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Correlation analysis between *JAK2*, *MPL*, and *CALR* mutations in patients with myeloproliferative neoplasms of Chinese Uygur and Han nationality and their clinical characteristics

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Abstract

Background: Genetic factors play a role in the etiology of BCR-ABL-negative myeloproliferative neoplasms (MPNs). This study explored the relationship between mutations in the Janus kinase 2 gene (*JAK2*), *MPL*, and the calreticulin gene (*CALR*) in Uygur and Han Chinese patients with BCR-ABL fusion gene-negative MPN and corresponding clinical features.

Methods: A total of 492 BCR-ABL-negative MPN patients treated in our hospital from May 2013 to August 2016 were enrolled. Genomic DNA was extracted from peripheral blood and used for PCR amplification and DNA sequencing. Mutations including *JAK2* V617F, *MPL* W515L/K, and those in *JAK2* exon 12 and *CALR* were analyzed and compared with patient clinical characteristics.

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Results: Of the 492 MPN patients, 169 were Uygur and 323 were Han. In these two patient groups, *JAK2* mutations were detected in 39.64% and 52.63%, respectively, *CALR* mutations were detected in 10.06% and 20.43%, respectively, and *MPL* mutations were detected in 0.93% of Han patients. The age, white blood cell count, platelet levels, and hemoglobin levels in *JAK2* in Han patients were higher than those in Uygur patients.
Conclusion: Han MPN patients harboring *JAK2* mutations had higher level of age, WBC, PLT, and Hb than Uyghur patients with the same mutations.

Keywords
JAK2, *MPL*, *CALR*, myeloproliferative neoplasms, primary thrombocytosis, single nucleotide polymorphism

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Introduction

The myeloproliferative neoplasms (MPNs) are a group of heterogeneous diseases caused by the aberrant proliferation of bone marrow hematopoietic cells (BMHCs).[1] BCR-ABL-negative MPNs include primary myelofibrosis (PMF), essential thrombocythemia (ET), and polycythemia vera (PV).[2] Prominent clinical symptoms of MPN patients include the overproduction of erythrocytes and blood platelets, splenomegaly, myelofibrosis, and thrombogenesis.[3]

Baxter et al. first identified the *Janus kinase 2* gene (*JAK2*) V617F mutation in MPN patients in 2005,[4] and since then the role of *JAK2* mutations in MPN has become a hotspot for research. Additionally, calreticulin gene (*CALR*) and MPL mutations have previously been identified in some ET and PMF patients.[5] As a multi-functional protein, CALR plays an important role in a variety of biological processes including calcium dynamic equilibrium regulation, new synthetic glycoprotein folding, cell proliferation, differentiation, and apoptosis.[6] MPL, the receptor of thrombopoietin, is crucial for megakaryogenesis, platelet production, and hematopoietic stem cell homeostasis.[7]

The main population groups in the Xinjiang region of China are Uygur and Han, but it is unclear whether *JAK2*, *MPL*, and *CALR* mutations differ between Uygur and Han MPN patients. In this study, we investigated *JAK2*, *MPL*, and *CALR* mutations in MPN patients of Chinese Uygur and Han nationality, and analyzed the effect of these mutations on clinicopathologic features in the two ethnic groups.

Materials and methods

Patients and samples

A total of 492 patients with BCR-ABL1-negative MPN at the People's Hospital of Xinjiang Uygur Autonomous Region were recruited for this study between May 2013 and August 2016, including 104 cases of PMF, 240 cases of ET, and 148 cases of PV. Diagnosis was according to the World Health Organization Classification of Tumours of Haematopoietic and

Lymphoid Tissues (2016).[8] Informed consent was obtained from all subjects and the study was approved by the Local Ethical Committee of the People's Hospital of Xinjiang Uygur Autonomous Region (Urumqi, China; approval no.: LL20150114).

Methods

We collected clinical data from MPN patients, including white blood cell (WBC) counts, and levels of hemoglobin (Hb), platelets (PLT), and lactate dehydrogenase (LDH). We also conducted bone marrow biopsy analysis of myelofibrosis and Acuson 128XP/10 ultrasound detection of thrombogenesis. Genomic (g)DNA was isolated from peripheral blood using the QIAmp DNA Blood Mini blood kit (Qiagen, Hilden, Germany). The extracted gDNA was sent to Sunny Biological Technology (Shanghai, China) for DNA sequencing of mutations. The mutation sites analyzed included *JAK2* V617F, *JAK2* K539L, *JAK2* exon 12 N542-E543del, *MPL* W515L/K, and *CALR* L367fs*46 and K385fs*47. Sequencing primers were synthesized as previously described.[9–11]

Statistical analysis

SPSS 20.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. Differences between two groups were evaluated using the t test or Kruskal–Wallis test. Differences between multiple groups were evaluated using one-way analysis of variance. The Chi-square test was used to calculate the significance of enumeration data between two groups. Values of P below 0.05 were considered to be significant.

Results

Prevalence of JAK2 V617F, JAK2 exon 12, MPL, and CALR mutations

gDNA sequencing identified 234 (47.56%) cases with *JAK2* V617F mutations, three (0.61%) with *JAK2* exon 12 mutations, 83 with *CALR* mutations (16.87%), and three with *MPL* W515L/K mutations. No patient displayed all four mutation types. Sequencing results also showed that no mutation was found in the remaining 169 (34.35%) patients with BCR-ABL-negative MPN.

Among the 104 patients with PMF, 46 (44.23%) had *JAK2* V617F, and 16 (15.38%) had *CALR* mutations. The remaining 42 cases were negative for all types of mutation. The 240 patients with ET included 97 (40.42%) with *JAK2* V617F, 67 (27.92%) with *CALR* mutations, 3 (1.25%) with *MPL* W515L/K mutations, and 73 with non-mutated *JAK2*, *CALR*, and *MPL*. The group of 148 patients with PV comprised 91 (61.49%) with *JAK2* V617F, and three (3.06%) with mutations in *JAK2* exon 12, including one (0.68%) with K539L and two (1.35%) with N542-E543del. No mutation was detected in the remaining 54 PV patients.

DNA sequencing chromatograms of mutations in *JAK2*, *CALR*, and *MPL* are shown in Figure 1. The distributions of *JAK2*, *MPL*, and *CALR* mutations in patients with each type of MPN are shown in Figure 2.

JAK2, MPL, and CALR mutations in MPN patients of Han and Uygur nationality

Among the 429 patients with MPN, 169 (34.35%) had Uygur nationality, and 323 (65.65%) were Han. In Uygur patients, *JAK2* V617F and *CALR* mutations were detected in 39.64% (67/169)

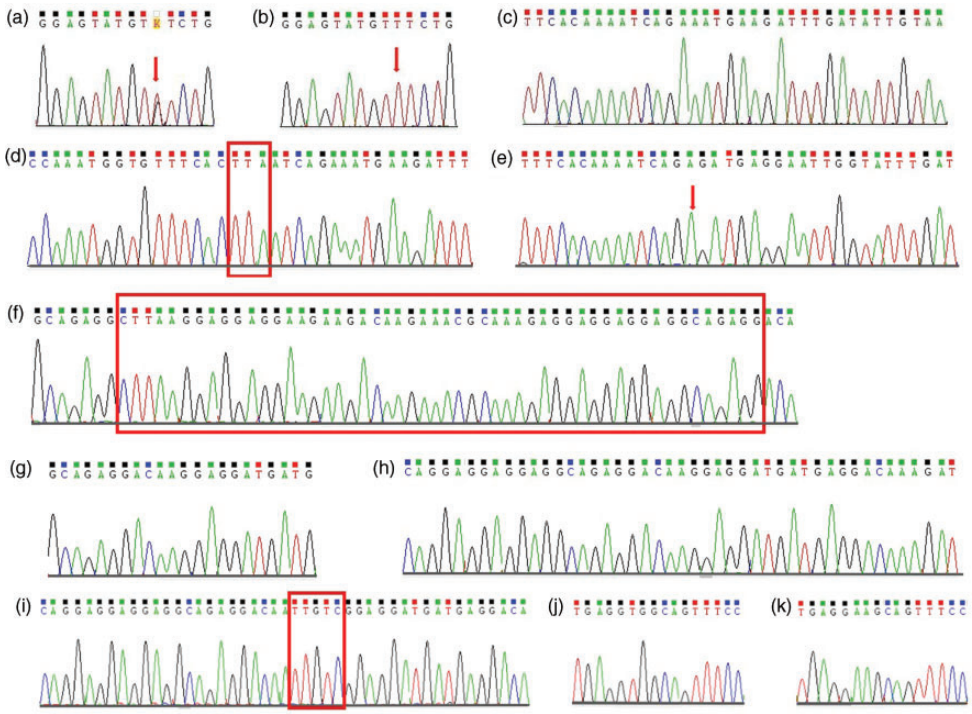

Figure 1. Sequencing chromatogram of mutations in *JAK2*, *CALR*, and *MPL*. (a) Heterozygous mutation "G to A" in *JAK2* V617F. (b) Wild-type *JAK2* V617F sequence. (c) Wild-type *JAK2* exon 12 sequence. (d) K539L mutation in *JAK2* exon 12. (e) *JAK2* exon 12 N542-E543del. (f) Wild-type *CALR* L367fs*46. (g) Mutant *CALR* L367fs*46. (h) Wild-type *CALR* K385fs*47. (i) Mutant *CALR* K385fs*47. (j) TGG to TTG conversion in *MPL* W515L. (k) TGG to AAG conversion in *MPL* W515K.

and 10.06% (17/169), respectively. No *JAK2* V617F or *CALR* mutations were detected in the remaining 85 Uygur patients (50.30%).

For Han MPN patients, *JAK2* mutations were detected in 52.63% (170/323), while *CALR* and *MPL* mutations were seen in 20.43% (66/323) and 0.93% (3/323), respectively. *JAK2* and CALR mutation rates were significantly higher in Han MPN patients than in those with a Uygur nationality ($P = 0.006$). However, no significant difference was found in the rate of *MPL* mutations between Han and Uygur groups. Moreover, the frequency of *JAK2* mutations was significantly higher than that of *CALR* and *MPL* mutations ($P < 0.01$) (Table 1).

Correlation between JAK2, CALR, and MPL mutations in MPN patients and clinical characteristics

In patients with PMF, significant differences in clinical features, including Hb concentrations and LDH levels, were observed among patients who were *JAK2* mutant-positive, *CALR* mutant-positive, and the *JAK2*, *CALR* non-mutation group ($F = 8.954$; $F = 42.351$, $P < 0.001$; and $F = 37.954$, $P < 0.001$, respectively). In ET patients, the age, WBC count,

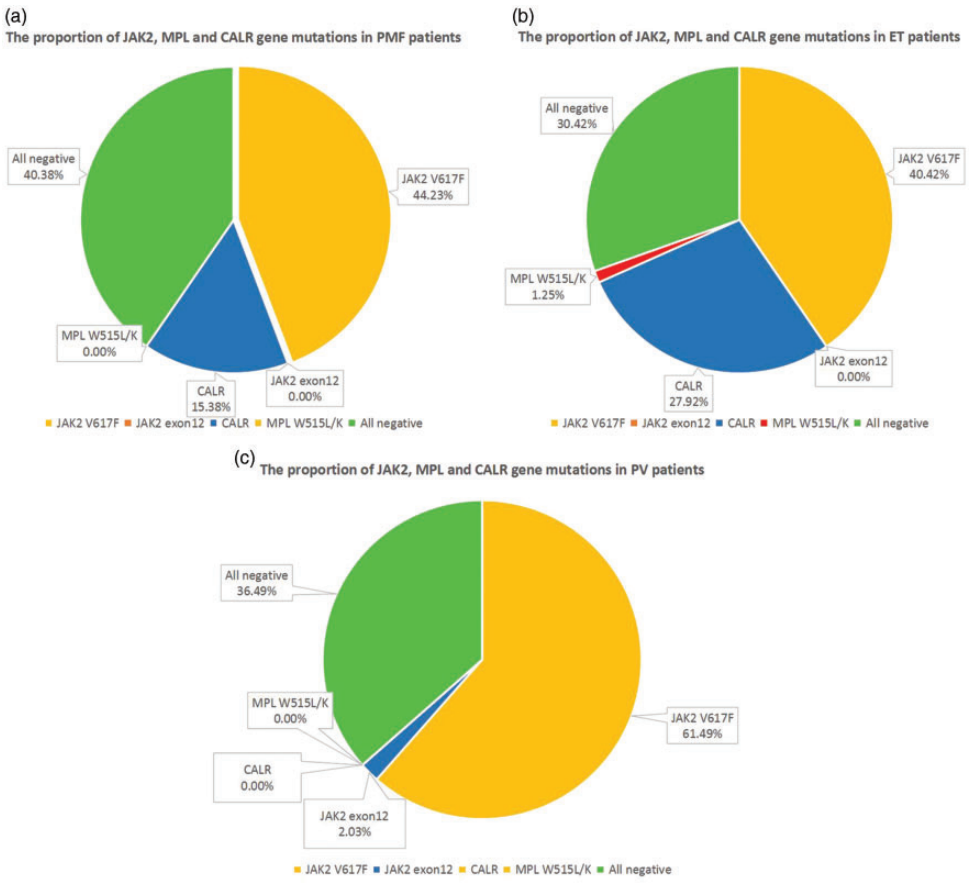

Figure 2. Mutation percentages of *JAK2*, *MPL*, and *CALR* in patients with PMF, ET, and PV. (a) Gene mutation proportion in PMF patients. (b) Gene mutation proportion in ET patients. (c) Gene mutation proportion in PV patients.

Table 1. Comparison of *JAK2*, *MPL*, and *CALR* mutations in MPN patients of Han and Uygur nationality.

Nationality	*JAK2*	*CALR*	*MPL*	Mutation-negative	χ^2 value	*P* value
Uygur (n=169)	67 (39.64%)	17 (10.06%)	0 (0)	85 (50.30%)	31.589	0.000
Han (n=323)	170 (52.63%)	66 (20.43%)	3 (0.93%)	84 (26.01%)		
χ^2 value	7.495	8.515	1.579	29.027		
P value	0.006	0.004	0.209	0.000		

concentration of Hb, PLT level, and LDH level were significantly different among these same patient groups ($F=8.774$; $F=12.153$, $P<0.001$; $F=8.652$; $F=15.654$, $P<0.001$; and $F=23.452$, $P<0.001$, respectively) (Table 2). In PV patients, the age, WBC count, concentration of Hb, PLT level, and LDH level were significantly different among *JAK2* mutant-positive patients and the *JAK2*, *CALR*

Table 2. Relationship between *JAK2* and *CALR* mutations in patients with different types of MPN and clinical features.

Parameter	PMF (n = 104)			ET (n = 240)			PV (n = 148)		
Sex	JAK2 (−) CALR (−)	JAK2 (+)	CALR (+)	JAK2 (−) CALR (−)	JAK2 (+)	CALR (+)	JAK2 (−) CALR (−)	JAK2 (+)	CALR (+)
Male	20	24	7	35	33	32	29	49	0
Female	22	22	9	41	64	35	25	45	0
Age, years	48.74±8.72▲	52.35±9.37	47.65±5.43▲	49.01±8.45▲	58.52±6.73	52.35±7.25▲	49.84±14.31▲	57.43±9.43	/
WBC ($\times 10^9$/L)	3.04±2.25	3.51±2.71	3.25±2.34	10.43±2.01▲	18.71±4.89	7.94±2.15▲	5.53±1.25▲	15.79±5.12	/
Hb (g/L)	87.45±12.43▲	136.87±13.24	68.54±14.28▲	124.27±14.79▲	147.25±25.37	125.73±18.94▲	204.15±16.04▲	187.55±17.38	/
PLT ($\times 10^9$/L)	170.25±20.45	166.38±18.76	171.35±17.95	1215.87±280.65▲	929.75±265.87	892.54±273.24▲	225.17±43.54▲	518.55±63.51	/
Thrombosis									/
+	18	15	4	30	35	25	27	46	/
−	24	31	12	46	62	42	27	48	/
Myelofibrosis									/
0	26	36	10	42	65	45	46	65	/
+	11	8	5	25	24	12	8	25	/
++	5	2	1	9	8	10	0	4	/
LDH(U/L)	370.54±32.15▲	385.75±24.37	254.32±45.73▲	270.54±85.37▲	310.54±75.89	253.43±50.14▲	214.05±43.22▲	365.43±57.63	/

WBC, White blood cell count; Hb, Hemoglobin; PLT, Platelet; LDH, Lactate dehydrogenase; ▲$P < 0.05$, compared with *JAK2* mutation group.

Table 3. Clinical characteristics in MPN patients of Uygur and Han nationality, subdivided according to *JAK2* or *CALR* mutation status.

Parameter	Uygur (n = 169)			Han (n = 323)		
Sex	JAK2 (−) CALR (−)	JAK2 (+)	CALR (+)	JAK2 (−) CALR (−)	JAK2 (+)	CALR (+)
Male	39	30	5	42	83	30
Female	46	37	12	45	87	36
Age, years	47.65±7.92$^{\triangle}$	52.45±6.44$^{\triangle}$	48.04±7.12$^{\triangle}$	50.10±7.93	59.15±7.35	53.24±7.66
WBC ($\times 10^9$/L)	3.12±1.31$^{\triangle}$	8.47±2.85$^{\triangle}$	4.31±1.25	5.43±2.01	16.79±5.12	8.77±3.21
Hb (g/L)	155.15±19.57	157.47±22.77$^{\triangle}$	161.42±23.54	158.43±19.44	167.42±21.52	159.71±20.34
PLT ($\times 10^9$/L)	581.45±121.35$^{\triangle}$	567.43±119.87$^{\triangle}$	572.54±118.75	824.75±180.65	829.73±175.52	835.26±183.62
Thrombosis						
+	35	23	2	40	75	25
−	50	44	15	47	95	41
Myelofibrosis						
0	50$^{\triangle}$	47	13	69	105	51
+	26$^{\triangle}$	14	3	14	48	13
++	9$^{\triangle}$	6	1	4	17	2
LDH(U/L)	374.95±37.54	383.62±27.31	255.79±35.77	381.55±55.42	315.44±65.37	261.57±54.38

WBC, White blood cell count; Hb, Hemoglobin; PLT, Platelet; LDH, Lactate dehydrogenase; $^{\triangle}P < 0.05$, compared with Han patients harboring the same mutation.

non-mutation group (t = 5.388, 23.683, 8.539, 43.651, and 25.565, respectively; all $P < 0.001$).

Correlation between JAK2 and CALR mutations in Uygur and Han patients and clinical characteristics

The WBC count, PLT levels, and the age of Han MPN patients with non-mutated *JAK2*/*CALR* were significantly higher than in Uygur patients with non-mutated *JAK2*/*CALR* ($t = 3.256$, 9.863, and 17.736, respectively; all $P = 0.001$). Among Han MPN patients with *JAK2* mutations, the age, WBC count, and PLT level were significantly higher than those of Uygur MPN patients harboring *JAK2* mutations ($t = 10.009$, 23.145, and 19.529, respectively; all $P < 0.001$). Moreover, the age, WBC count, and PLT level of Han MPN patients with *CALR* mutations were significantly higher than in Uygur MPN patients with *CALR* mutations ($t = 7.323$, 21.987, and 19.170, respectively; all $P < 0.001$).

Among Han MPN patients, the level of Hb in *JAK2* mutant patients was significantly higher than in Uygur patients ($t = 4.773$, $P < 0.001$) while the myelofibrosis rate in the *JAK2*/*CALR* non-mutation group was lower than in Uygur patients ($\chi^2 = 8.53$, $P = 0.01$). However, the gender, age, and degree of thrombosis were not significantly different between Han or Uygur *JAK2*- or *CALR*-mutant patients. Findings of the statistical analysis are shown in Table 3.

Discussion

BCR-ABL-negative MPN originates from the aberrant proliferation of BMHCs, and is accompanied by the excessive proliferation of myeloid cells, peripheral blood granulocytes, platelets, and erythrocytes. BCR-ABL-negative MPN patients usually

have a higher risk of developing thrombogenesis, myelofibrosis, and acute leukemia than those with the BCR-ABL fusion gene.[12,13] The molecular pathology of BCR-ABL negative MPN has been closely studied in recent years, with many mutation sites identified as diagnostic targets, including those in *JAK2*, *MPL*, and *CALR*.[5,14,15]

China has a large population and a wide ethnic diversity, but few studies have investigated the relationship between clinical features and *JAK2*, *MPL*, and *CALR* mutations in BCR-ABL fusion gene-negative MPN patients of different nationalities. However, this work is highly relevant to the clinical diagnosis of BCR-ABL1-negative MPN.

Because of a small sample source, the present study only compared MPN patients of Chinese Han and Uyghur nationalities. We recruited a total of 492 BCR-ABL-negative MPN patients, of whom 169 (34.35%) were Uyghur and 323 (65.65%) were Han. We analyzed the frequency of *JAK2*, *MPL*, and *CALR* mutations, detecting the *JAK2* V617F mutation in 47.56% (234/492) of patients, *JAK2* exon 12 mutations in 0.61% (3/492), *CALR* mutations in 16.87% (83/492), and *MPL* W515L/K in only 0.61% (3/492). These findings were in accordance with a previous study conducted by Ouyang et al indicating that our selection of study cases was representative.[16]

In MPN patients of Uyghur nationality in our study, the mutation rates of *JAK2* and *CALR* were 39.64% (67/169) and 10.06% (17/169), respectively, while 52.63% (170/323), 20.43% (66/323), and 0.93% (3/323) Han MPN patients harbored *JAK2*, *CALR*, and *MPL* mutations, respectively. This compares with a study by Ojeda et al of 439 BCR-ABL1-negative MPN patients, in which *JAK2* mutations were detected in 74.9% of cases, slightly higher than in the Han group. This earlier study also detected a *CALR* mutation rate of 12.3%, lower than in the Han group, and a *MPL* mutation rate of only 2.1%, which was not significantly different from that in the Han group.[17]

A previous study showed that the *JAK2* V617F mutation rate in PV patients ranged from 65% to 98%, which was higher than our detected frequency of only 61.49%. This variation may be caused by population differences among ethnic backgrounds. Indeed, our statistical analysis revealed the *JAK2* and *CALR* mutation rate in patients of Han nationality to be significantly higher than that of Uyghur patients. Han and Uyghur individuals are known to have major differences in eating habits, living conditions, and genetic factors acquired over many years, and we believe that this may contribute to the different mutation rates.

In patients with PMF and ET in our study, the mutation rate of *JAK2* V617F was 40.40% to 44.23%, suggesting that differences in *JAK2* V617F mutation rates exist among patients with different types of MPN. Moreover, in PMF and ET patients, *CALR* mutations were the next most common mutation after *JAK2* V617F, indicating the importance of *JAK2* V617F and *CALR* mutation detection in the diagnosis of MPN patients, especially those with PMF and ET. Age and Hb and LDH levels were significantly different among *JAK2*-mutant, *CALR*-mutant, and *JAK2*, *CALR* non-mutation PMF patients, while the age of patients with *JAK2* V617F mutations was significantly higher than that of *CALR*-mutant, and *JAK2, CALR* non-mutation patients. These results revealed that patients of different ages had different mutations, which is consistent with the findings of Wu et al.[18]

Some studies[19,20] showed that *JAK2*, *MPL,* and *CALR* mutations are mutually exclusive. In support of this, our sequencing data showed that no more than one gene was mutated in the same patient. However, another study detected both *JAK2* V617F and *CALR* mutations in a small number

of patients, as well as both *JAK2* V617F and *MPL* W515L/S mutations.[21] Any correlation among the three mutations in MPN patients remains to be identified. Interestingly, the age of the *JAK2*-mutant group was significantly higher than the *CALR*-mutant group and the *JAK2*, *CALR* non-mutation group in both Han and Uyghur populations. This supports the fact that age, but not nationality, is the key factor associated with *JAK2* mutation rate, and is consistent with previous findings.[17] We propose that the higher mutation rate in older individuals reflects physical decline and a weakened immune response. We also compared differences in the parameters of MPN patients with the same mutation from different ethnic groups. These results showed that age, WBC count, and PLT levels were significantly higher in Han patients with or without *JAK2* and *CALR* mutations than in Uyghur patients ($P < 0.05$). In Han patients with MPN, the level of Hb in those with *JAK2* mutations was significantly higher than in Uygur patients ($P < 0.05$), but bone fibrosis in Han patients with *JAK2* or *CALR* mutations was significantly lower than in Uyghur patients with corresponding mutations. The molecular mechanism underlying this remains to be confirmed.

A limitation of this study was that it did not include all relevant mutation types because of the restricted sample source. Therefore, the sample size should be expanded for further analysis. Moreover, difficulties in follow-up and the lack of relevant research data meant that the survival index was not included in the scope of the study, so should be explored in future work. Finally, only 0.61% (3/492) of Han and Uyghur patients carried *MPL* mutations, so it was difficult to determine the relationship between *MPL* mutation rate and clinical characteristics of MPN patients.

In summary, *JAK2* and *CALR* mutation rates in Han MPN patients were higher than in Uyghur patients. Moreover, Han MPN patients harboring *JAK2* mutations had higher age, WBC count, and levels of PLT and Hb than Uyghur patients with the same mutations.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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