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TET2 rs2454206, *TET2* rs12498609 and *ASXL1* rs3746609 single nucleotide polymorphisms in patients with myelodysplastic syndromes

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ABSTRACT

To study the association between *TET2*rs2454206, *TET2*rs12498609 and *ASXL1*rs3746609 and Myelodysplastic syndromes (MDS), a total of 90 MDS patients and 143 healthy volunteers were included. The clinical data, bone marrow samples of patients and peripheral blood samples of volunteers were obtained. We found *TET2*rs2454206 G/A genotype, *TET2*rs12498609 G/C genotype and *ASXL1*rs3746609 A/G genotype in 13.3%, 11.1%, 10.1% MDS patients and in 42.7%, 22.4%, 23.8% healthy volunteers ($P < 0.001$; $P = 0.029$; $P = 0.009$, respectively). *TET2* rs2454206 G/A genotype was associated with higher serum LDH level in MDS ($P = 0.025$). Patients with *TET2*rs12498609 G/C genotype were characterized with higher frequency of mutated SRSF2 gene ($P = 0.042$) and lower occurrence rate of anemia ($P = 0.026$) than those with C/C genotype. *ASXL1*rs3746609 A/G genotype linked with higher thrombocyte counts ($P = 0.02$) and percent of total T lymphocyte ($P = 0.029$), whereas with lower percent of NK cell ($P = 0.032$) and B lymphocyte ($P = 0.007$). None of these three SNPs had impact on the overall survival and disease progression to AML. We concluded that People with *TET* rs2454206 G/A genotype, *TET2*rs12498609 G/C genotype or *ASXL1*rs3746609 A/G genotype were related to lower prevalence of MDS. All of the three SNPs were associated with certain laboratory features in MDS patients.

1. Introduction

Myelodysplastic syndromes (MDS) are highly heterogenous clonal disorders of hematopoietic stem cells (HSC) characterized by dysplastic myeloid, ineffective hematopoiesis and an increased risk of transformation to acute myelocytic leukemia (AML) [1]. Transformation of MDS to AML is one of major contributors of lethality in MDS patients [2]. The pathogenesis and mechanism of leukemic transformation are complex and still unclear. With the continuous innovation in the field of next-generation sequencing, a variety of genetic aberrations have recently been identified in MDS, including epigenetic modifiers (*TET2, ASXL1, DNMT3A* and *EZH2*), spicing factors (*SF3B1, SRSF2*and *U2AF1*), transcription factors (*RUNX1* and *BCOR*) and oncogenes et al. [3]. Recent data suggest that the perturbations of these epigenetic regulators are a common genetic event in myeloid malignancies and have been revealed to be involved in myeloid transformation in functional studies [4].

Epigenetic regulatory gene *TET2* (Ten-Eleven Translocation 2) plays an important part in DNA demethylation, truncations or catalytic domain mutants of which can affect its binding with α-KG and/or Fe^{2+} and lead to the status of hypermethylation [5]. It has been demonstrated in vitro that loss of function in *TET2* can cause the increasement of self-renewal and impairment of normal hematopoietic differentiation, which in turn lead to a progressive expansion of the progenitor c-kit+ compartment, companied with myeloid and erythroid expansion [6,7], while restoration of *TET2* reverses aberrant self-renewal of hematopoietic stem and progenitor cell (HSPC) and block disease progression [8]. Somatic mutations of *TET2* are frequently found in hematologic malignancies and are related to high risk of transformation in MDS and worse outcome in AML [8,9]. *ASXL1* (Additional sex *comb*-like 1) belongs to the enhancer of trithorax and polycomb group (ETP) genes which can either repress or activate Hox genes. Its protein includes several nuclear receptor binding motifs and a carboxy-terminal plant homeodomain (PHD) that is involved in chromatin modification. Mutations in *ASXL1* were identified firstly in MDS [10], and then in other myeloid malignancies, like CMML and AML [11,12]. The ASXL1 mutations are now generally acknowledged to be dominant-negative or gain-of-function mutations [13], which are uniformly associated with poor prognosis in a variety of myeloid neoplasms, including AML, MDS, myeloproliferative neoplasm (MPN) or MDS-MPN, and chronic myeloid

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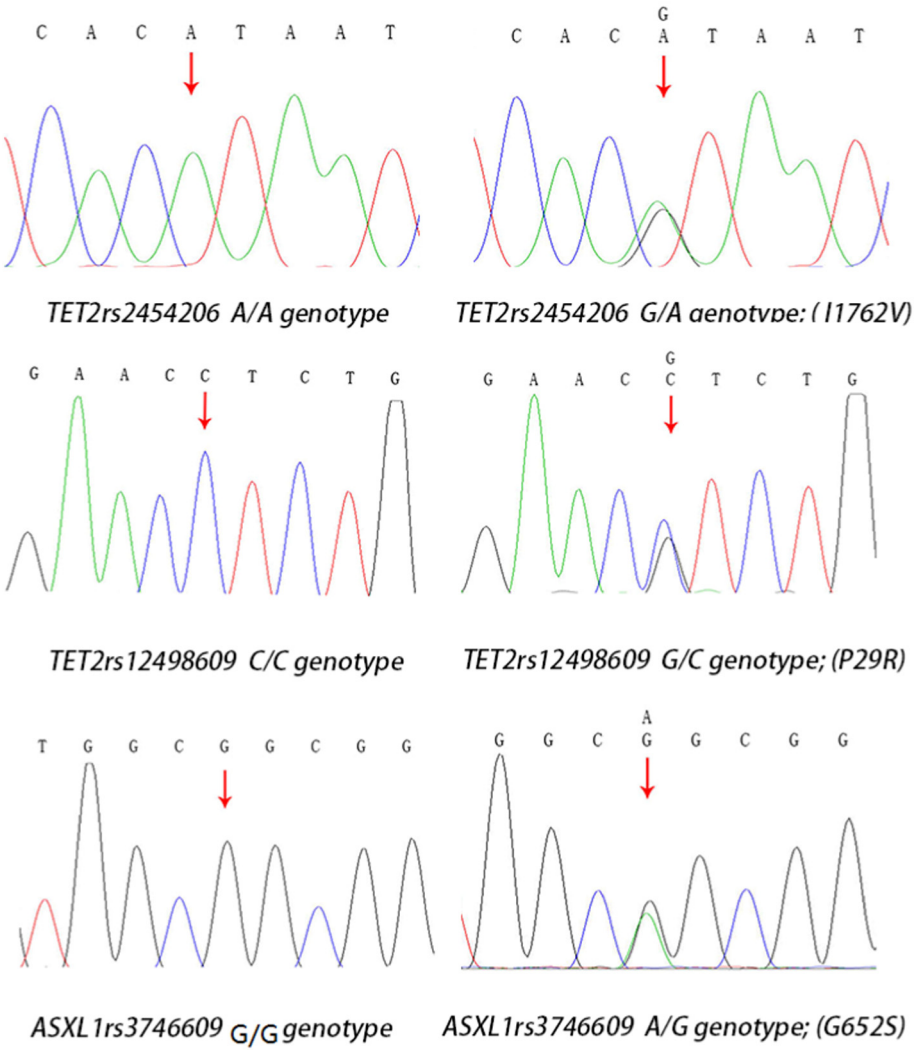

Fig. 1. Representative DNA sequence of TET2 rs2454206 genotype, TET2 rs12498609 genotype and ASXL1 rs3756609 genotype.

leukemia [3,14,15].

Growing evidence shows that germline single-nucleotide polymorphisms (SNPs) may also influence clinical features and predict outcomes [16–19]. Currently, three SNPs in *TET2* and *ASXL1* gene were noticed during the detection of gene mutations of MDS patients in our center, including *TET2* rs2454206, *TET2* rs12498609 and *ASXL1* rs3746609. All of the three SNPs belong to nonsynonymous SNPs in the coding region of the gene and result in the changes of the amino acid sequence such as TET2 I1726V, TET2 P29R and ASXL1 G652S, which may influence the function of the protein. To date, the influence of the three SNPs in MDS remains controversial and unclear. In this study, we focused on the three SNPs in *TET2* and *ASXL1* genes, and aimed to ascertain the genotype distribution, and clinical prognostic impact in MDS patients.

2. Materials and methods

2.1. Participants

Bone marrow samples were obtained from 90 newly diagnosed MDS patients enrolled in the department of Hematology, the Second Hospital of Shanxi Medical University from January 2015 to December 2017 (50 males, 40 females, age median 62 years old, range from 14 to 82 years old). Peripheral blood samples from 143 healthy volunteers were used as control (90 males, 53 females, age median 56 years old, range from 40 to 88 years old). All samples were collected under informed consent of the subjects. The diagnose of MDS patients was performed literally on the base of the criteria of World Health Organization classification of MDS [1]. In this subject group, there were 3 cases of MDS-SLD, 27 cases of MDS-MLD, 5 cases of MDS-RS, 1 case of MDS with 5q- only, 24 cases of MDS-EB1, 29 cases of MDS-EB2 and 1 case of MDS-u. Every patient was followed-up till 1st March 2018 by collecting patients' record and telephone call visit. Overall survival (OS) is defined as the period from diagnose to death or the last contact.

2.2. DNA extraction

The genomic DNA was extracted with Blood DNA Kit (OMEGA, USA), which was controlled both in concentration and purity, detected by protein nucleic acid analyzer (Bio Tek, USA), and stored at −80 °C for use.

2.3. SNP gene typing

For each of the three studied polymorphisms, all DNA samples were genotyped by Polymerase chain reaction (PCR) amplification with specific primers, direct sequenced and analyzed. The sequences of primers for PCR amplification were as follows:

TET2 rs2454206

Forward Primer: 5′-A/ACTTTTGCGACTTTCAG/GAC/C3-′

Reverse Primer: 5′-CTTTTCA/AGTGAG/GTA/AC/CA/ACA/A-3′
TET2 rs12498609
Forward Primer: 5′-TAGAG/GGCAGC/CTTGTG/GAT-3′
Reverse Primer: 5′-GCTTGAG/GTGTTCTGACATTG/G-3′
ASXL1 rs3746609
Forward Primer: 5′-CTCTGA/ATG/GTGTGTTATG/GCG-3′
Reverse Primer: 5′-GCTTGAG/GTGTTCTGACATTG/G-3′

PCR cycling conditions were: 95 °C for 2 min for enzyme activation, 35 cycles of 95 °C for 30s (denaturation), 60 °C for 30s (annealing) and 72 °C for 70s (extension), finally the products were stored at 4 °C and sequenced by General Biosystems Company, China.

2.4. Statistical analyses

Hardy-Weinberg equilibrium (HWE) was performed on each SNP. The consistence of the genotypes between MDS cases and controls was compared by Pearson's Chi-square test or Fisher's exact test when data were sparse. The Mann-Whitney *U* test was used to perform univariate analysis. OS curves were estimated using the Kaplan-Meier method, compared using a log-rank test. SPSS Version 20.0 was used for all statistical tests and $P < 0.05$ was defined as statistically significant in every test.

3. Results

3.1. The distribution of the studied polymorphisms in MDS patients

To find out whether *TET2* rs2454206, *TET2* rs12498609, *ASXL1* rs3746609 genotype were related to the predisposition to MDS, the distribution of the selected SNPs was analyzed by PCR-direct sequencing in 90 MDS patients and 142 healthy controls. Typical DNA sequencing of their genotype was shown in Fig. 1. Table 1 shows detailed data about the distribution of them in MDS patients and healthy volunteers. The distributions of the studied polymorphisms in control group were in Hardy-Weinberg equilibrium (HWE) ($P > 0.05$).

The rs2454206 G/A genotype was detected in 12 of 90 MDS patients (12/90; 13.3%), and the rest possessed the rs2454206 A/A genotype. The rs2454206 G/A genotype also existed in 56 of 143 healthy controls (56/143; 40.6%). In order to exclude the impact of classic *TET2* mutations on G/A variant, we sequenced remaining encoding exons of *TET2* (including exon 3–8, 10, 11) and found that none of patients with the G/A genotype harbored *TET2* mutations. The results showed that the G/A genotype was less frequent in the MDS group compared to controls ($P < 0.001$; Odd Ratio (OR) = 0.208; 95% Confidence Interval (CI) = 0.098–0.442), suggesting that *TET2* rs2454206A/A genotype rather than G/A genotype was associated with high risk of MDS. Similar association was also found in *TET2* rs12498609 G/C genotype ($P = 0.046$; OR = 0.431; 95% CI = 0.186–0.998) and in *ASXL1* rs3746609 A/G genotype ($P = 0.017$; OR = 0.361; 95% CI = 0.153–0.854). Additionally, there were 2 cases harboring both *ASXL1* rs3746609 A/G genotype and mutations (including p.H630fs and p. R693X). Except for the 2 case, *ASXL1* rs3746609 G/G genotype was still associated with high risk of MDS. ($P = 0.005$; OR = 0.281; 95% CI = 0.111–0.711). In addition, MDS patients involved in our study are matched with volunteers of the control group both in age and gender ($P = 0.076$, $P = 0.263$, respectively), and all of these participants come from China. The results indicated that the genotype differences observed between patients and control were not from the control selection bias.

Table 1
The frequency of *TET2* rs2454206, *TET2* rs12498609 *and ASXL1* rs3746609 in MDS patients.

	MDS		Controls		P^a	OR (95% CI)
	N	%	N	%		
TET2 rs2454206 Genotype						
A/A	78	86.7	82	57.3	< 0.001	0.207(0.103–0.413)
G/A	12	13.3	61	42.7		
TET2 rs12498609 Genotype						
C/C	80	88.9	111	77.6	0.029	0.434(0.201–0.933)
G/C	10	11.1	32	22.4		
ASXL1 rs3746609 Genotype						
G/G	80	89.9	109	76.2	0.009	0.361(0.164–0.794)
A/G	9	10.1	34	23.8		
ASXL1 rs3746609[b] Genotype						
G/G	80	92.0[b]	109	76.2	0.002[b]	0.281(0.118–0.665)[b]
A/G	7[b]	8.0[b]	34	23.8		

Abbreviations: CI, confidence interval; OR, odd ratios; a, χ^{2b} test; b, exclude the cases with ASXL1mutations. HWE: controls: $P = 0.69$ for TET2rs2454206, $P = 0.99$ for TET2rs12498609, $P = 0.54$ for ASXL1 rs3746609.

3.2. Clinical features of MDS patients between different allele genotypes

The clinical and laboratory characteristics of 90 MDS patients were analyzed to examine the association between the gene polymorphisms and parameters such as age, sex, disease subsets, expression level of *WT1* gene, peripheral blood cells, bone marrow cells, serum LDH (lactate dehydrogenase) level, proportion of immune cell subpopulation (Tables 2(1), 2(2), 2(3)). According to the rs2454206 genotype stratification, there was no significant difference in clinical phenotypes and hematological parameters between two groups except the LDH level (Tables 2(1), 2(2), 2(3)). As showed in the Table 2(3), LDH level was higher in MDS patients with *TET2* rs2454206 G/A genotype (M = 299 U/L, QR = 291 U/L) than those with A/A genotype (M = 195.0 U/L, QR = 80.7 U/L; $P = 0.025$). As for rs12498609 G/C genotype, patients with it had lower frequency of anemia (72.7% vs 96.0%, $P = 0.026$) and higher frequency of mutation of SRSF2 than with C/C genotype (37.5% vs 5.3%, $P = 0.042$) (Table 2(2), Fig. 3). Meanwhile, patients with *ASXL1* rs3746609 A/G genotype were characterized by higher thrombocyte counts ($83.0\hat{}10^9$/L ($35.0\hat{}10^9$/L) vs $53.0\hat{}10^9$/L($68.3\hat{}10^9$/L), $P = 0.041$) and higher proportion of total T cell (85.3% (13.1%) vs 75.2% (14.2%), $P = 0.029$), lower proportion of NK cells (3.2% (8.9%) vs 8.9% (9.6%), $P = 0.032$) and CD19 positive B lymphocyte cells (5.1% (2.6%) vs 10.2% (9.6%), $P = 0.007$) than the counterpart with *ASXL1* rs3746609 G/G genotype, respectively.

3.3. Clinical outcomes of MDS patients with different SNP allele genotype

In this study, we also wondered if there was association between different genotypes and the clinical outcomes of MDS patients such as transformation to AML and overall survival (OS).

There were totally 14 patients observed to have progression to AML during our study. As the Table 3 shows, there was no significantly statistical association between the selected SNPs genotype and disease progression in the given scale. Among these 14 patients, only one case was with *TET2* rs12498609 G/C genotype and two cases with *TET2* rs2454206 G/A genotype. Noticeably, none of patients harboring *ASXL1* rs3746609 A/G genotype transformed to AML (OR = 0.880,95%CI = 0.809–0.957). Then we also performed univariate analysis of overall survival (OS) on the patients by the Kaplan-Meier method, showing that there was no significant difference between MDS patients with major allele genotype of selected polymorphisms and those with minor one respectively (Fig. 2).

4. Discussion

Myelodysplastic Syndromes (MDS) are highly heterogenous

Table 2(1)
Clinical features of MDS patients with *TET* rs2454206, *TET2* rs12498609 and *ASXL1* rs3746609 genotype.

	*TET2*rs2454206		*P*	*TET2*rs12498609		*P*	*ASXL1*rs3746609		*P*
	A/A	G/A		C/C	G/C		G/G	A/G	
Ag	63(53–72)	58(45–80)	0.955	63(52–72)	64(58–75)	0.509	61(51–68)	60(32–77)	0.885
Sex (M:F)	74(38:36)	12(9:3)	0.127	75(39:36)	11(8:3)	0.334	64(32:32)	7(6:1)	0.151
Disease subtype									
SLD	3	1	0.774	4	0	0.225	4	0	0.344
MLD	24	5		26	3		22	1	
EB1	21	2		17	6		14	4	
EB2	24	4		26	2		23	2	
Others	2	0		2	0		1	0	

It's defined as statistically significant when $P < 0.05$ for all tests. "SLD" includes patients diagnosed as MDS-SLD and MDS-RS-SLD; "MLD" includes patients diagnosed as MDS-MLD and MDS-RS-MLD; "Others" refers to MDS patients with only 5q- and those with MDS-U.

disorders affected by complicated molecular pathogenesis. Additionally, single nucleotide polymorphisms of many genes were also determined to have association with the risk and clinical features of hematological disease or/and have impact on the outcomes of patients, should be considered in personalized drug treatment and development programs.

Mutations of *TET2*, common in many kinds of hematological diseases, predominantly result in loss of function of its coding protein [20], while the restoration of *TET2* function can block its effects on stem and progenitor cell self-renewal and leukemia progression [8]. *TET2* rs3733609 C/T variant was found to be a novel hereditary susceptible factor for the development of MPN [21]. A study of two independent clinical trials reported that *TET2* rs2454206 G/A genotype was an independent factor of survival and was associated with differences in non-relapse mortality (NRM) (particularly due to infection) in pediatric AML patients, even when analyzed with potential influence factors such as cytogenetic/molecular risk factors and race [16]. This is somewhat consistent with the results of two other studies based on children's AML. One showed that *TET2* rs2454206 G/A genotype was extremely associated with improved OS and EFS in AML patients with intermediate-risk cytogenetics features [22], while the other study indicated that OS of patients with G/A genotype had no difference with those with A/A genotype, but 10-year survival rate of patients between two genotypes was different (48.4% vs. 25.7%, $P = 0.049$) when analyzed by Chi-square test [23]. Due to the low frequency of *TET2* rs12498609 in pediatric AML patients, there were no significant results in these studies. However, the impact of the *TET2* rs2454206 and other SNPs in MDS patients is still unclear. In this study, we identified the distribution of three SNPs (*TET2* rs2454206, *TET2* rs12498609 and *ASXL1* rs3746609) in MDS patients. These three SNPs were all associated with predisposition of MDS but not with the outcomes such as progression to acute myelocytic leukemia and overall survival. Notably, all of patients with rs12498609 G/C genotype were alive until the last contact.

As for clinical and laboratory features, the prevalence of the SNP genotype varies in different ethnic groups. In our study based on Chinese population, these three SNPs were present at a higher frequency in healthy volunteers than MDS patients. There was no

Table 2(2)
Clinical features of MDS patients with *TET2* rs2454206, *TET2* rs12498609 and *ASXL* rs3746609 genotype.

	TET2 rs2454206		P^a	*TET2* rs12498609		P^a	*ASXL1* rs3746609		P^a
	A/A	G/A		C/C	G/C		G/G	A/G	
	N	*N*		*N*	*N*		*N*	*N*	
Mutated gene									
SRSF2	2/74	0/12	0.586	4/75	3/8	**0.042**	4/60	0/7	0.654
SF3B1	2/74	1/11	0.367	3/75	0/11	0.660	3/64	1/6	0.346
DNMT3A	2/74	0/12	0.739	2/75	0/11	0.759	1/64	0/7	0.901
RUNX1	3/74	2/10	0.141	5/75	0/11	0.496	3/61	0/7	0.729
Ferritin									
High	31/60	4/11	0.514	30/63	5/8	0.478	27/52	2/6	0.670
Low	29/60	7/11		33/63	3/8		25/52	4/6	
Folic acid									
High	21/58	4/11	0.371	23/61	2/8	0.796	15/50	4/6	0.242
Normal	26/58	3/11		25/61	4/8		23/50	2/6	
Low	11/58	4/11		13/61	2/8		12/50	0/6	
Vit-B12									
High	13/59	1/11	0.397	13/61	1/9	0.084	9/51	1/6	1.000
Normal	34/59	6/11		32/61	8/9		28/51	4/6	
Low	12/59	4/11		16/61	0/9		14/51	1/6	
Erythropoietin									
High	29/58	6/11	1.000	29/61	6/8	0.259	24/50	2/6	0.675
Low	29/58	5/11		32/61	2/8		26/50	4/6	
Anemia									
Yes (Hb < 100 g/L)	70/74	10/12	0.195	72/75	8/11	**0.026**	59/64	7/7	1.000
No (Hb ≥ 100 g/L)	4/74	2/12		3/75	3/11		5/64	0/7	

[a] P based on Mann-Whitney *U* test.

Table 2(3)
Clinical features of MDS patients with *TET2*rs2454206, *TET2*rs12498609 and *ASXL*rs3746609 genotype.

	TET2 rs2454206		P[a]	*TET2* rs12498609		P[a]	*ASXL1* rs3746609		P[a]
	A/A	G/A		C/C	G/C		G/G	A/G	
	M(*QR*)	*M*(*QR*)		*M*(*QR*)	*M*(*QR*)		*M*(*QR*)	*M*(*QR*)	
WT1 (WT1/*10^4Copies)	29.3(455.5)	103.5(551.8)	0.915	29.3(456.5)	73.9(711.45)	0.537	17.0(451.95)	47.05(287.7)	0.912
Bone marrow									
Granulocyte (%)	42.5(21.3)	40.0(25.7)	0.645	43.1(21.5)	36.0(20.0)	0.387	42.0(21.0)	31.0(25)	0.063
Blast (%)	5.5(9.0)	2.8(9.7)	0.467	5.0(9.1)	6.0(7.5)	0.207	5.0(9.0)	6.0(2.5)	0.486
Erythrocyte (%)	34.0(20.0)	42.0(30.5)	0.157	35.3(18.1)	37.0(41.5)	0.508	36.0(21.0)	26.5(23.5)	0.352
NAP (%)	61.5(44.2)	45.5(24.2)	0.124	58.5(40.5)	55.5(45.3)	0.124	64.0(40.0)	70.0(31.1)	0.787
NAP scores	112(123)	78(65)	0.724	109(111.5)	100(120.5)	0.724	112(117.3)	153(78.2)	0.259
Peripheral blood									
WBC (*10^9/L)	2.39(1.57)	3.00(1.91)	0.955	2.55(1.50)	2.40(3.55)	0.655	2.71(1.69)	2.12(1.67)	0.623
PLT (*10^9/L)	54.5(73.2)	87.5(72.5)	0.073	55.0(73.0)	79.0(91.2)	0.383	53.0(68.3)	83.0(35.0)	**0.041**
NEU (*10^9/L)	1.0(1.1)	2.1(1.2)	0.238	1.0(1.0)	1.0(1.1)	0.894	1.2(1.0)	0.2(1.1)	0.080
RET (%)	0.01(0.02)	0.01(0.02)	0.446	0.01(0.02)	0.00(0.01)	0.118	0.01(0.02)	0.01(0.025)	0.692
Th (%)	43.9(14.5)	43.6(21.4)	0.859	44.2(14.2)	33.3(26.2)	0.266	43.3(12.2)	44.8(12.7)	0.413
Ts (%)	27.1(14.4)	24.2(12.6)	0.432	27.1(14.0)	27.5(12.3)	0.237	27.1(12.5)	35.6(11.6)	0.102
Th/Ts	1.49(1.29)	1.83(1.46)	0.412	1.65(1.35)	1.19(1.30)	0.16	1.56(1.31)	1.18(0.98)	0.432
Total T (%)	77.5(15.0)	74.5(22.6)	0.302	78.2(15.0)	75.4(18.9)	0.938	75.2(14.2)	85.3(13.1)	**0.029**
NK (%)	6.9(9.6)	10.4(25.4)	0.469	7.0(10.1)	8.7(31.3)	0.6	8.9(9.6)	3.2(8.9)	**0.032**
19 + (%)	8.9(9.6)	9.2(12.9)	0.902	9.7(10.0)	4.5(10.1)	0.114	10.2(9.6)	5.1(2.6)	**0.007**
LDH (U/L)	195.0(80.7)	299.0(291.0)	**0.025**	196.5(78.5)	216.0(215.5)	0.545	207.0 (155.0–250.0)	146.0 (115.4–489.0)	0.292

[a] P based on Mann-Whitney *U* test.

Table 3
Transformation to AML in MDS patients with different SNPs genotype.

	Yes	No	Rate	*P*	OR (95%CI)
	N	N			
*TET2*rs2454206 Genotype					
A/A	12	66	15.4%	1.000	1.100 (0.214–5.660)
G/A	2	10	16.7%		
*TET2*rs12498609 Genotype					
C/C	13	67	11.8%	0.959	0.573 (0.067–4.915)
G/C	1	9	7.1%		
*ASXL1*rs3746609 Genotype					
G/G	14	66	17.5%	0.377	0.880 (0.809–0.957)
A/G	0	9	0		

Abbreviations: CI, confidence interval; OR, odd ratios.

difference in age, sex, WBC, blast percentage, FAB groups, cytogenetic groups, disease risk group or mutations of WT1, CEBPA and FLT3-ITD between patients with *TET2* rs2454206 G/A and *TET2* A/A genotypes [16]. There was a lower incidence of *NPM1* mutations with *TET2* G/A compared with *TET2* A/A (2.8% vs 9.5%, $P = 0.009$) [16]. Xingjuan Wang's team found the higher prevalence of *RAS* mutation in the *TET2* SNP rs2454206G/A subgroup compared to that in the *TET2* SNP rs2454206A/A subgroup [22]. Here, the rs2454206 genotype was not related to clinical characters except for higher LDH level in MDS patients, which is known as an indicator of tumor burden and plays a crucial role in tumor maintenance. Catalytic activity of lactate dehydrogenase was demonstrated to be generally increased among hematological malignancies [24], and high level of it in serum at diagnose was associated with poorer clinical outcome [24,25]. Interestingly, we found higher occurrence frequency of *SRSF2* mutation with *TET2* rs12498609 G/C genotype than with C/C genotype, in concert with that *TET2* mutations showed positive correlations with *SRSF2* mutations [3]. Besides, *TET2* rs12498609 G/C genotype also showed relation with lower frequency of anemia which is prognostic indicator for poor outcome.

However, we did not found study involved in *ASXL1* rs3746609. In our study, higher thrombocyte counts, higher portion of total T cell, lower portion of NK cells and 19^+ cells were observed in patients with *ASXL1* rs3746609 A/G genotype, indicating that *ASXL1* rs3746609 A/G genotype may have effect on the subpopulation of immune cells. It should be noted that there was no one of patients with *ASXL1* rs3746609 A/G genotype observed to transform to AML. Kotsianidis and his colleagues' findings indicated that the specific subpopulation of lymphocyte was involved in the pathophysiology of MDS, and increased regulatory T cell activity could promote leukemic clone progression in part of MDS patients [26]. Therefore, we come up with the hypothesis that the status of immune system combined with the *ASXL1* rs3746609 A/G genotype may be involved in preventing the transformation from MDS to AML, which need further research to confirm. Besides, a recent study showed that the abnormal populations and function of NK cells may result in ineffective antitumor activity in MDS [27], which was coincident in part with our observation that patients with the *ASXL1* rs3746609 A/G genotype had lower proportion of NK cells and had higher percentage of blast cells in their bone marrow at the same time. In another study in our center (not published data), among myeloproliferative neoplasms (MPNs) patients harboring *CALR* mutation, those with the *ASXL1* rs3746609 A/G genotype have higher counts of thrombocytes than their counterparts, which has the same tend as our study. However, it's unclear whether this coincidence comes from the effect of the nonsynonymous SNP or just from occasionalism and needs further study.

5. Conclusion

In conclusion, we found that people with *TET2* rs2454206 G/A genotype, *TET2* rs12498609 G/C genotype or *ASXL1* rs3746609 A/G genotype may be related to lower prevalence of MDS. Besides, they are all associated with laboratory features in MDS patients. Further study

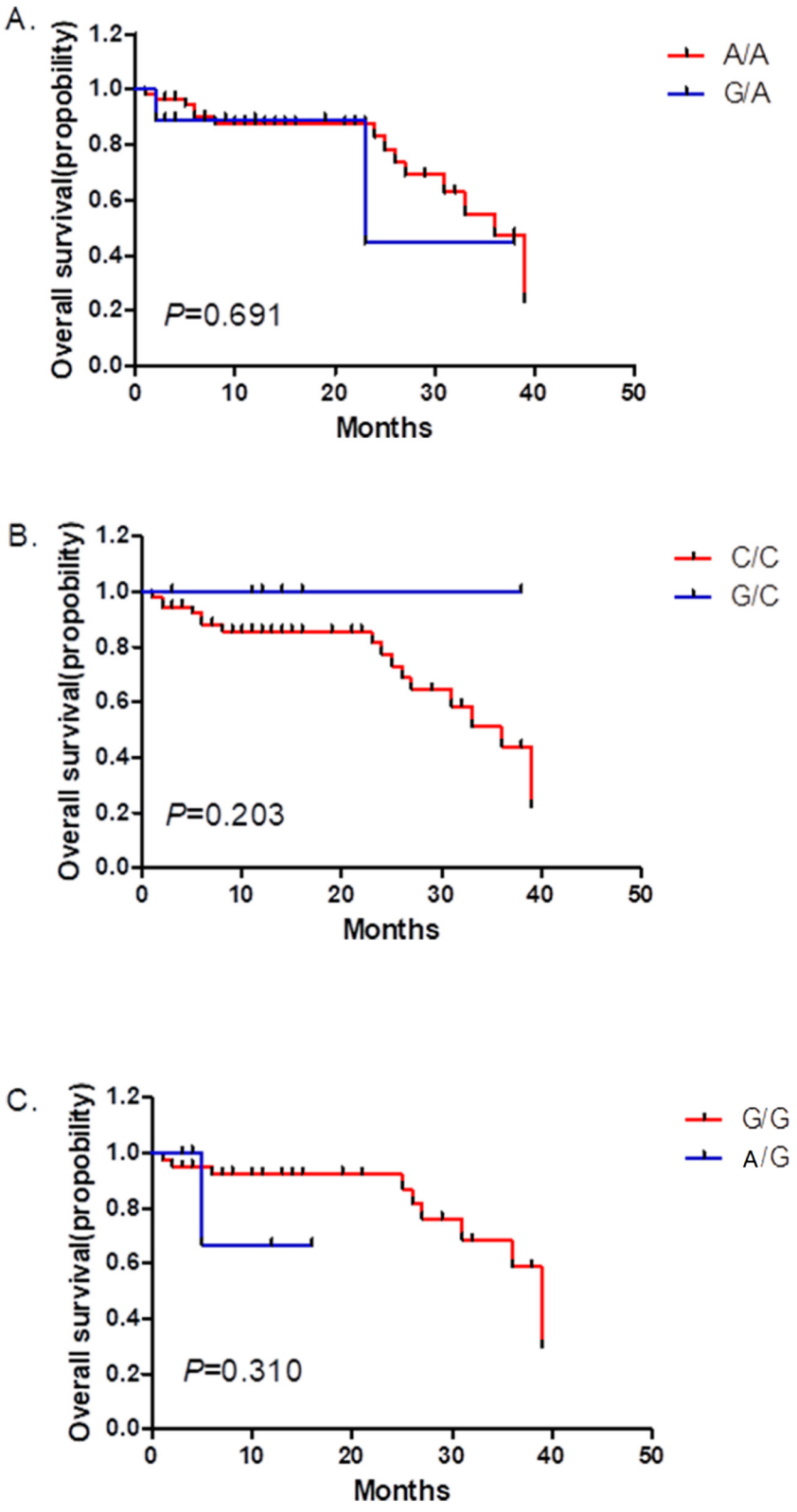

Fig. 2. Overall survival (OS) according to SNP genotype.
(A)Overall survival (OS) according to *TET2* rs2454206
The red line: overall survival of patients with *TET2* rs2454206 A/A genotype;
The blue line: overall survival of patients with *TET2* rs2454206 G/A genotype
(B)Overall survival (OS) according to *TET2* rs12498609
The red line: overall survival of patients with *TET2* rs12498609 C/C genotype;
The blue line: overall survival of patients with *TET2* rs12498609 G/C genotype
(C)Overall survival (OS) according to *ASXL1* rs3746609
The red line: overall survival of patients with *ASXL1* rs3746609 G/G genotype;
The blue line: overall survival of patients with *ASXL1* rs3746609 A/G genotype. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

will be necessary to define the mechanism.

Conflict of interests

The authors declare no conflict of interest.

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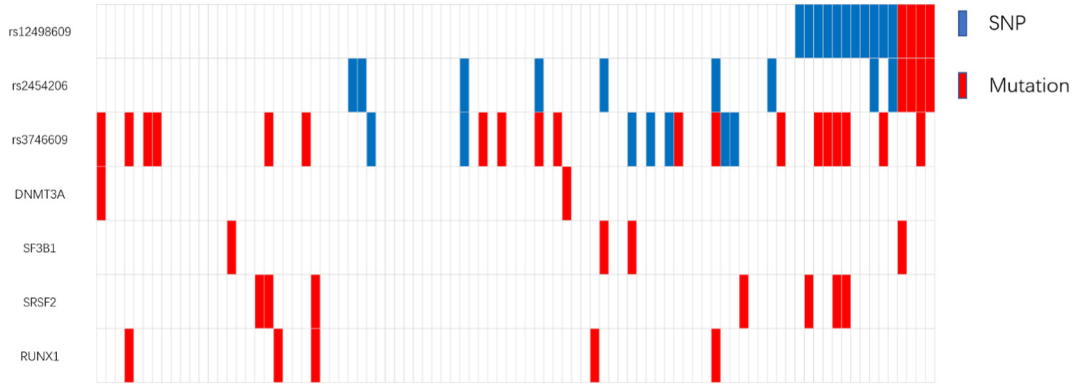

Fig. 3. Mutational data for 90 MDS cases sequenced by direct sequencing.

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