML susceptibility, induction chemotherapy response and prognosis, there were also limitations in our study. The number of patients is a major limitation of this study, as is the difficulty in obtaining specimens, and more multicentre studies are needed to enhance the accuracy and reliability of the data by increasing the number of patients. Moreover, we selected preliminary diagnosed AML patients rather than refractory and relapsed patients, which may have led to a selection bias that affects the accuracy of the results. Furthermore, our study lacked cell and animal experiments to further clarify the function of these SNPs and the mechanisms by which they function.

In summary, this study showed that SNPs of key molecules in the cGAS-STING pathway might be linked to susceptibility, induction chemotherapy response and prognosis of AML. In our study, cGAS rs311678 could play a vital role in AML onset, STING rs7380272 might be associated with induction chemotherapy response, and IKKB rs3747811 could be significant in the prognosis of AML. These findings offer biological insights into the management of AML patients. In addition, the functional SNP of IKKB rs3747811 may serve as a potential biomarker for identifying high-risk AML patients and for directing their treatment accordingly. Since this SNP is relatively common among the Chinese population (over 5%), this biomarker could be widely applied. It is necessary, however, to replicate these results in independent cohorts, and to perform functional experiments to verify the predictions about how these SNPs will affect AML.

5 Conclusion

In conclusion, our study showed that SNPs of pivotal molecules in the cGAS-STING pathway could be significantly associated with AML susceptibility, sensitivity to induction chemotherapy and prognosis in Chinese patients. Based on our findings, SNPs in cGAS-STING pathway molecules may be useful in diagnosing and evaluating AML outcomes. It is necessary to demonstrate the definite effect of the cGAS-STING signalling pathway in AML through more research, but our data add evidence to the role of the cGAS-STING signalling pathway in AML, which may be an expected therapeutic target.

Abbreviations

CI	Confidence interval
AML	Acute myeloid leukaemia
SNP	Single-nucleotide polymorphisms
cGAS	Cyclic GMP-AMP synthase
cGAMP	Cyclic GMP-AMP
GMP	Cyclic guanosine monophosphate
AMP	Adenosine monophosphate
STING	Stimulator of interferon genes
NF-κB	Nuclear factor kappa B
IRF3	Interferon regulatory factor 3
TBK1	TANK-binding kinase 1
OS	Overall survival

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Authors' contributions
DMW and JJY designed the research. DMW, YCM, FL, MYL, GQM, YYW and MYC performed experiments. DMW and YCM wrote the manuscript. TS, JJY, FL and CYJ provided supervision. All authors reviewed the paper. The author(s) read and approved the final manuscript.

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Availability of data and materials
All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Declarations

Ethics approval and consent to participate
The study was conducted in accordance with the Declaration of Helsinki and approved by the Medical Ethics Committee of Qilu Hospital, Shandong University. The patients/participants provided their written informed consent to participate in this study.

Consent for publication
Not applicable.

Competing interests
The authors have declared that no competing interest exists.

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