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Genetic Profile of Patients with Classical Ph-negative Chronic Myeloproliferative Diseases in the Republic of Sakha (Yakutia)

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Abstract

Background: Mutations in the *JAK2*, *CALR*, and *MPL* genes are key factors of the classical Ph-negative CMPD pathogenesis with demonstrated diagnostic and prognostic value. The aim of this research was to study the prevalence of *JAK2*, *CALR,* and *MPL* mutations in patients with CMPD and healthy individuals in the Republic of Sakha (Yakutia) (RS(Y)).

Methods and Results: The study included patients with previously confirmed diagnoses of PV (n=15), ET (n=16), and PMF (n=11) and 68 people with peripheral blood changes, suspected to have CMPD. The control group included 184 healthy volunteers. All patients and participants in the control group were genotyped according to the following SNPs: the *JAK2* rs77375493 SNP, the *CALR* rs765476509 SNP, the *CALR* rs1450785140 SNP, the *MPL* rs121913616 SNP, and the *MPL* rs121913615. The prevalence of the *JAK2*V617F mutation among PV patients in the RS(Y) was 90.9%. Patients with ET in 61.3% of cases were carriers of the *JAK2*V617F mutation, in 6.4% of *CALR* mutations, and in 3.2% of the *MPL*W515L mutations. In PMF patients, the *JAK2*V617F mutation was detected in 64.7% of cases, and the Type 1 *CALR* mutation was detected in 17.6% of cases. Carriage of the *JAK2*V617F mutation was revealed in 1.1% of healthy individuals and in 4.4% of individuals with initial signs of a myeloproliferative process.

Conclusion: Early molecular genetic testing will improve the timely diagnosis of CMPD and possibly reduce the number of complications. (**International Journal of Biomedicine. 2020;10(1):54-57.**)

Key Words: chronic myeloproliferative diseases • gene • mutations • single nucleotide polymorphism

Abbreviations

AS-PCR, allele-specific polymerase chain reaction; **CMPD**, chronic myeloproliferative diseases; **CALR**, Calreticulin; **ET**, essential thrombocythemia; **PV**, polycythemia vera; **PMF**, primary myelofibrosis; **SNPs**, single nucleotide polymorphisms

Introduction

Recent decades have been marked by a major breakthrough in understanding of the classical Ph-negative CMPD pathogenesis. In 2005, a sense mutation V617F (also known as *JAK2*Val617Phe) at exon 14 of the Janus Kinase *(JAK)2* gene (a valine-to-phenylalanine substitution in position 617) was described. This mutation can also be described using the SNP ID rs77375493 (the wild-type (normal) allele is rs77375493(G), and the (very rare) variant allele is rs77375493(T)). The mutation results in loss of autoinhibition of JAK2 tyrosine kinase, its hyperactivation and cytokine-independent differentiation of myeloid cells.[1] A little later, mutations in the MPL, CALR genes were described,

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which play a key role in the pathogenesis of megakaryocyte proliferation. Among mutations in the MPL gene encoding the thrombopoietin receptor, mutations W515L (a tryptophan-to-leucine substitution in position 515) and W515K (a tryptophan-to-lysine substitution in position 515) have a major clinical importance.[2] They lead to spontaneous activation of the MPL receptor and increase its sensitivity to thrombopoietin. The role of *CALR* mutations, in particular, Type 1 (52-bp deletion; p.L367fs*46) mutation and Type 2 (5-bp TTGTC insertion; p.K385fs*47) mutation, include the loss of KDEL signal sequence due to a shift of the reading frame by 1 nucleotide.[3]

The diagnostic value of the *JAK2*, *CALR*, and *MPL* mutations has been determined; they are included in the WHO criteria for the diagnosis of classical Ph-negative CMPD.[4] In addition, many studies have been published that describe the impact of mutational status on the clinical course, the risk of complications, and disease outcome.[5] Despite the progress, the problem of early diagnosis of diseases and the prevention of thrombotic complications remains unresolved. According to findings in the literature, at the time of diagnosis thrombotic complications are recorded in 12%-39% of PV patients, 7.14%-26.3% of ET patients and 4%-7% of PMF patients.[6] In recent years, we have been getting more information about the prevalence of the *JAK2*V617F mutation among individuals with thrombosis of different localization. In addition, the observed frequency of the *JAK2* (0.3%-3.1 %) and *CALR* (0.16 %) gene mutations among healthy populations in different countries exceeds the officially recorded incidence of chronic myeloid neoplasm (0.002%-0.02%).[7,8] In this regard, the *JAK2*V617F mutation may be an early diagnostic criterion for identifying individuals with latent clonal hematopoiesis of the myeloid germline, and the role of the *CALR* and *MPL* mutations remains unclear.

The aim of this research was to study the prevalence of *JAK2*, *CALR,* and *MPL* mutations in patients with CMPD and healthy individuals in the RS(Y).

Materials and Methods

The study included patients with previously confirmed diagnoses of PV (n=15), ET (n=16), and PMF (n=11) and 68 people with peripheral blood changes, suspected to have CMPD. All patients underwent outpatient consultation in the Republican Hospital №1 "National Center of Medicine"; the diagnosis was verified based on WHO diagnostic criteria valid at the time of diagnosis.[4] The control group included 184 healthy volunteers.

The study was approved by the Ethics Committee of the Yakut Science Center of Complex Medical Problems (YSC CMP). Written informed consent was obtained from each research participant.

The average age of patients ranged from 50 to 60 years: 52±16.63 for ET, 60±12.84 for PV, and 50±21.17 for PMF. In all investigated subgroups of patients, women predominated (80.6% in ET, 54.5% in PV and 52.9% in PMF). Further analysis of ethnic groups demonstrated a predominance of Yakuts among ET patients (67.7%) and Russians among patients with PV and PMF (54.5% and 52.9%, respectively). The median follow-up time was 48 months (from 0 to 252 months).

The experimental part of the work was carried out in the Department of Molecular Genetics at YSC CMP. All patients and participants in the control group were genotyped according to the following SNPs: the *JAK2* rs77375493 SNP, the *CALR* rs765476509 SNP, the *CALR* rs1450785140 SNP, the *MPL* rs121913616 SNP, and the *MPL* rs121913615.

DNA was isolated from peripheral blood lymphocytes with a commercial DNA-isolation kit (Excell Biotech Corporation; Yakutsk, Russia). SNP was determined using AS-PCR. Amplification of the gene region containing the polymorphic variant was carried out using standard pairs of primers produced by SybEnzime (Novosibirsk, Russia).[9-11] Primer sequences and conditions for amplification are presented in Table 1.

Table 1.
Primers and conditions for PCR

SNP	Primers	Amplicon length	Annealing temperature
JAK2 rs77375493	Forward mutant-specific 5'-AGCATTTGGTTTTAAATTATGGAGTATATT-3'	Allele T – 364 bp and 203 bp Allele G – 364 bp	56°C
	Forward 5'-ATCTATAGTCATGCTGAAAGTAGGAGAAAG-3'		
	Reverse 5'-CTGACACCTAGCTGTGATCCTG-3'		
MPL rs121913616 rs121913615	Forward 5'-GCCGAAGTCTGACCCTTTTT-3'	Wild-type – 209 bp Mutation W515L– 124 bp Mutation W515K – 125 bp	55°C
	Reverse 5'-ACAGAGCGAACCAAGAATGCCTGTTTACA-3'		
	Forward mutant-specific for W515L 5'-GGCCTGCTGCTGCTGAAGTT-3'		
	Reverse mutant-specific for W515K 5'-TGTAGTGTGCAGGAAACTGCTT-3'		
CALR rs765476509 rs1450785140	Forward 1 5'-GCAGCAGAGAAACAAATGAAGG-3' Forward 2 5'-GCAGAGGACAATTGTCGG-3' Reverse 5'-AGAGTGGAGGAGGGGAACAA-3'	Wild-type – 357 bp Type 1 mutation – 302 bp Type 2 mutation – 272 bp	56°C

Detection of PCR products was carried out on a 3% agarose gel stained with ethidium bromide using a standard Tris-acetate buffer at 120V for 45 minutes.

Statistical analysis was performed using Microsoft Excel 2010. For descriptive analysis, results are presented as mean±standard deviation (SD). The Mann-Whitney U Test was used to compare the differences between the two independent groups. Differences in the allele distribution between the two groups were assessed by $\chi 2$- test with Yates correction. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. A probability value of $P<0.05$ was considered statistically significant.

Results and Discussion

According to the results of genotyping studied groups, the T allele of the *JAK2*V617F mutation was detected in 48.2% of patients and 1.1% of healthy individuals. Mutations in the *CALR* gene were detected in 4.5% of patients, of which 3.6% had a mutation of Type 1, and 0.9% had a mutation of Type 2. Mutations in the *MPL* gene were detected in 1.8% of patients. Among the healthy group, mutations in the *CALR* and *MPL* genes were not detected (Table 2).

Table 2.

Prevalence of JAK2, CALR, MPL mutations in patients and control group

Alleles/ Type of mutation	Patients (n=110) %	Control (n =184) %	$\chi 2$	OR (95% CI) *P*
JAK2				
T	48.2)	1.1	197.7	84.614 (30.504-234.708) *P*=0.000
G	51.8	98.9		
CALR				
Wild-type	95.4	100	6.007	*P*=0.015
1 type mutation	3.6	0		
2 type mutation	0.9	0		
MPL				
Wild-type	98.2	100	1.215	*P*=0.271
W515L mutation	1,8	0		
W515K mutation	0	0		

The genetic profiles of patients with verified diagnoses (n=70) demonstrated that PV patients in 90.9% of cases carry the *JAK2*V617F mutation. Among ET patients the *JAK2*V617F mutation was detected in 61.3% of cases, *CALR* mutations in 6.4%, and *MPL* mutation in 3.2%. PMF patients were carriers of the *JAK2*V617F mutation in 64.7% of cases, and *CALR* mutations in 17.6% of cases (Table 3). Mutations in the *CALR* and *MPL* genes were found in patients with PV. In 3 *JAK2*V617F-positive young patients (24, 29, and 33 years old) with borderline changes in the peripheral blood count, clinical and laboratory parameters did not meet the criteria for the diagnosis of CMPD; therefore, they were recommended for dynamic follow-up.

Table 3.

Prevalence of JAK2, MPL, CALR mutations in patients with CMPD

Mutation	PV, n=22 %	ET, n=31 %	PMF, n=17 %
JAK2V617F	90.9	61.3	64.7
CALR, total	0	6.4	17.6
CALR Type 1	0	3.2	0
CALR Type 2	0	3.2	17.6
MPL, total	0	3.2	0

Next, we evaluated the impact of the *JAK2*V617F mutation in clinical presentation of patients with ET and PMF. It was found that the average age of the *JAK2*V617F(T allele)-positive ET patients was significantly higher than that of carriers of the wild-type G allele (58.9±13.25 and 44.3±16.33, $P<0.05$) (Table 4). In patients with PMF, the *JAK2*V617F(T allele)-positive individuals demonstrated a higher level of leukocytosis ($20.2\times10^9\pm11.01$ vs $10.2\times10^9\pm6.24$, $P<0.05$).

Table 4.

The JAK2V617F mutation and clinical presentation of patients with ET and PMF

Variable	ET (n=31)			PMF (n=17)		
	JAK2V617F positive	JAK2V617F negative	*P*	JAK2V617F positive	JAK2V617F negative	*P*
Age, yrs	58.9±13.25	44.3±16.33	<0.05	54±16.95	54±15.39	>0.05
RBC, $\times10^{12}$/L	4.8±1.03	4.6±0.89	>0.05	5.1±1.47	4.0±0.96	>0.05
Hb, g/L	130±20.94	140±22.75	>0.05	125±26.59	125±30.98	>0.05
Hct, %	39.9±6.65	42.5±6.13	>0.05	40.2±9.26	38.9±8.06	>0.05
WBC, $\times10^9$/L	9.2±4.26	11.2±9.40	>0.05	20.2±11.01	10.2±6.24	<0.05
Platelets, $\times10^9$/L	934.4±278.3	940.9±172.9	>0.05	868±576.21	671.8±577.94	>0.05
Spleen, cm^2	42.1±21.2	40.4±17.60	>0.05	60±31.23	103.2±64.83	>0.05

RBC - Red Blood Cells; Hb - Hemoglobin; Hct - Hematocrit; WBC- -White Blood Cells

Our results are comparable with well-known data and confirm the diagnostic value of genetic testing. According to the results of numerous studies, the prevalence of the *JAK2*V617F mutation among patients with PV is more than 95%, with ET and PMF – 60%. Among patients with ET and PMF, *CALR* mutations were detected in 20%–25% of cases, *MPL* in 5%, and triple-negative status in 5%–10%.[12]

In clinical practice, mutations in the *JAK2*, *CALR*, and *MPL* genes play a role in predicting the clinical course of diseases and stratifying the risk of thrombotic complications. The *JAK2*V617F mutation is known to significantly increase the risk of thrombotic complications among patients with ET and PMF. The *JAK2*V617F-positive patients with ET are primarily among the elderly. They are characterized

by a high level of hemoglobin, leukocytosis and moderate thrombocytosis. Extreme trombocytosis, a lower risk of thrombotic complications and a high risk of transformation into secondary myelofibrosis are characteristic of *CALR* mutation-positive patients. In cases of PMF, patients with the *JAK2*V617F mutation have a worse prognosis than those with the *CALR* mutation. Leukocytosis and a lower incidence of severe anemia is common for *JAK2*V617F-positive PMF patients.[13]

The results of numerous epidemiological studies that include patients with thrombosis at different sites led to the hypothesis that the *JAK2*V617F mutation can be used as a universal marker of thrombogenic risk in various clinical conditions.[14] A high prevalence of mutation was observed among individuals with cerebral vascular thrombosis (3.8%–6.6%), splanchnic vein thrombosis (16%), and Budd–Chiari syndrome (up to 40%).[7] A screening study of Russian donors revealed carriage of the *JAK2*V617F mutation in 0.65% of cases, and the highest frequency was recorded in the Danish population – 3.1%.[7,8] Long-term monitoring of mutation carriers has demonstrated that the *JAK2*V617F-positive individuals have a higher risk of developing not only myeloid neoplasms, but also solid tumors.[15]

In conclusion: The prevalence of the *JAK2*V617F mutation among PV patients in the RS(Y) was 90.9%. Patients with ET in 61.3% of cases were carriers of the *JAK2*V617F mutation, in 6.4% of *CALR* mutations, and in 3.2% of the *MPL*W515L mutations. In PMF patients, the *JAK2*V617F mutation was detected in 64.7% of cases, and the Type 1 *CALR* mutation was detected in 17.6% of cases. Carriage of the *JAK2*V617F mutation was revealed in 1.1% of healthy individuals and in 4.4% of individuals with initial signs of a myeloproliferative process. The results of clinical evaluation demonstrated that the *JAK2*V617F mutation affects disease phenotype. For the *JAK2*V617F-positive ET patients, disease manifestation in older age is characteristic, and in the case of PMF, the *JAK2*V617F mutation was associated with higher leukocytosis. Early molecular genetic testing will improve the timely diagnosis of CMPD and possibly reduce the number of complications.

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Competing Interests

The authors declare that they have no competing interests.

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