Pedigree Investigation, Clinical Characteristics and Prognosis Analysis of Hematological Disease Patients with Germline TET2 Mutation

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Abstract

Background: More and more germline gene mutations have been discovered in hematological malignances with the development of next gene sequencing (NGS). Tet methylcytosine dioxygenase 2 (TET2) is one of the most common mutation genes in hematological neoplasms. We aimed to analyzed whether germline TET2 mutation has a family aggregation or is a tumor predisposition gene. Further we compared its impact with somatic TET2 mutation in hematological diseases.

Methods: A total cohort of 103 hematological patients with TET2 mutation were included from December 2016 to December 2019. Data were extracted from hematology department of West China Hospital of Sichuan University. Bone marrow (BM) or peripheral blood (PB) as somatic DNA origin to be detected by next-generation sequencing (NGS), and nails and hairs as germline DNA origin to be detected by Sanger sequencing, respectively. Further, we compared the clinical characteristics between the patients with germline and somatic TET2 mutation.

Results: 103 patients were included, including 33 (32.03%) patients with germline TET2 mutation and 70 (67.97%) patients with somatic TET2 mutation. Variant allele frequency (VAF) of germline TET2 mutation was more stable in our study, ranging from 40% to 55% and mutation sites were more concentrated. Patients with germline TET2 mutation were younger with median age 48 (range, 16-82) (P=0.0078). Further, patients with germline TET2 mutation were mainly myelodysplastic syndromes (MDS) (n=13, 39.4%), while patients with somatic TET2 mutation were acute myeloid leukemia (AML) (n=28, 40.0%) (P=0.0003). Germline TET2 mutation affected the distribution of peripheral blood cell count and the proportion in bone marrow (P<0.05). Germline TET2 mutation was a poor prognosis factor in MDS patients via univariate analysis (HR=5.3, 95%CI: 0.89-32.2, P=0.0209), but not in multivariate analysis by Cox regression model (P=0.062).

Conclusions: Some family members were asymptomatic carriers, which indicated germline TET2 mutation might have a family aggregation. More importantly, TET2 gene may be a predisposition gene of hematological malignant when the other gene mutations as the second hit. The VAF of germline TET2 mutation is more stable. At the same time, the germline TET2 mutation may be an adverse factor for the MDS patients.

Background

The role of germline gene mutations in the tumor has been increasingly recognized since the occurrence and wide application of next-generation sequencing (NGS), especially in hematological neoplasms(1). Woo-Joo Song, *et al* first reported germline RUNX1 mutation is associated with the familial platelet disorder predisposition to acute myeloid leukemia (FPD/AML), in 1999(2). However, due to the difficult in collecting the samples of germline DNA origin, the development and exploration of germline gene mutations in hematological disease is relatively slower than that in solid tumor. Although, the skin fibroblasts are the gold specimens for germline mutation test (3), nails are recently reported to be as a reliable source of germline DNA(1, 4, 5), which largely contribute to the recognition of germline mutations in hematological disease. For instance, germline CEBPA and DDX41 mutations are found to be relative to the family predisposition myelodysplastic syndrome (MDS) and AML (6-8), germline RUNX1 mutation is associated with family inherited platelet disease and high risk of transformation to MDS/AML(2, 9), and germline GATA2, ANKRD26 and ETV6 mutations are reported to relate to the family inheritable hematological malignancies (HM) (10-12). Therefore, the 2016 World Health Organization (WHO) classification has proposed a new and distinct entity that myeloid neoplasms with germline predisposition(13). Given that, our center conducted an investigation on hematological disease patients with germline mutation last year and found that 33 (15.8%) patients had germline tet methylcytosine dioxygenase (TET2) mutation among 209 patients with hematology diseases, which was only following the ZRSR2 (17.7%) (unpublished data).

TET2, a gene involving in DNA demethylation, mainly catalyzes the conversion of 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) to contribute DNA demethylation(14). TET2 is highly expressed in the hematopoietic stem cell (HSC) and significantly impacts the self-renew, differentiation and proliferation of HSCs(15). TET2 mutation is common in the hematological neoplasms, which can occur in 30% myelodysplastic syndrome (MDS), 20% myeloproliferative neoplasms (MPNs), 30% secondary acute myeloid leukemia (sAML), 17% do novel AML and 50-60% chronic myelomonocytic leukemia (CMML)(16-19). Furthermore, many studies on DNA demethylation medicines, like Decitabine (DAC) or 5-azacitidine (AZA), have been reported, especially in hematological myeloid neoplasms (20-22).

However, the germline mutation of the TET2 has not been reported so far. Considering the updated 2016 WHO classification, the significant role of TET2 mutation in hematological neoplasms and data from our previous research, we performed this study mainly on whether germline TET2 mutation has a family aggregation phenomenon and is a tumor predisposition gene. Further we compared its impact in hematological disease with somatic TET2 mutation, including their mutation sites, variant allele frequency, diagnosis distribution, blood cell count, prognosis and survival and so on.

Methods

Patients and sample collection

We received approval from ethics committee of West China Hospital of Sichuan University and obtained the informed consent from all patients or their family members in accordance with the Declaration of Helsinki. All the patients accepted the 34 myeloid genes panel test (**S1 Table**). We totally screened out 103 in-hospital or out-hospital hematological patients with TET2 mutation, including 33 (32.03%) patients with germline mutation and 70 (67.97%) patients with somatic mutation in our center from December 2016 to December 2019. The patients were diagnosed by hematologists by combining morphology, cytogenetics, immunophenotyping, and molecular genetics according to the 2008 and 2016 WHO guideline, and AML classification adopted French-American-British (FAB) criteria.

We collected bone marrow (BM) or peripheral blood (PB) as somatic DNA origin, and nails or hairs as germline DNA origin(1, 4, 23). All patients offered both the BM/PB sample and nails/hairs to carried out somatic and germline TET2 mutation tests, respectively. Meanwhile, we collected nails/hairs sample of family members of proband patients with germline TET2 mutation to test the statue of TET2, and the peripheral blood samples as much as possible.

Sequence

The collected sample was sent to Wuhan Kindstar Global Esoteric Test Service Work. For somatic mutation, extracting the genomic DNA (gDNA) from bone morrow aspirate or peripheral blood to perform next-generation sequencing (NGS) (Ion Ampliseq technology), which captures 34 genes (34 Myeloid Panel, 34-MP) (**S1 Table**) to be detected at one time. Then using the Ion Proton semiconductor of the constructed Life Technologies platform to finish the test. For the germline mutation, using gDNA extracted from nails/hairs to perform Sanger sequencing. The sequencing depth was at least 500 reads. The TET2 detection aera was mainly from exon 3 to exon 11 for the patients.

For the germline TET2 mutation, if the allele frequency (AF) in the 1000genome and Exome Aggregation Consortium (ExAC) is more than 1% in the whole people will be considered possibility of single nucleotide polymorphism (SNP) and removed(24, 25). If the minor allele frequency (MAF) is less than 1% or no reported in that two public databases will be considered a rare variate and be included to analyze(26).

Statistical analyses

Statistical calculations of materials were performed using SPSS version 24.0 and GraphPad Prism 7. Value of *P* is two-tail, and less than 0.05 is considered statistically significant. All the tests were two-sided. Categorical variables were described with count and relative frequency (percentage) and continuous variables with median and range. Comparison of categorical variables were performed by Fisher's exact test or X^2 test, and continuous variables by Mann-Whitney's U test. Survival analyses were calculated by Kaplan-Meier test for univariate analyses (log-rank test). A Cox proportional hazard model for multivariate analyses. OS was based on death from any cause. (lost to follow or still alive considered as censored).

Results

The basic characteristic of the patients with germline and somatic TET2 mutation

As was shown in **Table 1**, we finally included 33 patients with germline TET2 mutation, including 18 males (54.5%) and 15 females (45.5%). The median age of them was 48 (rang, 16-82). The median variate allele frequency (VAF) of germline TET2 mutation was 50.58% (range, 40-55%). The most common mutation was missense mutation, accounting for 90.9%. 11 mutation sites detected mainly distributed at the exon 3 to11, among them eight mutation sites were specifically at the exon 3, one at the exon 6 and two not sure. Furthermore, three mutation sites were highly recurrent, including c.2604T>G(p.Phe868Leu), c.3116C>T(p.Ser1039Leu) and c.2440C>T(p.Arg814Cys). The frequency of that three sites was 12 (36.36%), 9 (27.27%) and 4 (12.12%), respectively, and the others occurred only once. AF of six mutation sites were less than 1% in 1000genome and ExAC databases, and five mutation site's AF were not be reported in that two databases. Among the 33 patients, 18 (54.5%) patients had normal chromosomal karyotype, two (6.1%) patients had complex karyotypes, six (18.2%) had other abnormal karyotypes and seven (21.2%) had no available results of karyotype. Summarizing the distribution of disease in 33 patients, we found that 13 (39.4%) patients were MDS, four (12.1%) were aplastic anemia (AA), three (9.1%) were AML, five (15.1%) were diagnosed with other diseases and eight (21.2%) were undiagnosed (**Table 1**). We then classified the MDS according to the 2008 and 2016 WHO guideline, and AML according to FAB guideline (**Table 1**).

Table 1 Clinical characteristics of patients with TET2 gene germline mutation

Patient	Gender	Diagnosis	VAF	Mutation type	Mutation site	Exon	Karyotype	MAF	1000 genome	ExA
Age (<=60 years)										
1	F	?	0.5048	missense	c.2604T>G(p.Phe868Leu)	3	46,XY[20]	0.0024	0.0024	0.00
2	M	Neutropenia	0.5234	missense	c.2604T>G(p.Phe868Leu)	3	46,XY[20]	0.0024	0.0024	0.00
3	F	?	0.493	missense	c.455G>A(p.Ser152Asn)	3	NA	NR	NR	NR
4	M	MDS-U	0.5258	missense	c.2604T>G(p.Phe868Leu)	3	46,XX[20],+(8), UPD(11p)	0.0024	0.0024	0.00
5	M	?	0.509	missense	c.3116C>T(p.Ser1039Leu)	3	NA	0.0012	0.0012	0.00
6*	M	CML	0.5416	missense	c.2604T>G(p.Phe868Leu)	3	NA	0.0024	0.0024	0.00
7	F	AA	0.5285	missense	c.3116C>T(p.Ser1039Leu)	3	46,XX[20]	0.0012	0.0012	0.00
8	M	?	0.4902	missense	c.2604T>G(p.Phe868Leu)	3	NA	0.0024	0.0024	0.00
9	M	HC	0.502	missense	c.3116C>T(p.Ser1039Leu)	3	NA	0.0012	0.0012	0.00
10	F	AA	0.511	missense	c.3116C>T(p.Ser1039Leu)	3	46,XY[20]	0.0012	0.0012	0.00
11	F	AML-M2	0.475	missense	c.3728A>G(p.Lys1243Arg)	6	46,XX,t(8;21)(q22;q22)	NR	NR	NR
12	F	?	0.4841	missense	c.3116C>T(p.Ser1039Leu)	3	NA	0.0012	0.0012	0.00
13	M	AA	0.482	NA	c.3106C>Tp.His1036Tyr	3	46,XY[15]	NR	NR	NR
14	F	IDA	0.5224	missense	c.2604T>G(p.Phe868Leu)	3	46,XY[20]	0.0024	0.0024	0.00
15	M	MDS-U	0.5094	missense	c.2604T>G(p.Phe868Leu)	3	46,XY[20]	0.0024	0.0024	0.00
16	F	AML-M4	0.4867	missense	c.2440C>T(p.Arg814Cys)	3	46,XY[20]	0.0014	0.0014	0.00
17	F	MDS-SLD	0.5023	NA	c.5816A>G(p.Tyr1939Cys)	3	46,XX[20]	NR	NR	NR
18	M	?	0.512	missense	c.1712G>A(p.Arg571His)	3	NA	NR	NR	0.00
19	M	MDS-EB2	0.4984	missense	c.2604T>G(p.Phe868Leu	3	44~45,XY,-6,-7,+13,-17,-21,-22 +r,+3-, 4mar,inc[cp4]/46,XY[1]	0.0024	0.0024	0.00
20	M	MDS-SLD	0.5332	missense	c.3116C>T(p.Ser1039Leu)	3	46,XY[20]	0.0012	0.0012	0.00
21	F	AML-M2	0.5253	missense	c.2604T>G(p.Phe868Leu)	3	46,XX,t(6;11)(q27;q23)[19]/46,xx[1]	0.0024	0.0024	0.00
22	F	MDS-EB2	0.5061	missense	c.427G>A(p.Asp143Asn)	3	46,XX[20]	NR	NR	0.00
Patient Num	Gender	Diagnosis	VAF	Mutation type	Mutation site	Exon	Karyotype	MAF	1000 genome	ExA
Age (>60 years)										
23	F	MDS-RA	0.5058	missense	c.2440C>T(p.Arg814Cys)	3	46,XX[20]	0.0014	0.0014	0.00
24	F	MDS-SLD	0.4912	missense	c.2440C>T(p.Arg814Cys)	3	47,XX,+add(1)(p11.2)[20]	0.0014	0.0014	0.00
25	F	AA	0.5078	missense	c.2440C>T(p.Arg814Cys)	3	46,XY[20]	0.0014	0.0014	0.00
26	F	MDS-U	0.4835	missense	c.218G>A(p.Arg73His)	3	46,XX,-20,+mar[20]	NR	NR	0.00
27	M	MDS-EB2	0.55	missense	c.2604T>G(p.Phe868Leu)	3	46,XY,der(7)t(1;7) (q10;p10)[20]	0.0024	0.0024	0.00
28	M	MDS-MLD	0.5125	missense	c.3116C>T(p.Ser1039Leu)	3	46,XX[20]	0.0012	0.0012	0.00
29	M	?	0.4965	missense	c.3116C>T(p.Ser1039Leu)	3	46,XY[20]	0.0012	0.0012	0.00
30	M	MDS-RAEB1	0.4845	NA	c.4183G>A(p.Val1395Ile)	3	46~48,XY,+1,-5,del(5) (q13q33),+8,-9,-18,-20, +2~4mar1,+mar2[cp20]	NR	NR	NR
31	M	MDS-RAEB2	0.5259	missense	c.2604T>G(p.Phe868Leu)	3	46,XX[20]	0.0024	0.0024	0.00
32	M	ITP	0.4985	missense	c.3116C>T(p.Ser1039Leu)	3	46,XY[20]	0.0012	0.0012	0.00
33	M	?	0.5002	missense	c.2604T>G(p.Phe868Leu)	3	46,XX[13]	0.0024	0.0024	0.00

Abbreviation: patient No, patient's number; VAF, variate allele frequency; MAF, minor allele frequency; ExAC, Exome Aggregation Consortium; M, male; F, female; HC, hepatic cirrhosis; IDA, iron deficiency anemia; NA, no available; NR, no report; ?, undiagnosed; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; MDS-RA, MDS with refractory anemia; MDS-SLD, MDS with single lineage dysplasia; MDS-U, MDS unclassifiable; MDS-RAEB1, MDS with refractory anemia and excess blast-1; MDS-RAEB-2, MDS with refractory anemia and excess blast-2; MDS-EB2, MDS with excess blast-2; (BM>10-19% or PB 5-19%); MDS-MLD, MDS with multilineage dysplasia; CML, chronic myeloid leukemia; AA, aplastic anemia.

A total of 70 patients with somatic TET2 mutation were included in our study, including 45 (64.29%) male patients and 25 (35.71%) female patients. The median age of the patients was 65 (range, 24-89). The median VAF was 44.87% (rang, 5.45-95.9%). Unlike germline mutation, the mutation patterns of somatic mutation were diversity, including frameshift mutation, missense mutation, nonsense mutation, intron mutation and other mutations. The frameshift mutation was the most common. A total of 86 mutation sites were detected among 70 patients, one patient had three mutation sites and 14 patients had two mutation sites. The recurrence of the mutation sites was relatively rare, including: c.3409+42G>T, c.3595-4G>A, c.2290C>T(p.Gln764Ter). Among the 70 patients, 48 (68.6%) patients had normal karyotype, one patient (1.4%) only had -Y, four patients (5.7%) had complex karyotypes, and three patients (5.7%) patients had one more chromosome 8, five patients (7.1%) had other abnormal karyotypes, and eight patients (11.4%) had unknown karyotypes. We also found that most of the patients in this group were AML, accounting for 40.0%, followed by 18 (25.7%) MDS patients, nine (12.9%) MDS/MPN patients, one (1.4%) AA patients and 14 (20.0%) undiagnosed patients. Additionally, among the 28 AML patients, most were AML-M2 and AML-M4, 14 (50%) and 7 (25%) respectively. Among the nine patients with MDS/MPN, the majority were CMML patients (n=6, 66.7%)

Pedigree investigation of patients with germline TET2 mutation

We collected hairs and nails of family members of nine proband patients to detect the statue of TET2, and two family members also completed the peripheral blood count test. The family number were consistent with patient number in **Table 1**, including family one, 11,17, 19, 22, 24, 25, 28, 31. Among the nine patients, including six patients with MDS, and one patient each with AML, AA and neutropenia. After testing the hair and nail samples of the family members, we found that some family members carried the germline TET2 mutation like proband patients with same mutation site and pattern, but without any hematological symptoms (**Fig 1**). What's more, peripheral blood count test of two family members were normal. All the asymptomatic carriers of germline TET2 mutation did not have any other germline or somatic gene mutation like the proband patients. The specific information of the pedigree was provided in **S Data**.

Comparison of patients with germline TET2 mutation alone and not

Among 33 patients, we found that 11(33.33%) patients had the germline TET2 mutation alone, while other 22(66.67%) patients had other gene germline or somatic mutations. The disease distribution and bone marrow blast percentage were significant different between the two subgroups (P=0.026, P=0.004, respectively). Two of 11 patients with germline TET2 mutation alone were MDS, others were unclearly diagnosed. While among 22 patients with other gene mutations, most were diagnosed with hematological neoplasms, including 11 MDS patients and 3 AML patients. The median of myeloblast percentage of the 11 patients was significantly lower than the 22 patients with other gene mutations (1% (0.5-4%) *vs.* 4% (1.0-48%), P=0.004). The other indicators, like age, VAF, chromosome karyotype, hemoglobin, white blood cell count (WBC), lymphocyte absolute count and so on were all not significant different. **(Table 2)**

Table 2. Comparison of patients with germline TET2 mutation alone and not

Patient's parameters	TET2 GM-Alone (n=11)	TET2 GM-Others (n=22)	*P*
Age(Y)median(range)	46(16-74)	55.5(27-82)	0.114
Male (%)	7(63.6%)	11(50.0%)	0.712
VAF (%) median(range)	50.23(48.2-54.16)	50.70(47.5-55.0)	0.611
Cytogenetics			0.384
Normal or -Y alone	6(54.5%)	12(54.5%)	
Complex	0(0.0%)	2(9.1%)	
Others	1(9.1%)	5(22.7%)	
unknown	4(36.4%)	3(13.6%)	
Diagnosis			**0.026**
MDS/AML	2(18.2%)	14(63.6%)	
Others	9(72.8%)	8(22.7%)	
Peripheral blood median(range)			
Hemoglobin (g/L)	73(52-160)	83(27.0-137)	0.711
WBC ($\times10^9$/L)	4.43(0.23-33.38)	4.72(1.26-13.25)	0.749
Platelet ($\times10^9$/L)	124(3-611)	62(8-348)	1
Absolute Neutrophils ($\times10^9$/L)	1.83(0.0-25.37)	1.53(0.19-8.86)	0.711
Absolute Lymphocyte ($\times10^9$/L)	1.80(0.2-3.0)	1.19(0.3-2.66)	0.223
Absolute Monocyte ($\times10^9$/L)	0.23(0.0-0.83)	0.24(0.0-0.760)	0.863
Total Eosinophils ($\times10^9$/L)	0.2(0-1.0)	0.01(0-0.49)	0.065
Bone marrow (%) median (range)			
Myeloblast	1(0.5-4.0)	4(1.0-48)	**0.004**
Mature Lymphocyte	21(5.0-70.0)	13.25(5.5-49)	0.145
Mature Monocyte	1.5(0.5-2.0)	1.5(0.5-21.0)	0.438
Basophilic Erythroblast	1.5(0.5-2.0)	1.5(0.5-5.0)	0.71
Polychromatophilic erythroblast	9.0(3.0-17.5)	8.5(1.5-25.5)	0.76
Acidophilic Erythroblast	16.5(4-39)	15.5(2.5-51.5)	0.89

Aberration: Age (Y), Age (year); n, number of patients; TET2 GM-Alone, TET2 germline mutation alone; TET2 GM-others, TET2 germline mutation simultaneously with other gene mutation; VAF, variate allele frequency; MDS/AML, myelodysplastic syndrome/acute myeloid leukemia.

Value of *P* less than 0.05 is statistic significant.

Comparison of germline and somatic TET2 mutation

As described previously, VAF in patients with germline TET2 mutation was more concentrated, the median was 50.58% (range, 40-55%), while the VAF was decentralized and the median was 44.87% (range, 5.45%-95.9%) in patients with somatic TET2 mutation, (*P*<0.0001) (**S1 Fig A**). The difference of the TET2 mutation sites also existed between the germline and somatic mutation. The germline mutation sites were more recurrent, which mainly concentrated on three mutation sites: c.2604T>G(p.Phe868Leu), c.3116C>T(p.Ser1039Leu) and c.2440C>T(p.Arg814Cys). However, the somatic mutation sites were more diversity, also only three sites were repeated with a less frequency. What's more, no common mutation sites were detected in the two group of patients (**S1 Fig B, C**). We further classified and compared the co-mutated genes according to the functional region without significant difference (P=0.976) (**S2 Fig**).

Among the 33 patients with germline TET2 mutation, 11 patients had the germline TET2 mutation alone and 22 patients also had other gene mutations. We found that the most common co-mutated genes with germline TET2 mutation were germline ZRSR2 mutation and somatic TP53 mutation, followed by ETV6, BCORL1, RUNX1 and NF1. In addition, we discovered that 13 patients only had one co-mutated gene, including IDH2, SF3B1, STAG2, SETBP1, PIGA, KIT, FLT3, EZH2, DNMT3A, CSF3R, CEBPA, CBL, WT1 genes. Among the co-mutated genes, STAG2, PIGA, CSF3R, CBL and BCOR were germline mutation, while IDH2, SF3B1, SETBP1, KIT, EZH2, FLT3, CEBPA, CBL were somatic mutation. However, 12 genes in our report were mutually exclusive with germline TET2 mutation, including U2AF1, SRSF2, PTPN11, KRAS, MPL, IDH1, ETNK1, CALR, PHF6, NRAS, NPM1, JAK2 **(Fig 2A)**.

For the 70 patients with TET2 somatic mutation, 64 (91.4%) patients also had other gene mutations and only six (8.6%) patients had somatic TET2 mutation alone. There were six genes mutually exclusive with somatic TET2 mutation, including CSF3R, ETNK1, PIGA, MPL, SETBP1 and IDH1, while 27 other genes were co-mutated with the somatic TET2 mutation. Among the co-mutated genes, ASXL1 was the most common co-mutated gene, followed by DNMT3A, ZRSR2, NPM1, CEBPA, BCOR, RUNX1, STAG2, SF3B1, NRAS and so on (**Fig 2B**). What's more, only ASXL1, ZRSR2, BCOR, RUNX1, PTPN11 and JAK2 had the germline mutation as a co-mutated gene.

In the total number of 103 patients with TET2 mutation, no MPL, ETNK1, IDH1 gene mutations were detected. While U2AF1, SRSF2, PTPN11, KRAS, CALR, PHF6, NRAS, NPM1, JAK2 gene mutations were detected in patients with germline TET2 mutation, but not in patients with somatic TET2 mutation **(S3 Fig)**.

Comparison of clinical features of patients with germline and somatic TET2 mutation

We collected the clinical characteristics of patients with germline and somatic mutation, respectively, and compared the indicators between the two groups, including age, diseases, peripheral blood cell count, and bone marrow smear cell proportion and so on. The age of the 33 patients with germline TET2 mutation was significantly younger than 70 patients with somatic TET2 mutation (48 (rang, 16-82) vs. 65 (rang, 24-89), P=0.0078). The distribution of the sex and chromosome karyotype was not significant different between the two groups (P=0.39 and P=0.415, respectively). Comparing the disease distribution, we found that the difference between the two groups was statistic significant (P=0.0003). Among the 33 patients with germline mutation were mainly 13(39.4%) MDS patients, while the 70 patients with somatic mutation were AML (n=28, 40%), followed by 18(25.7%) MDS patients. We further compared the peripheral blood cell count and found that only WBC was significant different between the two groups (4.69(0.23-33.38)×10^9/L *vs.*7.025(0.97-237.29)×10^9/L, P=0.038). However, the hemoglobin, platelet count, neutrophilic granulocyte, lymphocyte absolute count and monocyte absolute count were all not (**S4 Fig, Table 3).** For the proportion of cells in the bone marrow, the percentage of Myeloblast in patients with somatic TET2 mutation was higher than that in the germline mutation patients (P<0.05). However, patients with germline TET2 mutation had higher percentage of mature lymphocyte, total eosinophils and eosinophils than that of patients with somatic TET2 mutation (P<0.05) (**S5 Fig, Table 3**). For the ten common co-mutated genes, only mutation frequency of NPM1, ASXL1, and DNMT3A genes were significant different (P= 0.0152, 0.0104 and 0.0338, respectively) **(Table 3)**.

Table 3. Clinical characteristic of patients with germline and somatic TET2 mutation

Patient's parameters	Statue of TET2 mutation		*P*
	Germline mutation(n=33)	Somatic mutation(n=70)	
Age(Y)median(range)	48(16-82)	65(24-89)	**0.0078**
Male (%)	18(54.55%)	45(64.29%)	0.39
VAF (%) median(range)	50.58(40.48-55)	44.87(5.45-95.9)	**<0.0001**
Cytogenetics (%)			0.415
Normal or -Y alone	18(54.5%)	49(70.0%)	
Complex	2(6.1%)	4(5.7%)	
Others	6(18.2%)	9(12.9%)	
Unknown	7(21.2%)	8(11.4%)	
Diagnosis (n)			**0.0003**
MDS	13(39.4%)	18(25.7%)	
AML	3(9.1%)	28(40.0%)	
AA	4(12.1%)	1(1.4%)	
MDS/MPN	0(0%)	9(12.9%)	
Others	13(39.4%)	14(20.0%)	
Peripheral blood median(range)			
Hemoglobin (g/L)	74(27-160)	79.5(37-135)	0.568
WBC ($\times10^9$/L)	4.69(0.23-33.38)	7.025(0.97-237.29)	**0.038**
Platelet ($\times10^9$/L)	64(3-611)	61.5(6-1085)	0.785
Absolute Neutrophils ($\times10^9$/L)	1.58(0-25.37)	1.89(0.06-92.99)	0.5332
Absolute Lymphocyte ($\times10^9$/L)	1.33(0.2-3)	1.64(0.27-12.52)	0.0819
Absolute Monocyte ($\times10^9$/L)	0.24(0-0.83)	0.26(0-10.64)	0.3976
Bone marrow (%) median(range)			
Myeloblast	2(0.5-15.5)	7.5(0.5-86)	**0.0026**
Basophilic Erythroblast	1.5(0.5-5)	1.5(0.5-9)	0.9502
Polychromatophilic erythroblast	8.5(1.5-25.5)	6.5(0.5-36)	0.289
Acidophilic Erythroblast	16.5(2.5-51.5)	10(0.5-48.5)	0.1005
Mature Monocyte	1.5(0.5-21)	3(0.5-12)	0.1182
Mature Lymphocyte	16(5-70)	6.5(0.5-42.5)	**<0.0001**
Total Eosinophils	2(0.5-10.5)	1(0.5-9)	**0.0214**
ESG	1.5(0.5-5)	0.5(0.5-3)	**0.0061**
Mutate gene(n)			
CEBPA (+/-)	1/32(3.0%)	11/59(15.7%)	0.0973
FLT3 (+/-)	1/32(3.0%)	7/63(10.0%)	0.4311
NPM1 (+/-)	0/33(0.00%)	11/59(15.7%)	**0.0152**
RUNX1 (+/-)	2/31(6.1%)	10/60(14.29%)	0.3293
ASXL1 (+/-)	1/32(3.0%)	16/55(22.86%)	**0.0104**
TP53(+/-)	6/26(18.18%)	6/64(8.6%)	0.1923
DNMT3A (+/-)	1/32(3.0%)	13/57(18.6%)	**0.0338**
ZRSR2 (+/-)	6/28(18.18%)	11/59(15.7%)	0.7803
SF3B1 (+/-)	1/32(3.0%)	8/62(11.4%)	0.2654
SRSF2 (+/-)	0/33(0.0%)	6/64(8.6%)	0.1732

Aberration: Age (Y), Age (year); n, number of patients; VAF, variate allele frequency; ESG, Eosinophilic segmented granulocytes;

P value less than 0.05 indicate statistic significant.

We further selected the MDS patients from the two group of patients with germline and somatic TET2 mutation, respectively. Eventually, we included 13 MDS patients with germline TET2 mutation and 18 MDS patients with somatic TET2 mutation. Comparing the characteristic indicators, we discovered that only the difference of the VAF between the two subgroups was statistic significant. The differences of age, sex, cytogenetics, peripheral blood cell count, percentage of cell in bone marrow and the co-mutated genes were not significant (**S2 Table**).

Comparison of survival of patients with germline and somatic TET2 mutation

Till February 22th, 2020, a total of 30 patients with germline TET2 mutation and 59 patients with somatic TET2 mutation were included for survival analysis. Among the 30 patients with germline mutation, six (20.0%) patients died, 19 (63.3%) survived and five (16.7%) were lost. While among the 59 patients with somatic mutation, 23 (39%) died, 22 (37.3%) still survived and 14 (23.7%) were lost. The median survival time of patients with germline and somatic TET2 mutation was 33.3 months and 64 months, respectively. The two-year overall survival (2y-OS) was 83% and 71.8% (*P*=0.2706) (**Fig 3A**), respectively. We further selected out the patients without the ASXL1, NPM1 and DNMT3A gene mutations in the two groups for sub-analysis, and discovered no significant difference of OS between the two groups (**S6 Fig**). However, we found that the median survival time of MDS patients with germline TET2 mutation was significant shorter than that of MDS patients with somatic mutation (11.7 months vs. 64 months; 2y-OS: 72.7% vs. 91.7%, 95%CI: 0.89-32.2; *P*=0.0209) (**Fig 3B**). We further took the age (*P*=0.666), VAF (*P*=0.065) and IPSS-R (*P*=0.695) as covariates and adjusted via multivariate analysis of COX regression model, finding the difference of 2y-OS was not significant (95%CI: 0.9-77.5, *P*=0.062). What's more, we compared the survival of the patients with TET2 mutation alone in the two groups, including 11 patients with germline TET2 mutation alone and four patients with somatic TET2 mutation alone. Due to both no deaths in the two groups, the median survival was not available and no significant difference of survival between the two groups (P>0.9999).

Discussion

In our study, the allele frequency (AF) of germline TET2 mutation sites was all less than 1%. Although, some previous studies reported that it would more probability to be pathogenicity in two situations: the AF was less than 0.05% and 0.01% in the 1000genome and ExAC database respectively; or not reported in the two databases (27, 28). Indeed, the MAF of the germline TET2 mutation was all less than 1% in our study, indicating a rare mutation to exclude the likely of SNP(26). Therefore, we included all the patients with germline TET2 mutation to further analyze and compared with the patients with somatic TET2 mutation.

Collecting nails and hairs of the family members of proband patients with germline TET2 mutation, we found that these family members were all asymptomatic carriers. This phenomenon suggested that germline TET2 mutation might have family aggregation, but whether this similar to that of germline CEBPA, DDX41, RUNX1 mutations associated with hematological diseases needs further study(7, 29, 30). Among the 33 patients with germline TET2 mutation, a total of 11 patients only had germline TET2 mutation and 22 patients also had other gene mutations. Comparing the two subgroups, we discovered patients in the former group more presented with cytopenia, like thrombocytopenia, anemia, neutropenia or others. While only a few of them met the criteria of hematological tumors, and three of the 11 patients were diagnosed with hematological tumors including two MDS and one CML (BCR-ABL1+). However, 14 of another 22 patients were diagnosed with hematological tumors. This difference indicates that the germline TET2 mutation alone may not enough to induce the hematological neoplasms, and other gene co-mutations may be necessary for the initiation. This phenomenon was accordance with the previous reports that TET2 mutation alone often needs other gene mutations as a secondary hit to induce the occurrence and development of tumors(14, 15). This suggests that germline TET2 mutations are susceptible to hematological tumors.

We further compared the characteristic of patients with germline and somatic TET2 mutation. The median age of patients with germline mutation was younger than that of patients with somatic mutation (*P*=0.0078), which is similar to the previous reports about patients with germline CEBPA and RUNX1 mutation being younger(6, 30). However, the difference of median age in the two MDS subgroups was not significant, which may indicate that patients with germline TET2 mutation need a long incubation period to develop MDS or hematological tumors as sporadic patients, like the germline DDX41 mutation in hematological neoplasms(7). In the early stage, they most present with single lineage cytopenia and eventually develop into hematological neoplasms under the other gene mutations as a second hit. The difference of VAF was statistic significant (*P*=0.0001). The VAF of germline TET2 mutation ranged from 40.48% to 55%, which was consistent with the previous report that the germline gene mutation is more stable with a range from 40% to 60%(1). We also found that most patients of the two groups were normal karyotypes, being in accordance with the previous conclusion(31, 32). For the disease distribution, the patients with germline mutation were predominant MDS, while the patients with somatic mutation were AML (*P*=0.0003). For this result, we deduced that it may be related to the bias of the patient visits, and the short observation time was not enough to observe the progression of MDS or other diseases to AML. In fact, patients with somatic TET2 mutation were more likely to have ASXL1, NPM1 and DNMT3A gene co-mutations, which may be another potential factor for the disease differences. Besides, we also found that the 28 AML patients with somatic TET2 mutation were mainly M2 (14 (50%)), and among 9 MDS/MPN patients were more CMML (6(66.7%)). We hypothesized that this may be because TET2 impacts the differentiation of progenitor cells into myelomonocytic cells and neutrophilia, like the previous reports(33-35). Pan et al. reported that TET2 deletion induces mice to transform into myeloid tumors, mainly manifests as mononucleosis and neutrophilia(33). For other co-mutation genes, we discovered that IDH1/2 mutation rarely appeared in the total of 103 patients, which was almost mutually exclusive with the TET2 mutation. This phenomenon was similar to previous reports(36, 37).

For the peripheral blood parameters and the proportion of the bone marrow cell smear, we discovered that the WBC and the proportions of myeloblast were both significantly higher in patients with somatic mutations (*P*=0.038 and 0.0026, respectively). But this phenomenon did not exist in the MDS subgroup. Considering the difference of the disease distributions, we deduced that the result may be associated with it. While it is uncertain whether the differences of the WBC and myeloblast are affected by the difference of germline and somatic TET2 mutations, because previous articles reported that AML patients with TET2 mutation have a higher WBC(19, 32). We also observed that the proportion of bone marrow mature lymphocytes, total eosinophils and eosinophils in

patients with germline mutation were higher (*P*<0.05). Although the differences of the percentage of bone marrow mature lymphocytes and total eosinophils were not statistic significant in the MDS subgroups (P=0.052 and 0.055, respectively), the proportion of that two cells would higher than that in patients with somatic mutation. The difference of erythroid cells between two groups was not found in our study, which was.

The prognosis of TET2 mutation in hematological tumors is also controversial. Some articles reported that it has a prior prognosis in MDS patients or has no significant impact(17, 38), others reported it has no significant influence or an adverse impact in AML patients with normal karyotypes(39, 40). We compared the survival between the two groups and found no difference of 2y-OS. While in the MDS subgroup, the prognosis of patients with germline mutation was poorer than patients with somatic mutation (2y-OS: 2y-OS:72.7% vs 91.7%, HR=5.3, 95%CI: 0.89-32.2, P=0.0209) via K-M univariate analysis. We found only VAF had significant influence on the OS in the two groups of patients via univariate analysis (P<0.05). However, considering age and IPSS-R are common factors affecting the prognosis of MDS patients, we took these three factors as covariates to further compare the 2y-OS between two MDS subgroups by Cox regression model *(P*=0.062). Although the value of *P* was close to 0.05 rather than statistic significant, it was not still completely clear whether the germline TET2 mutation is an independent poor prognostic factor for MDS patients. According to IPSS-R risk stratification, 77% of patients with germline TET2 mutation were medium/high risk, while 61.1% of patients with somatic TET2 mutation were medium/high risk. We speculated that this risk difference might be another factor affecting the prognosis difference of MDS patients. In addition, patients with germline TET2 mutation may have a poor response to the traditional chemotherapeutics and need more active treatment measures in the early stage, such as hematopoietic stem cell transplantation (HSCT), which was similar to germline GATA2 and RUNX1 mutations (10, 30). In order to avoid the impacts of other gene mutations on the prognosis as much as possible, we compared the survival of patients only with germline and somatic TET2 mutation alone, but found no difference. Thus, whether the germline and somatic TET2 mutation have different effects on the prognosis of patients with hematological disease are still ambiguous.

Although our study firstly reported the role of germline TET2 mutation in the hematological diseases, there were still many limitations. Firstly, the sample size in our study was not enough, only including 33 patients with germline mutation and 70 patients with somatic mutation and there was a certain difference of sample sizes. Secondly, we couldn't completely avoid the impact of other gene mutations on clinical characteristics and prognosis. Thirdly, we did not collect a complete family history of every proband patients with germline mutation and only collected nail and hair specimens of family members of nine proband patients. Fourthly, due to the number of patients accepting demethylation drugs was small, we did not compare the response to demethylation drug between the two groups. Finally, some people were lost to follow-up and the follow-up time was not enough to observe some disease progression, so we did not make more analysis on the prognosis between the two groups.

Conclusion

we have shown that some family members are asymptomatic carriers, which indicates an aggregation of germline TET2 mutation and needs a longer time to observe whether they will develop into the hematology diseases. More importantly, TET2 gene may be a predisposition gene of hematological malignant when the other gene mutations as the second hit. The VAF of germline TET2 mutation is more stable. The germline TET2 mutation may play a role in the lymphocyte, WBC, myeloblast, eosinophils, incurring the difference between the two groups. At the same time, the germline TET2 mutation may be an adverse factor for the MDS patients, which We hope this phenomenon will provide a helpful and significant information for germline gene mutations in hematology diseases and to better the management of the unique patient population.

Abbreviations

TET2: Tet methylcytosine dioxygenase 2

NGS: Next generation sequencing

BM: Bone marrow

PB: Peripheral blood

VAF: Variant allele frequency

OS: Overall survival

HR: Hazard Ratio

CI: Confidential intervals

AML: Acute myeloid leukemia

MDS: Myelodysplastic syndrome

FPD/AML: Familial platelet disorder predisposition to acute myeloid leukemia

SNP: Single nucleotide polymorphism

WBC: White blood cell

HM: Hematological malignancies

WHO: World Health Organization

HSC: Hematopoietic stem cell

CMML: Chronic myelomonocytic leukemia

MPN: Myeloproliferative neoplasms

AA: Aplastic anemia

AF: Allele frequency

MAF: Minor allele frequency

ExAC: Exome Aggregation Consortium

DAC: Decitabine

AZA: 5-azacitidine

IPSS-R: International Prognostic Scoring System revised

HSCT: Hematopoietic stem cell transplantation

Declarations

Ethics approval and consent to participate

Due to the far distance and traffic inconvenience, we can't gather all the patients and their family members to sign the informed consents. Therefore, the informed consents were obtained from all patients and their family members by verbal in accordance with the Declaration of Helsinki. Ethic committees of our hospital all approved this procedure. This article does not contain any studies with animals performed by any of the authors.

Consent for publication

Participants gave informed consent for data to be published anonymously, provided that the researchers would not release any information that could be linked to them.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

Founding

The work was found by the Foundation of the Science & Technology Department of Sichuan Province in China (NO. 2019YFS0026). The founder was responsible for the design and revise of the study.

Author contributions

XW and YG designed the study. XW drafted the manuscript and reviewed the literature. XW, JD, NZ and WY collected the data. XW, TY, XL and XZ analyzed and guided interpretation the data. XW and YG revised the manuscript. All authors critiqued the manuscript and provided final approval.

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Figures

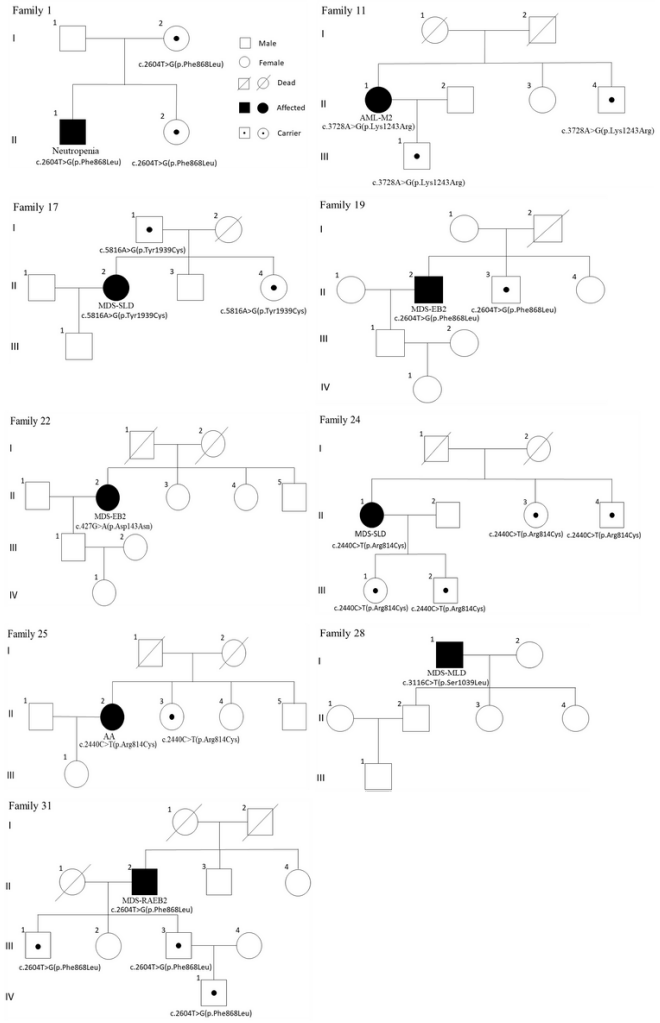

Figure 1

The pedigree of nine patients with germline TET2 mutation. Family number was accordance with the patient number. Nails and hairs as the germline DNA origin to test the TET2 statue. The more details of the pedigree investigation were supplied in the supplement manuscript.

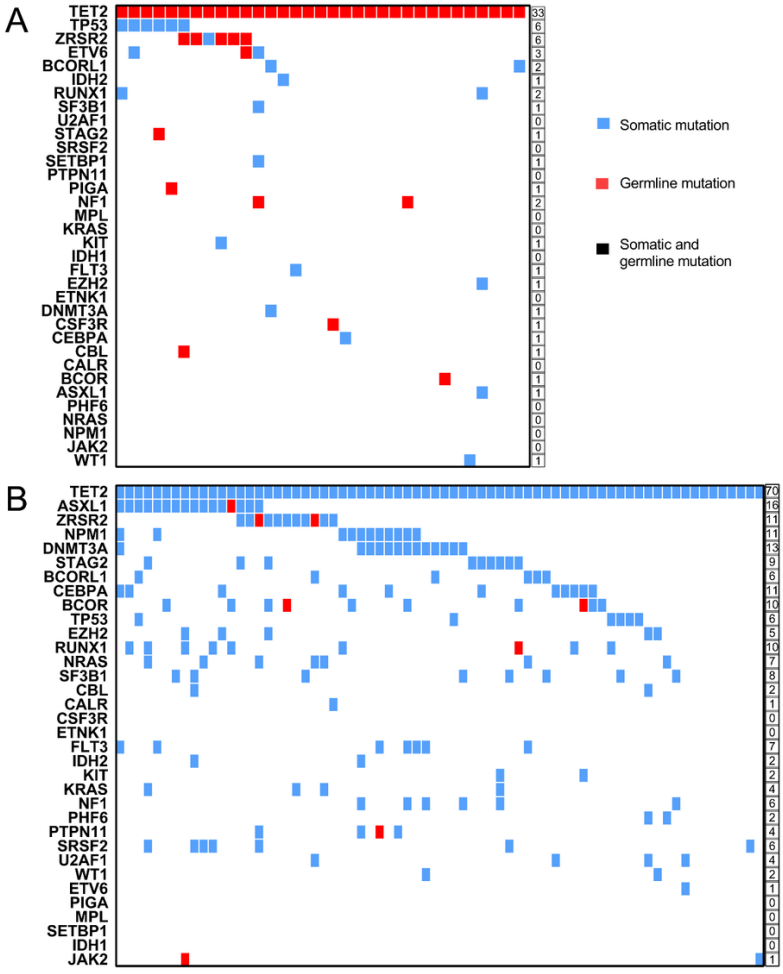

Figure 2

Co-mutated gene distribution in patients with germline and somatic TET2 mutation. (A) and (B) co-mutated gene distribution in patients with TET2 germline and somatic mutation, respectively. Each column in the figure represents a patient, and the digital at the right is the number of patients with each mutant gene. Germline mutation (blue), somatic mutation (red), germline and somatic mutation (black).

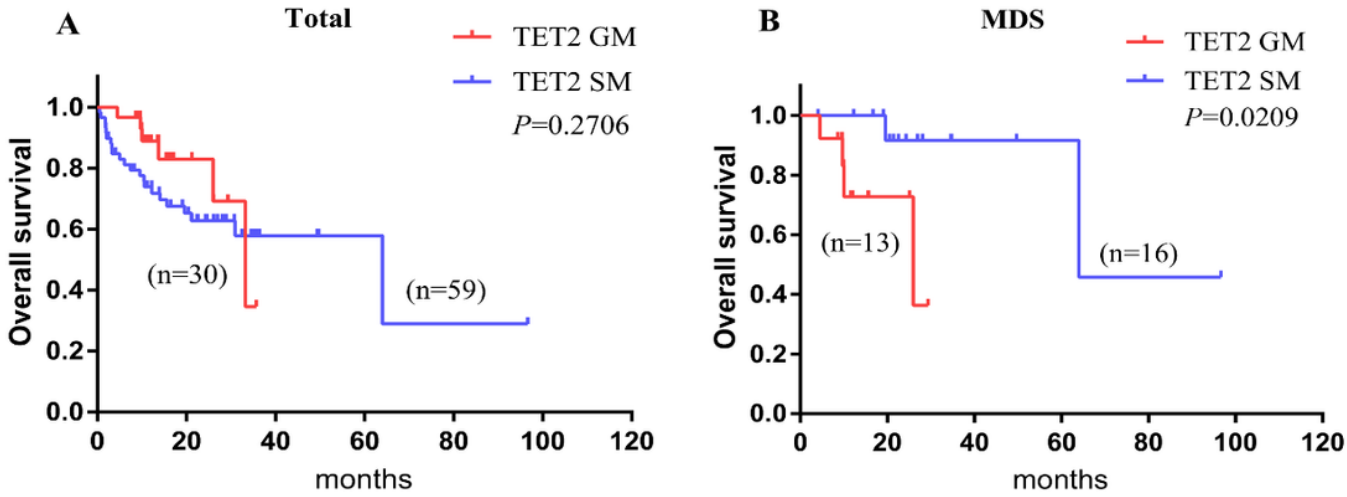

Figure 3

Survival outcomes in patients with germline and somatic TET2 mutation. Kaplan-Meier curves are stratified by TET2 mutation status: germline TET2 mutation (red), somatic TET2 mutation (blue). (A) OS in all patients with germline and somatic TET2 mutation. (B) OS in MDS patients with germline and somatic TET2

mutation.

Supplementary Files

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