JAK2 GGCC haplotype in *MPL* mutated myeloproliferative neoplasms

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***JAK2* (V617F) is associated with a genetic predisposition to its acquisition, as it is preferentially found in subjects with a common constitutional *JAK2* haplotype known as 46/1 or GGCC. A recent study suggests that a genetic predisposition to acquisition of *MPL* mutation may exist in sporadic patients, since an association was found with the *JAK2* 46/1 haplotype. We genotyped 509 patients with myeloproliferative neoplasms (MPN), 7% of which carrying a somatic mutation of *MPL* Exon 10. We found that the *JAK2* GGCC haplotype was closely associated with *JAK2* (V617F) (OR 1.84, $P < 0.001$) but not with *MPL* mutations (OR 0.98), suggesting a different genetic background for these molecular lesions.**

The *JAK2* (V617F) mutation, found in about two thirds of patients with a myeloproliferative neoplasm (MPN), is preferentially detected in subjects with a common constitutional *JAK2* haplotype known as 46/1 or GGCC [1–3]. The mechanism underlying this predisposition has not been elucidated so far. A first hypothesis, known as "hypermutability", is that the GGCC haplotype of *JAK2* is more susceptible to DNA repair defects or replication infidelity and, as a result of this genetic instability, is more likely to acquire somatic mutations such as *JAK2* (V617F) or Exon 12 mutations [4]. A second hypothesis is that mutations may arise on all *JAK2* haplotypes at equal rates, but that the GGCC haplotype positively interacts with *JAK2* mutations and thus provides a "fertile ground" for development of MPN.

About 15–25% of *JAK2* (V617F)-negative patients with essential thrombocythemia (ET) or primary myelofibrosis (PMF) carry activating somatic mutations of *MPL* Exon 10, mainly involving a W515 substitution [5–7]. By contrast, *MPL* mutations are rarely found in patients with polycythemia vera (PV) or post-PV myelofibrosis, and in the few cases described they coexisted with *JAK2* (V617F) [8].

Through a meta-analysis of several studies in MPN patients, Jones et al. [9] found an association between the *JAK2* 46/1 haplotype and *MPL* (W515) mutations, supporting the "fertile ground" hypothesis. This association was not confirmed in a study conducted on patients recruited from the Mayo Clinic database [10].

We evaluated patients with MPN followed at the Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Pavia, Italy. We used direct sequencing and high-resolution melt (HRM) analysis to identify mutations of *MPL* Exon 10 in 570 patients with MPN, and allele-specific PCR and deep sequencing to further characterize a subset of mutated patients. Somatic mutations were detected in 33/221 patients (15%) with *JAK2* (V617F)-negative ET or PMF [7].

We genotyped the rs10974944 SNP in 509 cases of MPN (389 ET, 96 PMF, and 24 post ET-myelofibrosis) and 203 control subjects and results are reported in Table I. As expected, the GGCC haplotype was more frequent in MPN patients compared to controls. When the mutational status of the MPN patients was considered, the GGCC haplotype was significantly over-represented in *JAK2* (V617F)-positive subjects ($P < 0.001$). By contrast, no difference was observed between *MPL*-mutated patients and the control cohort ($P = 0.95$). However, when we performed a meta-analysis including our patients and those reported by Jones et al. [9], a significant association became apparent (Table I). Nonetheless, the relationship between *JAK2* 46/1 or GGCC haplotype and *MPL* (W515) mutations was considerably weaker than that with *JAK2* (V617F) (Table I).

The most reasonable interpretation of these data is that, at variance with *JAK2* (V617F), mutations in *MPL* Exon 10 are not directly associated with the GGCC haplotype in MPN.

Methods

We evaluated patients with myeloproliferative neoplasms followed at the Department of Hematology Oncology, Fondazione IRCCS Policlinico San Matteo and University of Pavia, Pavia, Italy. This study was approved by the institutional ethics committee (Comitato di Bioetica, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy). The procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 2000, and samples were obtained after patients provided written informed consent.

Genomic T-lymphocyte DNA was genotyped for rs10974944, a tag SNP which acts as a surrogate for the GGCC haplotype (GG or GC = GGCC haplotype; CC = not GGCC haplotype) [4]. The commercially available C_31941696 TaqMan assay (Applied Biosystems, Foster City, CA) was used on the Rotor-Gene™ 6000 real-time PCR instrument in a final volume of 10 μL according to the manufacture's procedure.

Statistical analyses were performed using MedCalc 11.2.1.0 (MedCalc Software, Belgium). *P* values <0.05 were considered as significant.

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TABLE I. Genotyping Results for the *JAK2* GGCC Tag SNP rs10974944 in the Study Population and a Meta-analysis of Studies Published so far

Category	No. of cases	No. of GGCC alleles	No. of non-GGCC alleles	Frequency of GGCC alleles	*P* value vs. controls	OR (95% CI)
Study population						
All MPN patients	509[a]	370	648	0.36	0.0006	1.56 (1.21–2.01)
MPN patients carrying *JAK2* (V617F)	288	232	340	0.40	0.000013	1.84 (1.40–2.42)
Patients with ET, PMF or post-ET MF carrying *MPL* exon 10 mutations	34[b]	18	50	0.26	0.948	0.98 (0.55–1.76)
MPN patients carrying no mutation in *JAK2* or *MPL*	187	120	254	0.32	0.11	1.29 (0.95–1.75)
Controls	203	109	297	0.27	–	–
Meta-analysis[c]						
Patients with ET, PMF or post-ET MF carrying *MPL* exon 10 mutations	210	140	280	0.33	0.005	1.34 (1.08–1.66)
Controls	4,847	2,628	7,066	0.27	–	–

[a]Of the 513 MPN patients studied, 509 could be evaluated for the *JAK2* GGCC haplotype.
[b]One of these patients with *MPL* mutations also carried *JAK2* (V617F).
[c]Including genotyping data on the *JAK2* 46/1 haplotype from the study by Jones et al. [9] GGCC and 46/1 refer to the same haplotype.

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