CLINICS

CLINICAL SCIENCE

Evaluation of the association between the JAK2 46/1 haplotype and chronic myeloproliferative neoplasms in a Brazilian population

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OBJECTIVE: The JAK2 46/1 haplotype has recently been described as a major contributing factor to the development of myeloproliferative neoplasm, whether positive or negative for the JAK2 V617F mutation. The G allele, identified by a single-nucleotide polymorphism known as JAK2 rs10974944, is part of the JAK2 46/1 haplotype. The aim of this study was to verify the association between the presence of the G allele and the development of BCR-ABL-negative chronic myeloproliferative neoplasms in our population.

METHODS: Blood and oral mucosa swab samples were obtained from 56 patients of two local Brazilian hospitals who had previously been diagnosed with BCR-ABL-negative chronic myeloproliferative neoplasms. Blood samples from 90 local blood donors were used as controls. The presence of the G allele was assessed using a PCR-RFLP assay after extracting DNA from the samples.

RESULTS: The presence of the G allele was strongly associated with the presence of BCR-ABL-negative chronic myeloproliferative neoplasms ($p = 0.0001$; OR = 2.674; 95% CI = 1.630−4.385) in the studied population.

CONCLUSION: In agreement with previous reports, the JAK2 46/1 haplotype, represented in this study by the presence of the G allele, is an important predisposing factor in the oncogenetic development of these neoplasms in our population.

KEYWORDS: Myeloproliferative Disorders; JAK2 Human; Haplotype.

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INTRODUCTION

Chronic myeloproliferative neoplasms (cMPNs) are disorders that are characterized by the clonal proliferation of a single hematopoietic stem cell and result in increased peripheral blood counts of mature cells; they include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (MF) (1). The JAK2 V617F mutation was identified in more than 95% of PV patients and in approximately half of the patients with ET and MF but was not observed in healthy individuals (2-6). Consequently, JAK2 V617F mutation screening became a cornerstone in the molecular diagnostic approach for cMPN (1,7).

Even before identifying a common mutation associated with the development of cMPN, family clusters of patients sharing various types of these disorders had already been identified (8). Landgren et al. (9) studied the risk of PV, ET and MF development among the first-degree relatives of patients who had been previously diagnosed with cMPN. First-degree relatives were observed to have a five to seven times greater risk of developing cMPN, particularly in certain probands, suggesting that family members may share some of the oncogenes involved in myeloproliferative disorders.

Pardanani et al. (10) studied the genetic factors that could contribute to the phenotypic diversity of cMPN. The authors observed that some JAK2 gene SNPs and haplotypes occurred more frequently in PV patients than in ET or MF patients. They suggested that individual genetic factors could contribute to the diverse phenotypic presentation of cMPN.

Following the above findings, Jones et al. (11) analyzed several JAK2 SNPs. Considering uniparental disomy as the

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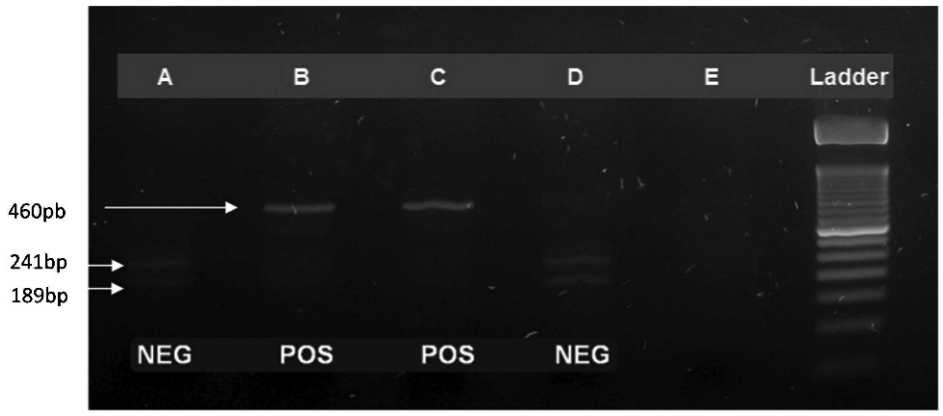

Figure 1 - JAK2 V617F screening using the PCR-RFLP technique. After digestion, the JAK2 V617F-positive samples presented a 460-bp band relative to a 50-bp molecular weight ladder. (A) An ET JAK2 V617F negative patient. (B) A PV JAK2 V617F positive patient. (C) A JAK2 V617F positive control. (D) A JAK2 V617F negative control. (E) Blank.

main cause of JAK2 V617F mutation homozygosity, the authors concluded that the JAK2 V617F mutation does not occur randomly but rather in a specific JAK2 gene haplotype. A comparison of JAK2 gene SNPs led to the identification of 92 different haplotypes. Two of them, referenced together as the 46/1 haplotype, were more frequently observed in patients carrying the JAK2 V617F mutation than in the general population.

Using genomic array techniques, Kilpivaara et al. (12) searched for SNPs that could predispose an individual to develop cMPN or that might act as phenotypic modifiers in these diseases. The JAK2 G allele, characterized by the presence of the rs10974944 SNP, occurred more frequently in PV than in ET patients. It was also more frequently observed in cMPN patients than in the general population. The C allele, characterized by the absence of the rs10974944 SNP, was considered to be the common allele that was most frequently observed in the general population. The G allele was also strongly associated with the JAK2 V617F mutation, with both occurring more frequently on the cis strand.

The aim of this study is to analyze the possible association between the G allele in cMPN patients and to verify its role in the development of these diseases in a Brazilian population.

METHODS

The study was submitted to and approved by the local ethics committee. All of the participants signed an informed consent. Blood and oral swab samples were collected from 56 patients who received treatment at one of two regional oncologic services (Hospital do Câncer de Maringá and Instituto do Câncer de Londrina); these patients were previously diagnosed with BCR-ABL-negative cMPN according to the 2008 WHO diagnostic criteria (1). Blood samples were also collected from 90 healthy individuals (blood donors from the Hemocentro Regional de Maringá) and used as controls, as the JAK2 V617F mutation is not found in healthy individuals (3-6,13,14). DNA was extracted from the biological specimens using a commercial kit according to the recommendations of the manufacturer (QIAamp® DNA Blood Mini Kit, Qiagen).

All of the blood samples obtained from the cMPN patients were genotyped for the JAK2 V617F mutation using a PCR-RFLP assay as previously described (14). To better visualize the post-digestion bands, we modified the described technique to include 2 U (1 µL) of BsaXI enzyme in each PCR amplicon digestion and used a 3% agarose gel to run the post-digestion electrophoresis. The JAK2 V617F-

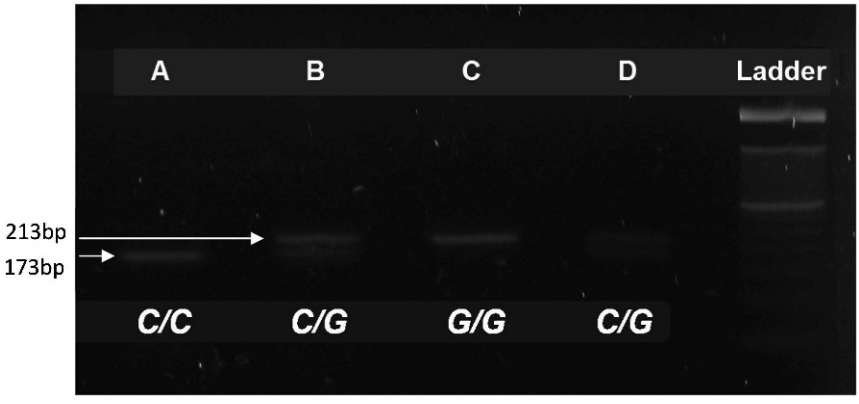

Figure 2 - JAK2 rs10974944 SNP allele screening using the PCR-RFLP technique. The presence of the G allele is characterized by the observation of a 213-bp band, whereas the presence of the C allele is noted by the observation of a 176-bp band, relative to a 50-bp molecular weight ladder. Lanes A-D represent patient blood samples. (A) Homozygous C allele. (B) Heterozygous C/G alleles. (C) Homozygous G allele. (D) Heterozygous C/G alleles.

Table 1 - General characteristics and JAK2 V617F mutational status of the cMPN patients.

Age (median range, in years)	22-83 (60.6)
Sex	
Male	25 (45%)
Female	31 (55%)
M:F	0.8:1
Diagnosis	
PV	17 (30%)
JAK2 V617F-positive	16 (94%)
JAK2 V617F-negative	1 (6%)
ET	22 (39%)
JAK2 V617F-positive	14 (64%)
JAK2 V617F-negative	8 (36%)
MF	12 (21%)
JAK2 V617F-positive	7 (58%)
JAK2 V617F-negative	5 (42%)
MPNu	5 (10%)
JAK2 V617F-positive	4 (80%)
JAK2 V617F-negative	1 (20%)
TOTAL (cMPN patients)	56

cMPN: chronic myeloproliferative neoplasms; PV: polycythemia vera; ET: essential thrombocytosis; MF: primary myelofibrosis; MPNu: myeloproliferative neoplasms, unclassifiable

positive samples presented a 460-bp band (Figure 1). The JAK2 V617F-negative samples contained two bands: 241 bp and 189 bp. The absolute and relative frequencies of the JAK2 V617F mutation in the cMPN patients were calculated.

Considering the possibility that a loss of heterozygosity occurred due to uniparental disomy, leading to a misinterpretation of the haplotype results, germline haplotypes (determined by oral swab) were compared to the results of the blood samples from the cMPN patients. Blood and oral swab samples obtained from cMPN patients and blood samples obtained from healthy subjects were submitted to JAK2 rs10974944 SNP screening using a PCR-RFLP assay developed by Trifa et al. (15,16). After digestion with the MboI enzyme, the presence of the G allele was detected by the observation of a 213-bp band, whereas the presence of the C allele is noted by the observation of a 176-bp band (Figure 2).

The allelic and genotypic frequencies were calculated using direct counts. The allelic and genotypic frequencies for the patients and the controls were compared using a Chi-Square test with Yates' correction, considering a 95% confidence interval (CI).

RESULTS

The overall characteristics of the cMPN patients enrolled in this study are shown in Table 1. After analyzing each of the cMPN patients alone, 16 (94%) of the 17 PV patients, 14 (64%) of the 22 ET patients and 7 (58%) of the 12 MF patients were positive for the JAK2 V617F mutation. Five (10% of 56) patients could not be classified with one of the three disease variants and were therefore classified as myeloproliferative neoplasms, unclassifiable (MPNu) according to the 2008 WHO diagnostic criteria (1). Some of those patients lacked information in their medical records or clinical history, and this missing data could have compromised the accuracy of the classification. The JAK2 V617F mutation screening in those patients indicated 80% (four of the five MPNu patients) positive results, which suggested that they should be placed into the BCR-ABL-negative cMPN group.

The distribution of JAK2 rs10974944 SNP genotypes and alleles is shown in Table 2, according to JAK2 V617F mutational status.

When comparing the JAK2 rs10974944 SNP genotypes and allelic frequencies between the patients and controls (Table 3), higher frequencies of the GG genotype and G allele were observed in the cMPN patients ($p = 0.0116$; OR = 3.079; 95% CI = 1.347−7.04 and $p = 0.0001$; OR = 2.674; 95% CI = 1.630−4.385, respectively). The CC genotype and C allele were observed more frequently in the healthy controls ($p = 0.0029$; OR = 0.331; 95% CI = 0.164−0.666 and $p = 0.0001$; OR = 0.374; 95% CI = 0.228−0.614, respectively).

A significant association was also observed between the JAK2 V617F mutation and the JAK2 rs10974944 SNP genotype or allele after comparing the patients who carried the mutation with the controls (Table 4). The GG genotype and G allele were more frequently observed in the patients who carried the mutation than in the healthy controls ($p = 0.006$; OR = 3.482; 95% CI = 1.454−8.339 and $p = 0.0001$; OR = 3.222; 95% CI = 1.884−5.510, respectively).

A comparison between the JAK2 rs10974944 SNP genotype or allele frequencies in the JAK2 V617F-negative patients and in the controls revealed no significant difference (data not shown). In addition, a comparison of the JAK2 rs10974944 SNP genotype or allele frequencies between the JAK2 V617F positive and negative patients did not reveal a significant difference in contrast to what has been previously described (11,12,16). This distinction may be explained by the modest number of patients enrolled in this study.

DISCUSSION

Recent reports have suggested that hereditary genetic factors, specifically the JAK2 haplotypes, can strongly contribute to the development of cMPN (11,12,16-18). These observations help explain previously known cMPN family clusters (8,9).

Table 2 - JAK2 rs10974944 SNP genotype/allele frequencies in cMPN patients and controls.

JAK2rs10974944 genotype/alleles	cMPN patients			Controls (n = 90)
	JAK2 V617F-positive (n = 43)	JAK2 V617F-negative (n = 13)	Total (n = 56)	
Genotype				
CC	11 (26%)	7 (54%)	18 (32%)	53 (59%)
CG	17 (40%)	3 (23%)	20 (36%)	25 (28%)
GG	15 (34%)	3 (23%)	18 (32%)	12 (13%)
Allele				
C	39 (45%)	17 (65%)	56 (50%)	131 (73%)
G	47 (55%)	9 (35%)	56 (50%)	49 (27%)

cMPN: chronic myeloproliferative neoplasms, SNP: single-nucleotide polymorphism

Table 3 - Correlation between JAK2 rs10974944 SNP genotype/allele frequencies and the presence of cMPN (cMPN patients vs. control group).

JAK2rs10974944 genotype/alleles	cMPN patients vs. control group			
	Patients (n = 56)	Controls (n = 90)	OR (95% CI)	*p*-value
Genotype				
CC	18 (32%)	53 (59%)	0.331 (0.164-0.666)	0.0029
CG	20 (36%)	25 (28%)	1.44 (0.706-2.953)	0.4
GG	18 (32%)	12 (13%)	3.079 (1.347-7.04)	0.0116
Allele				
C	56 (50%)	131 (73%)	0.374 (0.228-0.614)	0.0001
G	56 (50%)	49 (27%)	2.674 (1.630-4.385)	0.0001

cMPN: chronic myeloproliferative neoplasms, SNP: single nucleotide polymorphism.

The aim of this study was to analyze whether the genetic factors described above also play an important role in the development of cMPN in our population. The results of our study corroborate the hypothesis that JAK2 haplotypes are a major predisposing factor toward cMPN in the studied population. The GG genotype and G allele frequencies, representative of the JAK2 46/1 haplotype, were found to be significantly higher in our sample of patients.

It was observed that the JAK2 V617F mutation could occur in a homozygous or heterozygous fashion in the affected hematopoietic cells (2-5). Homozygosity frequently occurs for a specific allele through duplication of the mutated allele and consequent loss of the non-mutated allele (2). This event is called uniparental disomy. To rule out acquired uniparental disomy as a misleading factor in the genotype and allelic frequencies estimation, we analyzed oral swabs (data not shown) and verified that these results were concordant with the blood sample results.

The correlation between the JAK2 46/1 haplotype and the presence of the JAK2 V617F mutation has been well documented (11,12). Recent studies evaluating this correlation among other populations have also confirmed the JAK2 46/1 haplotype as a predisposing factor across various ethnic groups (19-20).

It was suggested that the JAK2 46/1 haplotype brings genetic instability to the JAK2 gene, favoring the emergence of JAK2 acquired mutations, such as the JAK2 V617F and JAK2 exon 12 mutations (10-12,18). Our data reinforces these findings because the G allele and the GG genotype were more frequent in the patients who carried the JAK2 V617F mutation than in the healthy controls.

As shown in Table 1, the characteristics of the studied patients are very similar to those observed in the literature (2-5), which indicates the comparability of our data and supports the assertion that the presence of the G allele is a pivotal factor in the development of cMPN in our population.

Various hypotheses have been proposed to explain the correlation between particular JAK2 haplotypes and the risk of cMPN. As mentioned above, one hypothesis is that a genetic instability brought by the presence of a specific inherited haplotype facilitated the emergence of an acquired JAK2 mutation (10-12,18). Another hypothesis suggests that the JAK2 46/1 haplotype could confer a proliferative or survival advantage to the neoplastic clone, which may explain the increased frequency of the JAK2 46/1 haplotype in different populations of cMPN patients (18). It is also possible that the various JAK2 haplotypes could produce different intensities of intracellular signaling, conferring the proliferative or survival advantage mentioned above (11,12). These hypotheses might coexist or even act synergistically in the process of cMPN oncogenesis.

The JAK2 V617F mutation also seems to play a role in other myeloproliferative and myelodysplastic diseases, reinforcing the importance of intracellular signaling pathways in the progression of disease (21).

The evaluation of genetic factors implicated in the development of chronic myeloproliferative disorders helps to identify acquired mutations, such as JAK2 V617F, and allows for the development of diagnostic assays. Recently, these molecular assays became a valuable tool in the evaluation of patients presenting with polycythemias, making diagnostic workup easier. In recently published data, inherited genetic factors were suggested to be important characters in the pathways of cMPN oncogenesis. More studies are needed to clarify the exact role these genetic factors play in modifying intracellular signaling and

Table 4 - Correlation between JAK2 rs10974944 SNP genotype/allele frequencies and the presence of the JAK2 V167F mutation (JAK2 V617F-positive patients vs. control group).

JAK2rs10974944 genotype/alleles	JAK2 V617F-positive patients vs. control group			
	JAK2 V617F-positive patients (n = 43)	Controls (n = 90)	OR (95% CI)	*p*-value
Genotype				
CC	11 (26%)	53 (59%)	0.24 (0.108-0.536)	0.0006
CG	17 (40%)	25 (28%)	1.7 (0.790-3.656)	0.244
GG	15 (34%)	12 (13%)	3.482 (1.454-8.339)	0.0078
Allele				
C	39 (45%)	131 (73%)	0.311 (0.182-0.531)	0.0001
G	47 (55%)	49 (27%)	3.222 (1.884-5.510)	0.0001

cMPN: chronic myeloproliferative neoplasms, SNP: single-nucleotide polymorphism.

perhaps to identify new targets for future diagnostic tools or even therapeutic agents.

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AUTHOR CONTRIBUTIONS

Pagliarini e Silva S and Sell AM were responsible for the statistical analysis. Pagliarini e Silva S, Santos BC, and Pereira EM designed the study and performed the molecular biological analysis. Pagliarini e Silva S, Ferreira ME, and Baraldi EC were responsible for the evaluation and collection of the clinical data. Pagliarini e Silva S, Sell AM, and Visentainer, JE were responsible for the manuscript writing and critical review.

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