onco

MDPI

Article

Association of JAK2 Haplotype GGCC_46/1 with the Response to Onco-Drug in MPNs Patients Positive for JAK2V617F Mutation

Michela Perrone [1,2,†], Sara Sergio [1,2,*,†], Amalia Tarantino [3], Giuseppina Loglisci [4], Rosella Matera [3], Davide Seripa [3], Michele Maffia [1,2,‡] and Nicola Di Renzo [4,‡]

[1] Laboratory of Clinical Proteomic, "V. Fazzi" Hospital, 73100 Lecce, Italy; michela.perrone2@studenti.unisalento.it (M.P.); michele.maffia@unisalento.it (M.M.)
[2] Laboratory of General and Human Physiology, Department of Experimental Medicine, University of Salento, 73100 Lecce, Italy
[3] Hematology Laboratory—Hematology and Stem Cell Transplant Unit, "V. Fazzi" Hospital, 73100 Lecce, Italy; amalia.tarantino@asl.lecce.it (A.T.); rosella.matera@asl.lecce.it (R.M.); davide.seripa@asl.lecce.it (D.S.)
[4] Hematology and Stem Cell Transplant Unit, Onco-Hematological Department, "V. Fazzi" Hospital, 73100 Lecce, Italy; giuseppina.loglisci@asl.lecce.it (G.L.); nicola.direnzo@asl.lecce.it (N.D.R.)
* Correspondence: sara.sergio@unisalento.it
† These authors contributed equally to this work.
‡ These authors contributed equally to this work.

Simple Summary: JAK2 V617F is a somatic mutation related with myeloproliferative neoplasm (MPN) and it is associated with the germline GGCC (46/1) haplotype. Nowadays, there are no studies focused on the association between the JAK2 haplotype GGCC_46/1 and the onset of onco-drug resistance. Thus, we looked for the haplotype 46/1 in JAK2V617F-positive patients, and we studied their therapy response. Most of the patients with onco-drug resistance had the C/G allele. Instead, the G risk allele, represented by few patients because of its reduced frequency in the general population, was shown to be associated with MPNs' evolution to myelofibrosis and to onco-drug resistance clinical parameters. These findings represent a significant advance in MPN management, although an in-depth analysis on a larger sample is required. We believe that the integration of a comprehensive genetic profiling into treatment decisions may influence the disease progression, prognosis, and treatment efficacy.

Abstract: Background: JAK2 V617F is a somatic mutation associated with myeloproliferative neoplasms (MPNs) including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). In MPNs, this mutation is associated with the germline GGCC (46/1) haplotype. Several studies associated JAK2 haplotype GGCC_46/1 with some MPNs clinical parameters, but not one explore the link between JAK2 haplotype GGCC_46/1 and onco-drug resistance. Thus, we assessed for the JAK2 46/1 haplotype's correlation with therapy response in JAK2 V617F-positive patients. Methods: Patients with MPN, selected by the Hematology Laboratory of "V. Fazzi" Hospital (LE), were analyzed with RLFP-PCR assay with rs10974944 SNP. Results: Results show how the majority of patients had PV (63%) or PMF (61%) and that 58% of patients who developed drug resistance had the C/G genotype, while only 11% had the G/G allele. While no direct correlation between JAK2 46/1 haplotype variants and drug resistance was found, the G/G allele was associated with disease progression to myelofibrosis and certain resistance-related clinical parameters ($p = 0.002449$, odds ratio = 3.701209). Conclusions: Although other analyses are required, due to the narrow cardinality of sample, our findings suggest how the G/G allele could be useful for MPNs diagnosis and for the prediction of the disease outcome.

Keywords: JAK V617F mutation; JAK2 haplotype GGCC_46/1; myeloproliferative neoplasm

Citation: Perrone, M.; Sergio, S.; Tarantino, A.; Loglisci, G.; Matera, R.; Seripa, D.; Maffia, M.; Di Renzo, N. Association of JAK2 Haplotype GGCC_46/1 with the Response to Onco-Drug in MPNs Patients Positive for JAK2V617F Mutation. *Onco* **2024**, *4*, 241–256. https://doi.org/10.3390/onco4030018

Academic Editor: Constantin N. Baxevanis

Received: 30 July 2024
Revised: 9 September 2024
Accepted: 20 September 2024
Published: 21 September 2024

1. Introduction

Janus Kinase 2 (JAK2) is a gene located on chromosome 9p24.1 [1] that encodes a non-receptor tyrosine kinase. This kinase plays a vital role in the JAK-STAT signaling pathway [2,3], which is essential for various cellular processes, particularly hematopoiesis [4,5].

The JAK-STAT pathway is activated by a variety of cytokines and growth factors. When a cytokine binds to its receptor, it activates JAK2, which then phosphorylates specific tyrosine residues on the receptor. This phosphorylation serves as a docking site for STAT (Signal Transducer and Activator of Transcription) proteins, which are then phosphorylated by JAK2. Once activated, STAT proteins dimerize and translocate to the nucleus, where they regulate the expression of target genes involved in cell survival, proliferation, differentiation, and immune responses [5,6].

Mutations in the JAK2 gene, particularly the JAK2 V617F mutation, are commonly associated with several myeloproliferative neoplasms (MPNs), such as polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). JAK2 V617F mutation is a somatic mutation, due to the substitution of valine to phenylalanine at codon 617 of JAK2 with gain of function and the resulting alteration of the JAK/STAT pathway [1,3]. This mutation leads to the constitutive activation of the JAK2 kinase, promoting abnormal proliferation of hematopoietic cells and contributing to the pathogenesis of these disorders [7].

Hematopoietic cells with JAK2V617F mutation are transformed into cytokine-independent growth and processes like tumorigenesis, tumor progression, and the resulting inflammation are promoted [1].

Thus, JAK2 is a crucial component in the regulation of blood cell formation and function, and its dysregulation due to mutations can lead to significant hematological diseases [8].

Ninety-five percent of PV patients and approximately half of patients with ET and MF [5] show JAK2 V617F mutation [1,3]. Recent advances in hematology are due to the identification of molecular markers and prognostic markers, allowing us to understand the molecular pathogenesis and genetics of MPNs. Consequently, JAK2 V617F mutation screening has become a cornerstone in the molecular diagnostic approach for MPNs.

Recent findings indicate that JAK2V617F is associated with a specific haplotype, the germline GGCC (46/1) haplotype [1,9], found in approximately 45% of the overall population [10–12].

This haplotype includes a set of genetic variations distributed along chromosome 9p.24.1, which covers the JAK2, INSL6, and INSL4 genes [1]. The latter two genes are not normally transcribed in the hematopoietic system [13]. The 46/1 haplotype includes a part called “GGCC”, that covers the most frequently mutated JAK2 exons: exon 14 (mainly, the V617F mutation), exon 12 (mutations and deletions), and to a lesser degree, exons 13 and 15 [14] Figure 1.

The “GGCC” part includes the JAK2 gene region between intron 10 and intron 15, which is characterized by four single nucleotide polymorphisms (SNPs) that replace three thymidines (T) and one cytosine (C) with two guanosines (G) and two cytosines (rs3780367:G, rs10974944:G, rs12343867:C, and rs1159782:C). All these SNPs are in complete linkage disequilibrium; thus, they are always inherited together [15].

In particular, research activities carried out in Europe, Japan, China, North America, and Brazil have demonstrated that all MPN patients, particularly those who carry the JAK2V617F mutation, have a higher frequency of the variant allele rs10974944 (G) than the control group [12,16–21]. The JAK2 G allele (rs10974944) is characterized by the substitution of a cytosine with a guanosine [21,22]; thus, the C allele is the common allele, whereas the G allele is the variant that represents the risk allele for MPNs. In fact, this haplotype has also been individualized as one of the factors that increases the risk of familial MPNs by more than five times [1].

The frequency of the JAK2 haplotype GGCC_46/1 in the healthy population is about 24%, but in patients with JAK2 V617F mutation, it was found in 40–80% of the cases [14,23].

The C allele has been shown to be associated with ET [24], while a prevalence of the heterozygous haplotype C/G was demonstrated in patients with PMF and PV [25].

Interestingly, JAK2 mutations are most commonly acquired in cis with the JAK2 predisposition haplotype, as demonstrated by many studies [10–12]. This preferential position is explained by two theories: the "hypermutability hypothesis" and the "fertile ground hypothesis". The first hypothesis supposes that the haplotype creates a regulatory environment that favors DNA damage or replication errors. Meanwhile, the second hypothesis suggests that the mutation occurs on all haplotypes, but the GGCC_46/1 haplotype provides a specific advantage for V617F mutation and the resulting MPN development. The latter hypothesis could also explain the acquisition of mutations in other genes that can cause MPNs. The understanding of the allele configuration can provide insights into the biological behavior of the disease and inform treatment decisions.

This type of disorder generally tends to become chronic. Treatment strategies aim to alleviate symptoms, reduce the risk of thrombotic events, and prevent disease progression. In detail, current approaches include a cytoreductive therapy using hydroxyurea (ATC: L01XX05), and the use of Ruxolitinib, a selective inhibitor of JAK1 and JAK2 [26,27].

The first one is an antimetabolite that inhibits DNA synthesis and may kill cancer cells or make them easier to kill with radiation therapy (National Cancer Institute) and is used as first-line treatment. Meanwhile, the second one acts on the JAK-STAT pathway, by reducing the phosphorylation and leading to a reduced cellular proliferation and to the induction of apoptosis [28]. Ruxolitinb is recommended when patients become refractory to hydroxyurea, as demonstrated by an Hct higher than 45% [29] and symptomatic splenomegaly [26] or intolerance.

Interestingly, some studies link certain JAK2 haplotypes to laboratory (increased platelet, leucocyte, hematocrit, and hemoglobin counts) and clinical (splenomegaly, splanchnic vein thrombosis, and Budd–Chari syndrome) results indicative of MPNs, but there is a lack of studies about the association between the haplotype tagged by rs10974944 single nucleotide polymorphism in JAK2 V617F-positive patients and their response to the onco-drugs used to treat the different MPNs. Thus, the aim of this work is to discover any association between haplotype(s) tagged by rs10974944 single nucleotide polymorphism in JAK2 V617F-positive patients and their response to onco-drugs, in a cohort of patients selected by the Hematology Laboratory of "V. Fazzi" hospital in Lecce.

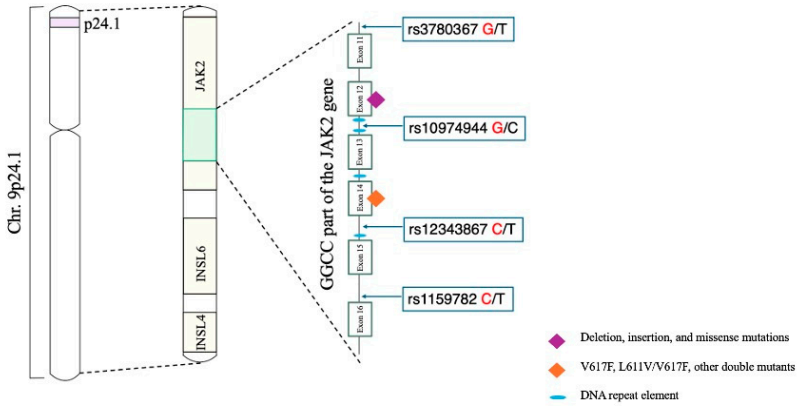


Figure 1. The 46/1 haplotype's localization on chromosome 9p.24.1. The 46/1 haplotype is a 280kb-long region of chromosome 9p which includes the entire JAK2, INSL6, and INSL4 genes. Most of the JAK2 mutations detected in Myeloproliferative Neoplasms (MPNs) are localized in the "GGCC" part of the JAK2 gene, as shown in the figure. The "GGCC" part is characterized by 4 single nucleotide polymorphisms (rs3780367, rs10974944, rs12343867, and rs1159782) that mark the haplotype and define the nomenclature based on the variant alleles, GGCC, as reported in literature [14].

2. Materials and Methods

2.1. Selection of Patients

A total of 665 samples were collected by the hematology laboratory at "V. Fazzi" Hospital (LE) from January 2020 to December 2023. These patients were previously diagnosed with the JAK2 V617F mutation. Among them, we selected only patients with a confirmed diagnosis of myeloproliferative syndrome (MPS), while we excluded individuals with no follow up available for many reasons (consultancy, occasional medical examination, patients who have changed hospitals, etc.,) and those with not enough data. Among the 547 remaining patients, we also excluded those with leukocytosis, erythrocytosis, thrombocytosis, and eosinophilia, and patients with thrombocytopenia, different types of polyglobulia, pancytopenia, and anemia were also excluded. Also, patients with leukemia, chronic lymphoproliferative syndrome (CLS), myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML) were removed. As a result, we obtained a corpus of 276 patients. Of these, however, 83 patients had no confirmed diagnosis of MPNs and were excluded. Among the 193 remaining patients, 91 were unclassifiable; thus, only 102 patients with certain diagnosis of MPNs were considered for this study.

Since there are three main classes of pathologies grouped under the name of MPNs, we analyzed the distribution of JAK2 V617F-positive and -negative patients in these three categories.

As a result, we selected for our study: 19 patients with polycythemia vera, 18 with primary myelofibrosis, and 13 with essential thrombocythemia. Samples analyzed include, for the major part, peripheral blood samples and just a few from bone marrow.

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of NOVARTIS PHARMACEUTICALS. ClinicalTrials.gov Identifier: NCT05548062, Novartis Reference Number: CINC424BIT01.

*2.2. DNA Extraction from Fresh Blood Samples with NucleoSpin DX Blood**

The NucleoSpin DX Blood* kit was used for the isolation and purification of genomic DNA from human whole blood samples for subsequent in vitro diagnostic purposes, following the supplier's instructions. After the extraction, DNA was quantified with a spectrophotometer. Samples extracted were stored at −20 °C.

2.3. JAK2 MutaQuant Analysis

JAK2 mutation V617F was detected with multiplex PCR using kit ipsogen® JAK2 MutaQuant® (QIAGEN, Hilden, Germany). It is a quantitative in vitro test for the accurate detection and quantification of the JAK2 V617F/G1849T allele starting from genomic DNA extracted from peripheral blood of patients with suspected MPN. PCR mix was prepared in multiwell PCR plates and put in 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems, Waltham, MA, USA). Patients who tested positive to V617F mutation were selected for the following phase.

2.4. PCR-RFLP Assay

For JAK2 rs10974944 SNP screening, a PCR-RFLP assay developed by Trifa et al. [30] was used. They developed a simple and inexpensive PCR-RFLP assay for studying the JAK2 rs10974944 SNP, a constituent of the putative JAK2V617F-predisposing haplotype. The primer sequences were as follows: Primer Rev 5′-CTGCTTGCTAGTGGGTGAAT-3′ (Eurofins Genomics, Ebersberg, Germany) and Primer Fw 5′-CAAGGGTCAACTGTAGTACATAA-3′ (Eurofins Genomics, Ebersberg, Germany).

The PCR reactions were set up in a 50 µL reaction volume, with the following composition: 44 µL PCR Master- Mix containing HotStartTaq® 5 units/µL (QUIAGEN, Hilden, Germany), $MgCl_2$ 25 mM (QUIAGEN, Hilden, Germany), dNTPs mix 10 mM (ThermoFisher Scientific, Waltham, MA, USA), 5 µL of each forward and reverse primer, PCR Buffer 10X

(QUIAGEN, Hilden, Germany), H_2O DNase-RNase free (Sigma-Aldrich, Milano, Italy), and 150 ng of genomic DNA.

The amplification procedure consisted of an initial activation step at 95 °C for 15 min. In this step, HotStartTaq was activated by the heating step. Then, 3-step cycling was performed as follows: denaturation at 94 °C for 1 min, annealing at 52 °C for 1 min (this step must be performed approximately 5 °C below Tm of primers), and finally, extension at 72 °C for 1.30 min. This 3 step cycling was repeated 35 times. At the end, there was a final extension at 72 °C for 10 min. In this way, a 243-bp amplicon was obtained, which was then incubated overnight (for 16 h) at 37 °C with 5 U of the restriction endonuclease MboI (ThermoFisher Scientific, Waltham, MA, USA), according to manufacturer's specifications. To visualize the fragments, 3% agarose gel was run, stained with Nucleic Acid Stain 20.000X-1 mL (EuroClone, Milano, Italy) and put in a GelDoc (Uvitec, Cambridge, UK).

If the common C allele was present, the 243-bp amplicon contained 3 restriction sites for MboI, giving rise to 4 fragments after digestion, as follows: 176, 37, 23, and 7 bp. In the presence of the G allele, one of the MboI restriction sites was abolished, such that the digestion with MboI would produce 3 fragments of 213, 23, and 7 bp. The fragments of 23 and 7 bp were not seen on the gel, but the delineation of the C and G alleles was made based on the difference between the fragments of 213 and 176 bp.

2.5. Statistical Analysis

For dichotomous variables, which are random variables that can take on only one of two values, differences between the groups were tested using the Fisher exact test with R project for statistical computing; this test can be used particularly when sample sizes are small or when the expected frequencies in the contingency table are too low for the chi-square test to be valid. Instead, when the variables are categoricals, we tested the indipendence between two variables or goodness of fit by using Pearson $\chi 2$ test, always performed with R project for statistical computing.

Two-sided p values < 0.01 were set as the threshold for statistical significance.

3. Results

3.1. Prevalence and Correlations of JAK2–V617F Mutation in MPN Diseases

A flow diagram illustrating the screening process for patient selection is presented in Figure 2.

To demonstrate the presence of a correlation between JAK2V617F mutation and the predisposition to MPNs, 665 patient samples were collected from the hematology laboratory at "V. Fazzi" Hospital (LE) and analyzed for their distribution according to JAK2 gene mutation and MPNs diagnosis. Among these patients, 445 tested positive for the JAK2V617F mutation, while 211 had a wildtype form of the gene (Figure 3A). The remaining 9 patients were undermined after the molecular biology analysis, due to technical and sampling issues, and were not considered in this study. Among the 665 patients tested for JAK2 mutation, 102 patients were diagnosed with myeloproliferative syndrome, while 472 patients included cases of polyglobulia, thrombocytopenia or thrombocytosis, anemia, myeloid or lymphoid leukemia, eosinophilia, leukocytosis, myelodysplasia, or suspected myeloproliferative syndromes not yet classified in a specific clinical picture (Figure 3B). We excluded 91 cases with unclassifiable diagnosis and those in which the diagnosis was not indicated in the medical records. Analyzing the correlation between JAK2V617F mutation-positive patients and patients with a diagnosis of MPN, a statistically significant correlation was found, as demonstrated by the p-value lower than 2.2×10^{-16}, calculated with Fisher analysis. Of the 102 MPNs patients involved, 41 (40%) had ET, 25 (25%) had PV, and 36 (35%) had PMF (Figure 4B). The JAK2-V617F mutation was found in 69 (69.6%) of the patients assessed (Figure 4A). This mutation was present in 25 out of 41 patients diagnosed with ET (61%; p-value = 0.07294), 23 out of 25 patients diagnosed with PV (92%; p-value = 0.009964), and 25 out of 36 patients diagnosed with PMF (69%; p-value = 0.8192) (Figure 4B), showing how the JAK2V6171F mutation is highly present

in all the MPN diseases with a prevalence in PV. All together, these data confirmed the presence of a strong correlation between the JAK2 V617F mutation and MPNs in the group of patients analyzed. Moreover, the analyses of the correlation between the JAK2 V617F mutation and each type of MPNs showed a statistically significant correlation in patients affected by PV but not in patients affected with ET or PMF. Thus, while the JAK2V617F mutation could be used as a diagnostic marker for PV, this is not true for ET and PMF, where it could be used only to support diagnosis.

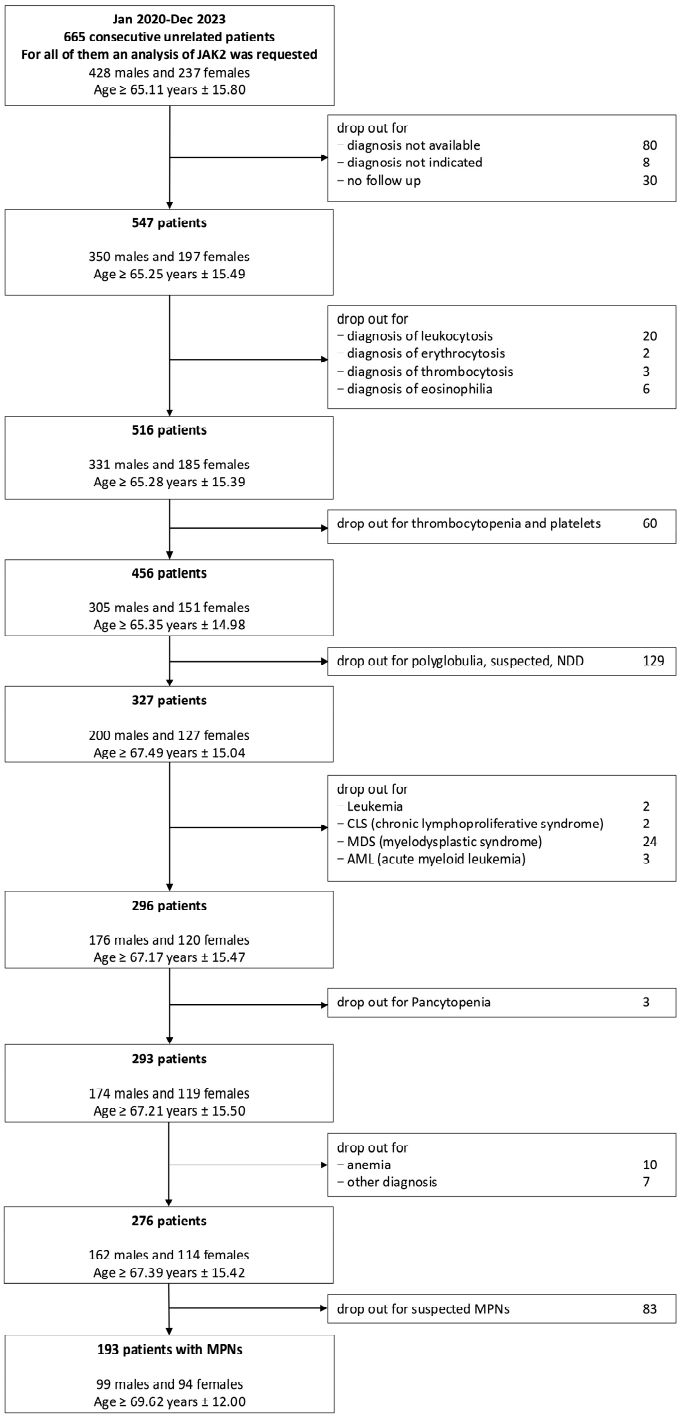


Figure 2. Flow diagram for selection of patients with MPNs.

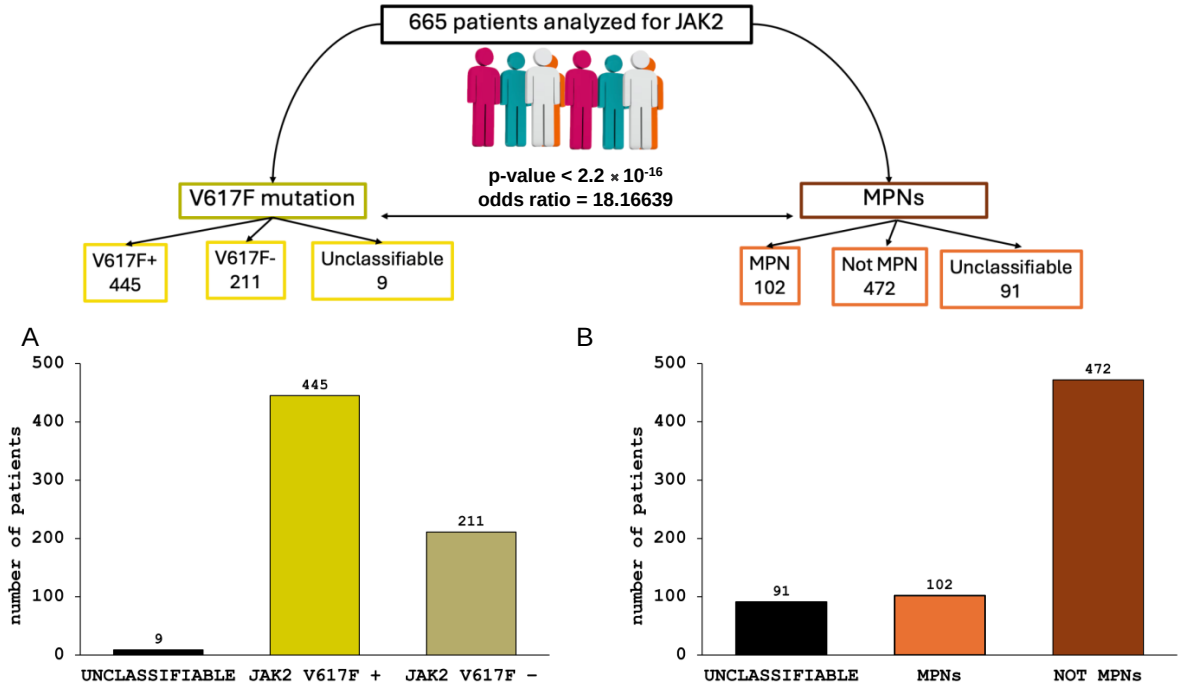


Figure 3. (**A**) This graph shows 665 patients analyzed for JAK2V617F mutation. In yellow, there are patients who tested positive or negative for V617F mutation. Nine samples were not evaluated by molecular analysis and are reported in graph as unclassifiable (i.e., poorly celluled sample, low concentration of extracted DNA that become undetectable with multiplex PCR assay). (**B**) Patients are classified based on the MPN diagnosis. Among the 665 patients analyzed, only 102 are classified as MPNs, thanks to clinical signs and molecular biology tests (orange), while 472 have other diagnoses (dark orange) and 91 are unclassifiable because their diagnosis was not indicated in the medical records. Differences between the groups were tested using the Fisher exact test with R project for statistical computing. p value < 0.01 was set as the threshold for statistical significance.

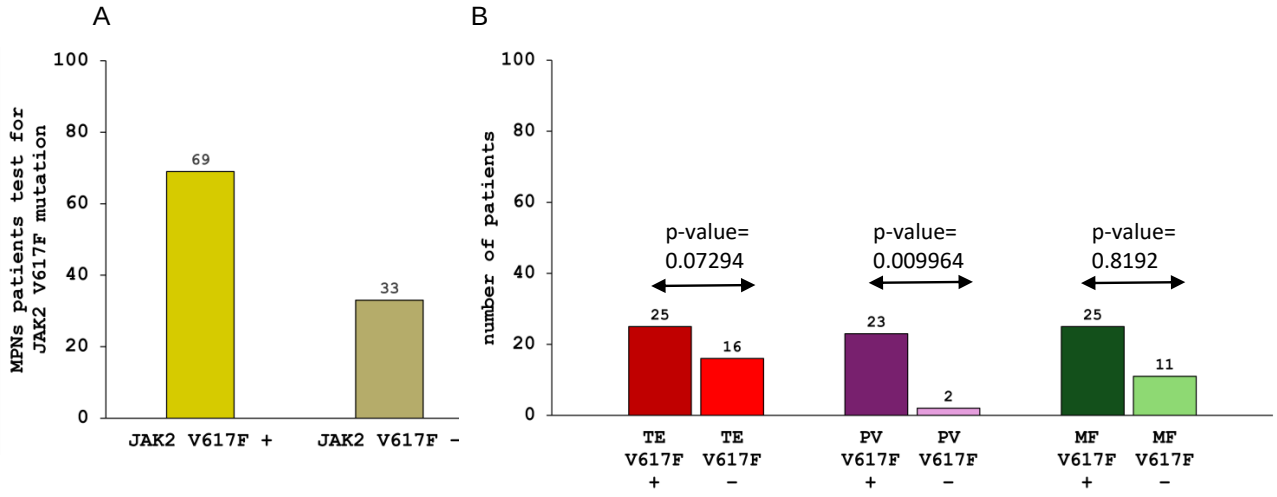


Figure 4. (**A**) This graph shows 102 patients analyzed for JAK2V617F mutation. In lighter yellow, there are patients who tested positive for V617F mutations, and in darker yellow, those who tested negative, thanks to multiplex PCR analysis. (**B**) This graph represents the prevalence of JAK2-V617F mutation in patients with MPNs. Red, purple, and green columns represent the prevalence ofV617F mutation in patients with essential thrombocythemia, polycythemia vera and myelofibrosis, respectively. TE: essential thrombocythemia; PV: polycythemia vera; MF: myelofibrosis. Differences between the groups were tested using the Fisher exact test with R project for statistical computing. p value < 0.01 was set as the threshold for statistical significance.

3.2. JAK2 Haplotype 46/1 Distribution in MPN Patients Tested Positive for JAK2 V617F Mutation

Subsequently, the 102 patients with a diagnosis of myeloproliferative disorder were tested for the presence of the JAK2 haplotype 46/1. From this group, patients subjected to analysis in 2020 were excluded, due to samples' lack of availability.

From this selection process, 50 samples were found suitable for the analysis, and they included 19 cases of PV, 18 cases of PMF, and 13 cases of ET. From all these patients, a blood sample was collected, and DNA was extracted. A RFLP-PCR assay was performed based on the protocol developed by Trifa et al. [30].

After a restriction enzyme digestion with MboI enzyme, a gel electrophoresis analysis was performed to test if the JAK2 46/1 haplotype existed in patients. Thus, JAK2 rs10974944 SNP was used as tag SNP to identify the haplotype. The aim of this analysis was to evaluate the presence of the rs10974944 (G) allele, as well as the heterozygous condition (G/C) and the common allele (C/C) (Figure 5).

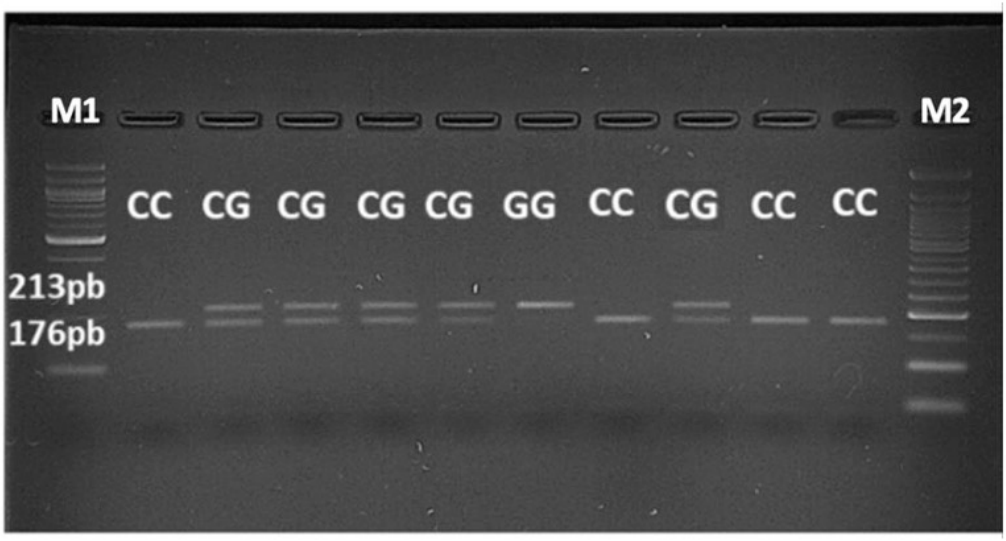


Figure 5. The image shows 3% agarose gel electrophoresis for JAK2 rs10974944 SNP alleles. GG = G allele homozygote; CG = heterozygote; CC = C allele homozygote; M1 = 100-bp molecular weight DNA marker; M2 = 50-bp molecular weight DNA marker.

As shown in Table 1, the C/G genotype was more common in JAK2 V617F-positive patients compared to the C/C common allele. Indeed, we found that the C/G genotype was present in 58% of JAK2 V617F-positive patients, with 36% of patients having the C/C common allele and only 6% of patients having the G allele.

Table 1. JAK2 rs10974944 SNP genotype frequencies in patients tested positive for JAK2 V617F mutation.

Genotype	MPN Patients Positive for JAK2V617F Mutation (*n* = 50)		
	PMF *n* = 18	**PV *n* = 19**	**ET *n* = 13**
CC (*n* = 18)	5/18 (28%)	7/19 (37%)	6/13 (46%)
CG (*n* = 29)	11/18 (61%)	12/19 (63%)	6/13 (46%)
GG (*n* = 3)	2/18 (11%)	0/19 (0%)	1/6 (8%)

This table shows 50 MPN patients tested positive for the JAK2V617F mutation classified based on their genotype and on the MPNs subtypes.

By considering the distribution of JAK2 rs10974944 SNP genotypes in the three different MPNs analyzed (TE, PMF, and PV), we found that the C/G genotype was prevalent in patients with myelofibrosis or with polycythemia vera, where it represented, respectively,

61% and 63% of total cases, compared to 46% of essential thrombocythemia cases (Table 1). Instead, the C/C common allele was found in 46% of ET cases, followed by 37% cases of PV and 28% of PMF (Table 1). The JAK2 rs10974944 G/G genotype was present in only 11% of myelofibrosis cases and in 8% of essential thrombocythemia patients.

All together, these data suggest that the heterozygous condition (C/G) is the most frequent in JAK2 V617F-positive patients (58%) and that this genotype is predominantly associated with PMF (61%) and PV (63%). Meanwhile, the C/C genotype is more often associated with ET (46%) and the G/G genotype with PMF (11%).

3.3. JAK2 Haplotype 46/1 Association with Therapy Response in MPNs Patients Positive for JAK2V617F Mutation

In this work, we studied, for the first time, the association between the JAK2 haplotype GGCC_46/1 and patients' response to therapy. According to the data reported in patients' medical records, all 50 patients with the JAK2 haplotype GGCC_46/1 were divided into three categories: those who did not do therapy, those who did not undergo change in therapy, and those who changed therapy.

The analysis of JAK2 haplotype GGCC_46/1 showed how the common allele (C/C) was present in 50% of subjects that did not receive therapy, 36% of subjects that received therapy without changes, and 29% of subjects that changed therapy due to drug resistance (Table 2). Meanwhile, in the heterozygous condition (C/G), 60% of patients received therapy without changes, 58% of patients presented drug resistance, and 50% of subjects received no therapy (Table 2). Finally, the G/G genotype in 4% of patients was associated with effective first-line treatment without the necessity of changes, while 11% of patients encountered onco-drug resistance. Meanwhile, we did not find the G/G genotype in patients that did not need therapy (Table 2). Analyzing the correlation between each genotype and each type of patient response to therapy, we found that there was no statistically significant correlation. In particular, no genotype correlated positively with the uprising in onco-drug resistance, although 58% of the subjects analyzed that underwent changes in therapy had the C/G genotype, compared to 29% with the C/C common genotype and 11% with the G/G genotype.

Table 2. JAK2 rs10974944 SNP genotype frequencies in MPN (JAK2 V617F) patients and their response to therapy.

Genotype	No Therapy $n = 8$	p-Value	Therapy $n = 25$	p-Value	Therapy Changed $n = 17$	p-Value
CC	4/8 (50%)	0.4357	9/25 (36%)	1	5/17 (29%)	0.7568
CG	4/8 (50%)	0.7058	15/25 (60%)	1	10/17 (58%)	0.7635
GG	0	1	1/25 (4%)	1	2/17 (11%)	1

This table shows 50 MPN patients tested positive for the JAK2V617F mutation classified based on their genotype and their response to therapy. p value < 0.01 is statistically significant.

3.4. JAK2 Haplotype 46/1 Association with Onco-Drug Resistance Symptoms in JAK2V617F-Positive MPN Patients

We decided to evaluate, only in the 17 patients who developed a drug resistance, whether or not there was a correlation between the presence of the JAK2 46/1 haplotype and some clinical parameters that could lead to onco-drug resistance: splenomegaly, leukocytosis, thrombocytosis, and hepatomegaly.

We found that 80% of patients with the (C/C) genotype presented splenomegaly, 60% and 40% of patients presented leukocytosis and thrombocytosis, respectively, and only 20% of the cases presented hepatomegaly (Table 3).

On the other hand, patients with the heterozygous (C/G) condition displayed, in about half of the cases, all the symptoms related to onco-drug resistance. In particular, we found thrombocytosis and leukocytosis in 50% of the cases and splenomegaly and hepatomegaly

in 40% of the cases (Table 3). The G/G genotype was identified in few patients because of the reduced frequency of the allele in the general population. It is interesting to observe that they presented thrombocytosis and leukocytosis in 100% of the cases and splenomegaly and hepatomegaly in 50% of the cases (Table 3). The data could suggest how subjects with the heterozygous condition (C/G) and those with the (G/G) genotype, in at least 50% of the cases, have the possibility of developing all the related symptoms of onco-drug resistance, although an in-depth analysis in a larger population is recommended to confirm this. In any case, it could indicate a predisposition for these patients to develop risk symptoms that could be taken into account during the choice of the therapy.

Table 3. Association of JAK2 rs10974944 SNP genotype frequencies with onco-drug resistance symptoms.

Genotype	Onco-Drug Resistance Symptoms			
	Splenomegaly ***n* = 17**	**Thrombocytosis** ***n* = 17**	**Leukocytosis** ***n* = 17**	**Hepatomegaly** ***n* = 17**
CC	4/5 (80%)	2/5 (40%)	3/5 (60%)	1/5 (20%)
CG	4/10 (40%)	5/10 (50%)	5/10 (50%)	4/10 (40%)
GG	1/2 (50%)	2/2 (100%)	2/2 (100%)	1/2 (50%)

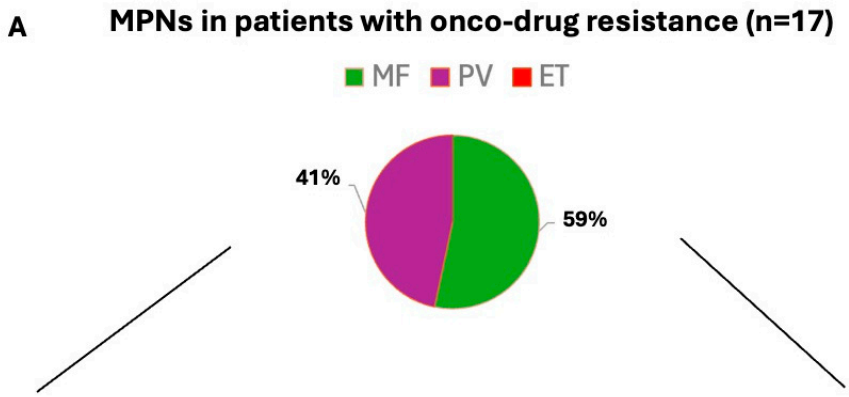
This table shows 17 MPN patients tested positive for the JAK2V617F mutation classified based on their genotype and their onco-drug resistance symptoms. p value < 0.01 is statistically significant.

3.5. JAK2 Haplotype 46/1 Contribution in MPN Patients with Drug Resistance

In 17 patients analyzed with onco-drug resistance, 58% suffered from PMF and 41% from PV, while there were no cases of ET. Thus, considering the role of the G allele, as a risk factor, in the outcome of MPNs we analyzed the JAK2 rs1097944 SNP genotype contribution.

According to the genotype frequencies, we found that the C/C common allele was present in 60% of patients with PMF and in 40% of patients with PV, while the heterozygous condition C/G showed the same percentage (50%) in both diseases (Figure 6A,B). Instead, we found that subjects with the G/G allele were affected only by PMF and that this condition was an evolution of different types of MPNs. Analyzing the other genotypes, 0% of patients with the C/C or C/G allele, affected by PMF, presented this condition. Fisher analysis revealed a statistically significant correlation between the G/G allele and the evolution of different kind of MPNs towards PMF, with a p-value = 0.002449 and an odds ratio of 3.701209, suggesting how the G/G allele of JAK2 haplotype 46/1 could be useful for the prediction of the disease severity.

To conclude, the data suggested how patients with onco-drug resistance were mostly affected by PMF and PV. Moreover, while, in these subjects, C/C and C/G genotypes were not statistically associated with a particular MPN disorder, the G/G condition could contribute to the development of PMF starting from other MPNs.



Genotype	MPN patients with onco-drug resistance *n* = 17		
	PMF	**PV**	**ET**
CC (*n* = 5)	3/5 (60%)	2/5 (40%)	0/5 (0%)
CG (*n* = 10)	5/10 (50%)	5/10 (50%)	0/10 (0%)
GG (*n* = 2)	2/2 (100%)	0/2 (0%)	0/2 (0%)

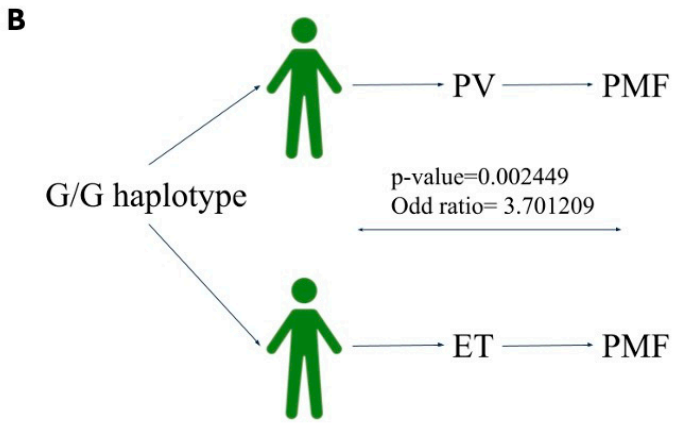


Figure 6. (**A**) This graph shows the distribution of MPNs in patients with onco-drug resistance (*n* = 17) and their classification according to their genotype and their MPN disorders. (**B**) Schematic representation, showing how G/G haplotype correlates with PMF evolution starting from different MPNs. *p* value < 0.01 was set as the threshold for statistical significance.

4. Discussion

MPNs are a group of bone marrow diseases with an excessive cell production, starting from a precursor of the myeloid lineage. It is widely believed that somatic acquisition of genetic aberrations may be one of the pathogenic mechanisms of the MPNs [18,31]. The identification of the JAK2 V617F mutation represented an important step in the understanding of the molecular mechanisms involved in MPN diseases, although these remain not completely understood.

Among the 665 patients selected by the hematology Laboratory at "V. Fazzi" hospital in Lecce, 102 received a confirmed diagnosis of myeloproliferative syndrome and 70% of these patients tested positive for the JAK2 V617F mutation.

By Fisher analysis, a statistically significant association between the JAK2V617F mutation and MPNs was found, with a p-value lower than 2.2×10^{-16} and an odds ratio of 18suggesting that these patients have a 18times higher risk of developing myeloproliferative disorders compared to JAK2V617F-negative patients. This finding corroborates previous studies showing the association between JAK2V617F mutation and the predisposition to MPNs [1,32]. In detail, we observed a prevalence of the JAK2 V617F mutation in patients with PV (92%), followed by those with PMF (69%) and those with ET (61%) [5,9,33]. A prevalence of JAK2 V617F-positive cases was previously reported in subjects affected by PV, because it is related to the specific pathogenesis of this hematologic malignancy and plays a key role in the constitutive activation of the JAK-STAT pathway [34].

Recently, different research groups reported, for the first time, that the JAK2V617F mutation tends to occur in a specific haplotype, called the 46/1 haplotype (GGCC haplotype). Indeed, the presence of the JAK2 haplotype GGCC_46/1 increases the risk of acquiring the JAK2V617F mutation from two to three times [1], making itself a valid biomarker for monitoring MPN patients.

The analysis of JAK2 haplotype distribution, in our cohort population, showed how the C/G genotype was present in 58% of patients with the JAK2V617F mutation, while 36% instead presented the C/C common allele and 6% had the G allele.

Our results were in agreement with the data reported in the literature that demonstrated how the C/G genotype is more common in JAK2 V617F-positive patients compared to the C/C [12,35] and G/G genotypes, which are the least represented [36].

Moreover, in V617F-positive patients, the heterozygous condition of the haplotype 46/1 was prevalently associated with the development of PV (63%) and PMF (61%). Different studies have analyzed the association between the haplotype 46/1 and certain clinical and laboratory findings [1], especially in patients with PV [18], but not one assessed the possible relationship between the haplotype 46/1 and the development of drug resistance. According to their medical records analysis and their therapy response, patients were divided into three main categories: those who did not receive treatment, those who did not change treatment, and those who changed treatment.

Although no statistically significant association was found between the presence of the JAK2 haplotype 46/1 variants and the different patients' therapy response, in patients with onco-drug resistance, we observed a prevalence of the heterozygous (C/G) genotype (58%) with about half of these patients developing all the related symptoms of onco-drug resistance: thrombocytosis, leukocytosis, splenomegaly, and hepatomegaly. However, the absence of a statistically significant correlation could be due to other factors that could bring on onco-drug resistance, such as dose-dependent drug-related cytopenias [37], the genetic variability of drug metabolizing enzyme such as P450 (CYP) enzyme CYP3A4 for Ruxolitinib [38,39], the bone marrow microenvironment, including stromal cells and cytokines, that supports the survival of malignant cells, reducing the effectiveness of drugs [40], or the alteration in the cell cycle that can lead to better survival of cell clones. It is also important to note that patient-specific factors, including age, overall health, and concurrent medications, may contribute to therapeutic responses. Furthermore, other mutations in genes associated with myeloproliferative neoplasms, such as MPL and CALR, may play a role in mediating resistance to onco-drugs. The presence of co-morbidities or pre-existing conditions might also influence the severity of symptoms and the effectiveness of treatment. In addition, considering the heterogeneity of tumors and the dynamic nature of cancer evolution, some patients may acquire new aberrations that confer drug resistance during the course of treatment. Thus, comprehensive genomic profiling and a deeper understanding of the molecular mechanisms involved in therapy resistance are essential for identifying potential biomarkers and developing targeted therapeutic strategies tailored to individual patients. In summary, while the JAK2 haplotype 46/1 variants may demonstrate a correlation with certain symptoms in onco-drug resistant patients, the interplay of multiple factors necessitates further investigation to elucidate the complex landscape of onco-drug resistance in these populations. Future studies should aim

to integrate genomic, transcriptomic, and clinical data to identify more robust predictors of treatment response and outcomes.

Most of the patients who experienced onco-drug resistance were mainly affected by PMF (58%) and PV (41%), while no cases of ET were detected. The absence of ET cases, in subjects with onco-drug resistance, could be explained considering that, normally, JAK2 V617F mutation in these patients causes not only a high risk of thrombosis but may also promote the progression to PV or PMF [41]. Moreover, ET patients are often treated simply by aspirin that is associated with a cytoreductive drug only in subjects with high risk.

Besides the prevalence of the heterozygous genotype in subjects with onco-drug resistance, an important finding came out from the analysis of the G/G genotype, even if it was present in only 11% of patients. Indeed, the G allele is the most studied, since it represents the risk allele for MPNs [16,36]. We found that subjects with the G/G allele were affected by PMF and 50% of the cases presented all the related symptoms of onco-drug resistance. Analyzing the clinical history of these patients, we discovered that their PMF was an evolution of different types of MPNs and that 0% of patients with the C/C or C/G allele, affected by PMF, presented this condition. Fisher analysis revealed a statistically significant correlation between the G/G allele and the evolution of different kind of MPNs towards PMF, with a p-value = 0.002449 and an odds ratio of 3.701209, thus suggesting how the G/G allele of JAK2 haplotype 46/1 could be useful for the prediction of the disease severity. In another study, it has been shown that the 46/1 haplotype could predispose PV patients to bone marrow fibrosis [42].

Thus, despite the narrow cardinality of the sample analyzed, we could assume that the presence of the G allele is a risk factor for the evolution of polycythemia vera and essential thrombocythemia in myelofibrosis.

Hence, despite the absence of a correlation between the JAK2 haplotype GGCC_46/1 and onco-drug resistance in patients with the C/G allele, the presence of the G/G allele seems to be related to a predisposition for MPNs' evolution to the myelofibrosis condition and to the development of clinical parameters that could cause onco-drug resistance. Considering the narrow cardinality of the studied sample, we believe that current efforts should be aimed at shedding light on the clinical role of the JAK2 haplotype GGCC_46/1, through an in-depth analysis, perhaps expanding the sample under study. Indeed, our data suggest that the presence of the JAK2 haplotype 46/1 could indicate a predisposition for these patients to develop risk symptoms that could be taken into account during therapy. Thus, understanding the presence of the GGCC haplotype can aid in personalizing treatment protocols. Tailoring the drug doses based on this genetic marker may enhance therapeutic outcomes and minimize adverse effects. Overall, understanding the implications of the GGCC haplotype can significantly inform treatment strategies, leading to better-tailored therapies that enhance efficacy while reducing the risk of adverse effects. Such findings underscore the importance of genetic testing and personalized medicine in improving treatment outcomes. The integration of a comprehensive genetic profiling into treatment decisions could mark a significant advancement in MPN management, influencing disease progression, prognosis, and treatment efficacy. Moreover, the association of the JAK2 haplotype GGCC_46/1 with the progression to medullary fibrosis can represent an innovative prognostic marker for the pathology evolution and could help in patients' follow up. Perhaps it could become a new therapy target to prevent the worsening of the disease.

By examining diverse populations, researchers can identify potential genetic, environmental, and lifestyle factors that may influence the expression and effects of the 46/1 haplotype. This comprehensive approach will facilitate the development of targeted therapies that account for individual variability, ultimately leading to more tailored interventions.

Furthermore, the integration of findings from these studies in clinical practice will enhance the ability to monitor disease progression and response to treatment in real-time. The establishment of standardized protocols for diagnosis and management based on this research will ensure consistency across healthcare settings, improving overall patient care.

In conclusion, ongoing research into the impact of JAK2 haplotype 46/1 on myeloproliferative dynamics is crucial for advancing our understanding of these conditions. The insights gained will empower healthcare providers to deliver personalized care, enhance patient outcomes, and foster a greater quality of life for those affected by myeloproliferative disorders.

Author Contributions: Conceptualization, M.P., S.S., D.S., M.M. and N.D.R.; methodology, M.P., S.S., A.T. and R.M.; validation, M.P., S.S. and D.S.; formal analysis, M.P., S.S., A.T. and G.L.; investigation M.P. and S.S.; resources, M.M. and N.D.R.; data curation, M.P., S.S., R.M., D.S. and G.L.; writing—original draft preparation, M.P. and S.S.; writing—review and editing, M.P., S.S., A.T., G.L., R.M., D.S., M.M. and N.D.R.; supervision, S.S., D.S., M.M. and N.D.R.; project administration, M.M. and N.D.R.; funding acquisition, M.M. and N.D.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by ALESSIA PALLARA Association. Via Messapia,13-Monteroni di Lecce (Le), Agreement's protocol number: 39601, 09/11/2020 (Unisalento DiSTeBA-Alessia Pallara Association).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of NOVARTIS PHARMACEUTICALS. ClinicalTrials.gov Identifier: NCT05548062, Novartis Reference Number: CINC424BIT01.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Acknowledgments: The authors are highly thankful to the Alessia Pallara Association for providing financial support for this study. Also, we duly acknowledge (i) Dott. Antonio Danieli, Laboratory of General Physiology, University of Salento, for its technical support and (ii) all medical stuff from the Hematology and Stem Cell Transplant Unit, Onco-Hematological Department, who contributed to this work with their data.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Paes, J.; Silva, G.A.V.; Tarragô, A.M.; Mourão, L.P.d.S. The Contribution of *JAK2* 46/1 Haplotype in the Predisposition to Myeloproliferative Neoplasms. *Int. J. Mol. Sci.* **2022**, *23*, 12582. [CrossRef] [PubMed]
2. Hu, X.; Li, J.; Fu, M.; Zhao, X.; Wang, W. The JAK/STAT signaling pathway: From bench to clinic. *Signal Transduct. Target. Ther.* **2021**, *6*, 402. [CrossRef] [PubMed]
3. Levine, R.L.; Pardanani, A.; Tefferi, A.; Gilliland, D.G. Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. *Nat. Rev. Cancer* **2007**, *7*, 673–683. [CrossRef] [PubMed]
4. Yamaoka, K.; Saharinen, P.; Pesu, M.; Holt, V.E.; Silvennoinen, O.; O'Shea, J.J. The Janus kinases (Jaks). *Genome Biol.* **2004**, *5*, 253. [CrossRef] [PubMed]
5. Staerk, J.; Constantinescu, S.N. The JAK-STAT pathway and hematopoietic stem cells from the JAK2 V617F perspective. *JAK-STAT* **2012**, *1*, 184–190. [CrossRef]
6. Torres, D.G.; Paes, J.; da Costa, A.G.; Malheiro, A.; Silva, G.V.; Mourão, L.P.d.S.; Tarragô, A.M. *JAK2* Variant Signaling: Genetic, Hematologic and Immune Implication in Chronic Myeloproliferative Neoplasms. *Biomolecules* **2022**, *12*, 291. [CrossRef] [PubMed] [PubMed Central]
7. Dunbar, A.J.; Bowman, R.L.; Park, Y.C.; O'Connor, K.; Izzo, F.; Myers, R.M.; Karzai, A.; Zaroogian, Z.; Kim, W.J.; Fernández-Maestre, I.; et al. Jak2V617F Reversible Activation Shows Its Essential Requirement in Myeloproliferative Neoplasms. *Cancer Discov.* **2024**, *14*, 737–751. [CrossRef]
8. Josil, J.; Thuillier, E.; Chambrun, L.; Plo, I. Décryptage de l'histoire naturelle des néoplasmes myéloprolifératifs grâce à une approche d'arbres phylogénétiques. *M S-Med. Sci.* **2024**, *40*, 209–211. [CrossRef]
9. Tefferi, A.; Lasho, T.L.; Mudireddy, M.; Finke, C.M.; Hanson, C.A.; Ketterling, R.P.; Gangat, N.; Pardanani, A. The germline*JAK2*GGCC (46/1) haplotype and survival among 414 molecularly-annotated patients with primary myelofibrosis. *Am. J. Hematol.* **2018**, *94*, 299–305. [CrossRef]
10. Jones, A.V.; Chase, A.; Silver, R.T.; Oscier, D.; Zoi, K.; Wang, Y.L.; Cario, H.; Pahl, H.L.; Collins, A.; Reiter, A.; et al. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat. Genet.* **2009**, *41*, 446–449. [CrossRef]

11. Olcaydu, D.; Harutyunyan, A.; Jäger, R.; Berg, T.; Gisslinger, B.; Pabinger, I.; Gisslinger, H.; Kralovics, R. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nat. Genet.* **2009**, *41*, 450–454. [CrossRef] [PubMed]
12. Kilpivaara, O.; Mukherjee, S.; Schram, A.M.; Wadleigh, M.; Mullally, A.; Ebert, B.L.; Bass, A.; Marubayashi, S.; Heguy, A.; Garcia-Manero, G.; et al. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nat. Genet.* **2009**, *41*, 455–459. [CrossRef] [PubMed]
13. Vannucchi, A.M.; Guglielmelli, P. The *JAK2* 46/1 (*GGCC*) MPN-predisposing haplotype: A risky haplotype, after all. *Am. J. Hematol.* **2018**, *94*, 283–285. [CrossRef] [PubMed]
14. Hermouet, S.; Vilaine, M. The JAK2 46/1 haplotype: A marker of inappropriate myelomonocytic response to cytokine stimulation, leading to increased risk of inflammation, myeloid neoplasm, and impaired defense against infection? *Haematologica* **2011**, *96*, 1575–1579. [CrossRef] [PubMed] [PubMed Central]
15. Anelli, L.; Zagaria, A.; Specchia, G.; Albano, F. The JAK2 GGCC (46/1) Haplotype in Myeloproliferative Neoplasms: Causal or Random? *Int. J. Mol. Sci.* **2018**, *19*, 1152. [CrossRef]
16. Pagliarini-e-Silva, S.; Santos, B.C.; Pereira, E.M.d.F.; Ferreira, M.E.; Baraldi, E.C.; Sell, A.M.; Visentainer, J.E.L. Evaluation of the association between the JAK2 46/1 haplotype and chronic myeloproliferative neoplasms in a Brazilian population. *Clinics* **2013**, *68*, 5–9. [CrossRef]
17. Macedo, L.C.; Santos, B.C.; Pagliarini-E-Silva, S.; Pagnano, K.B.B.; Rodrigues, C.; Quintero, F.C.; Ferreira, M.E.; Baraldi, E.C.; Ambrosio-Albuquerque, E.P.; Sell, A.M.; et al. JAK2 46/1 haplotype is associated with JAK2 V617F—Positive myeloproliferative neoplasms in Brazilian patients. *Int. J. Lab. Hematol.* **2015**, *37*, 654–660. [CrossRef]
18. Ohyashiki, J.H.; Yoneta, M.; Hisatomi, H.; Iwabuchi, T.; Umezu, T.; Ohyashiki, K. The C allele of JAK2 rs4495487 is an additional candidate locus that contributes to myeloproliferative neoplasm predisposition in the Japanese population. *BMC Med Genet.* **2012**, *13*, 6. [CrossRef]
19. Li, S.-L.; Zhang, P.-J.; Sun, G.-X.; Lu, Z.-J. The JAK2 46/1 haplotype (GGCC) in myeloproliferative neoplasms and splanchnic vein thrombosis: A pooled analysis of 26 observational studies. *Ann. Hematol.* **2014**, *93*, 1845–1852. [CrossRef]
20. Koh, S.P.; Yip, S.P.; Lee, K.K.; Chan, C.C.; Lau, S.M.; Kho, C.S.; Lau, C.K.; Lin, S.Y.; Lau, Y.M.; Wong, L.G.; et al. Genetic association between germline JAK2 polymorphisms and myeloproliferative neoplasms in Hong Kong Chinese population: A case-control study. *BMC Genet.* **2014**, *15*, 147. [CrossRef]
21. Paes, J.F.; Torres, D.G.; Aquino, D.C.; Alves, E.V.B.; Mesquita, E.A.; Sousa, M.A.; Fraiji, N.A.; Passos, L.N.M.; Abreu, R.S.; Silva, G.A.V.; et al. Exploring hematological alterations and genetics linked to SNV rs10974944 in myeloproliferative neoplasms among Amazon patients. *Sci. Rep.* **2024**, *14*, 9389. [CrossRef] [PubMed] [PubMed Central]
22. Zhang, X.; Hu, T.; Wu, Z.; Kang, Z.; Liu, W.; Guan, M. The JAK2 46/1 haplotype is a risk factor for myeloproliferative neoplasms in Chinese patients. *Int. J. Hematol.* **2012**, *96*, 611–616. [CrossRef] [PubMed]
23. Andrikovics, H.; Nahajevszky, S.; Koszarska, M.; Meggyesi, N.; Bors, A.; Halm, G.; Lueff, S.; Lovas, N.; Matrai, Z.; Csomor, J.; et al. JAK2 46/1 haplotype analysis in myeloproliferative neoplasms and acute myeloid leukemia. *Leukemia* **2010**, *24*, 1809–1813. [CrossRef] [PubMed]
24. Pardanani, A.; Lasho, T.L.; Finke, C.M.; Gangat, N.; Wolanskyj, A.P.; Hanson, C.A.; Tefferi, A. The JAK2 46/1 haplotype confers susceptibility to essential thrombocythemia regardless of JAK2V617F mutational status—Clinical correlates in a study of 226 consecutive patients. *Leukemia* **2009**, *24*, 110–114. [CrossRef] [PubMed]
25. Guglielmelli, P.; Biamonte, F.; Spolverini, A.; Pieri, L.; Isgrò, A.; Antonioli, E.; Pancrazzi, A.; Bosi, A.; Barosi, G.; Vannucchi, A.M. Frequency and clinical correlates of JAK2 46/1 (GGCC) haplotype in primary myelofibrosis. *Leukemia* **2010**, *24*, 1533–1537. [CrossRef]
26. Verstovsek, S.; Mesa, R.A.; Gotlib, J.; Levy, R.S.; Gupta, V.; DiPersio, J.F.; Catalano, J.V.; Deininger, M.; Miller, C.; Silver, R.T.; et al. A Double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N. Engl. J. Med.* **2012**, *366*, 799–807. [CrossRef]
27. Sobas, M.; Ianotto, J.-C.; Kiladjian, J.-J.; Harrison, C. Myeloproliferative neoplasms: Young patients, current data and future considerations. *Ann. Hematol.* **2024**, *103*, 3287–3291. [CrossRef]
28. Passamonti, F.; Maffioli, M. The role of JAK2 inhibitors in MPNs 7 years after approval. *Blood* **2018**, *131*, 2426–2435. [CrossRef]
29. Barbui, T.; Barosi, G.; Birgegard, G.; Cervantes, F.; Finazzi, G.; Griesshammer, M.; Harrison, C.; Hasselbalch, H.C.; Hehlmann, R.; Hoffman, R.; et al. Philadelphia-negative classical myeloproliferative neoplasms: Critical concepts and management recommendations from European LeukemiaNet. *J. Clin. Oncol.* **2011**, *29*, 761–770. [CrossRef]
30. Trifa, A.P.; Cucuianu, A.; Popp, R.A. Development of a reliable PCR-RFLP assay for investigation of the JAK2 rs10974944 SNP, which might predispose to the acquisition of somatic mutation JAK2V617F. *Acta Haematol.* **2009**, *123*, 84–87. [CrossRef]
31. Abdel-Wahab, O.; Levine, R. The Spliceosome as an indicted conspirator in myeloid malignancies. *Cancer Cell* **2011**, *20*, 420–423. [CrossRef] [PubMed]
32. Duletic, A.N.; Dekanic, A.; Hadzisejdic, I.; Kusen, I.; Matusan-Llijas, K.; Grohovac, D.; Grahovac, B.; Jonjic, N. JAK2-V617F Mutation is associated with clinical and laboratory features of myeloproliferative neoplasms. *Coll Antropol.* **2012**, *36*, 859–865. [PubMed]
33. Kucine, N. Myeloproliferative Neoplasms in Children, Adolescents, and Young Adults. *Curr. Hematol. Malign. Rep.* **2020**, *15*, 141–148. [CrossRef] [PubMed]
34. Tefferi, A.; Barbui, T. Polycythemia vera and essential thrombocythemia: 2021 update on diagnosis, risk-stratification and management. *Am. J. Hematol.* **2020**, *95*, 1599–1613. [CrossRef] [PubMed]

35. Ngoc, N.T.; Hau, B.B.; Vuong, N.B.; Xuan, N.T. JAK2 rs10974944 is associated with both V617F-positive and negative myeloproliferative neoplasms in a Vietnamese population: A potential genetic marker. *Mol. Genet. Genom. Med.* **2022**, *10*, e2044. [CrossRef]
36. Trifa, A.P.; Cucuianu, A.; Petrov, L.; Urian, L.; Militaru, M.S.; Dima, D.; Pop, I.V.; Popp, R.A. The G allele of the JAK2 rs10974944 SNP, part of JAK2 46/1 haplotype, is strongly associated with JAK2 V617F-positive myeloproliferative neoplasms. *Ann. Hematol.* **2010**, *89*, 979–983. [CrossRef]
37. Harrison, C.N.; Schaap, N.; Mesa, R.A. Management of myelofibrosis after ruxolitinib failure. *Ann. Hematol.* **2020**, *99*, 1177–1191. [CrossRef] [PubMed] [PubMed Central]
38. Shi, J.G.; Chen, X.; Emm, T.; Scherle, P.A.; McGee, R.F.; Lo, Y.; Landman, R.R.; McKeever, E.G.; Punwani, N.G.; Williams, W.V.; et al. The Effect of CYP3A4 inhibition or induction on the pharmacokinetics and pharmacodynamics of orally administered ruxolitinib (INCB018424 Phosphate) in healthy volunteers. *J. Clin. Pharmacol.* **2012**, *52*, 809–818. [CrossRef] [PubMed]
39. Appeldoorn, T.Y.J.; Munnink, T.H.O.; Morsink, L.M.; Hooge, M.N.L.-D.; Touw, D.J. Pharmacokinetics and Pharmacodynamics of Ruxolitinib: A Review. *Clin. Pharmacokinet.* **2023**, *62*, 559–571. [CrossRef] [PubMed] [PubMed Central]
40. Schepers, K.; Campbell, T.B.; Passegué, E. Normal and leukemic stem cell niches: Insights and therapeutic opportunities. *Cell Stem Cell* **2015**, *16*, 254–267. [CrossRef] [PubMed] [PubMed Central]
41. Rumi, E.; Cazzola, M. Diagnosis, risk stratification, and response evaluation in classical myeloproliferative neoplasms. *Blood* **2017**, *129*, 680–692. [CrossRef]
42. Tefferi, A.; Lasho, T.L.; Patnaik, M.M.; Finke, C.M.; Hussein, K.; Hogan, W.J.; Elliott, M.A.; Litzow, M.R.; Hanson, C.A.; Pardanani, A. JAK2 germline genetic variation affects disease susceptibility in primary myelofibrosis regardless of V617F mutational status: Nullizygosity for the JAK2 46/1 haplotype is associated with inferior survival. *Leukemia* **2009**, *24*, 105–109. [CrossRef] [PubMed]