No association between the XPD Lys751Gln (rs13181) polymorphism and disease phenotype or leukemic transformation in primary myelofibrosis

Philadelphia negative (Ph-) classical myeloproliferative neoplasms (MPNs), i.e. polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF), are clonal disorders of hematopoiesis with high frequency of recurrent somatic gene mutations, like *JAK2*V617F and *MPL*W515L, DNA copy variations, and chromosomal aberrations.[1,2] Consequently, the neoplastic process is thought to be initiated, maintained, and enhanced by acquired molecular lesions. However, germ-line variants, like the 46/1 haplotype of the JAK2 gene[3-6] or the A3669G single nucleotide polymorphism (SNP) of glucocorticoid receptor,[7] have been documented to predispose to the acquisition of the mutations or the diseases themselves, and to determine their phenotype and outcome.

Recently, Hernandez-Boluda and co-workers have identified a polymorphism in the xeroderma pigmentosum group D (*XPD*) gene, rs 13181, reporting it to be an independent risk factor for leukemic transformation in ET and PV.[8] Specifically, homozygous carriers for the minor allele (Gln/Gln) had an almost 5-fold higher risk of progression to acute myeloid leukemia (AML) compared with the other *XPD* genotypes. In addition, the Gln/Gln *XPD* variant was associated with a 4-fold increased risk of developing a new primary non-myeloid malignancy during follow up, regardless of the type of treatment given to the patients.

PMF shares with ET and PV an increased risk of leukemic transformation which occurs in 15-20% of patients;[9] more frequently, therefore, than in PV or ET where it occurs in 5-10% of patients.[10-12] We analyzed the *XPD* Lys751Gln (rs13181) polymorphism in a representative cohort of PMF patients in order to evaluate its contribution to the hematologic malignancy and whether it could influence the likelihood of developing AML. The cohort was made up of 456 consecutive patients with World Health Organization defined PMF enrolled in our patient database from 2000 to 2012.[13] The control group was made up of 138 Italian individuals without any hematologic malignancies. The ethics committee of the Policlinico San Matteo Foundation, Pavia, Italy, had approved the informed consent for PMF patients to donate samples for molecular research on their disease, and all patients signed this consent form before sampling. The normal control population was made up of healthy Italian subjects belonging to the bone marrow donor registry whose samples were made anonymous for the purpose of the study.

The genomic DNA was isolated from blood granulocytes of all patients and controls, obtained using the QIAamp DNA Blood Mini Kit (QIAGEN, Duesseldorf, Germany). The rs13181 SNP was genotyped by real-time polymerase chain reaction (PCR) using the TaqMan SNP genotyping on demand assay C-3145033-10 (Life Technologies Corporation, Paisley, UK). Assays were performed according to the manufacturer's instructions. *JAK2*V617F mutation and allele burden were determined by RTq PCR.[14]

The genotypes and allele distribution of the Lys751Gln SNP were compared between PMF patients and controls using Fisher's exact test. The association between candidate SNP and risk of leukemic transformation was assessed by logistical regression. The association of genotypes with PMF outcomes was assessed by Kaplan-Meier analysis and the differences by the log rank test. P=0.05 was considered statistically significant. STATISTICA software (Statsoft, Tulsa, OK, USA) was used for statistical analysis.

The C/C homozygous polymorphism genotype was the rarest seen in this study. Overall, 65 PMF patients (14.2%) and 18 controls (13%) harbored this genotype and the A/C and C/C genotypes were similarly distributed in PMF patients and controls (OR 0.78; 95%CI: 0.42–1.43; P=0.62) (Table 1). We then analyzed the distribution of the XPD Lys751Gln SNP in patients stratified according to *JAK2*V617F mutation status. Once again, the A/C or C/C genotypes of the SNP were similarly distributed in *JAK2* V617F-positive (n=262, 58.6%) compared to *JAK2* V617F-negative (n=185, 41.4%) patients (OR 1; 95%CI: 0.5–2.2; P=1). In conclusion, we found no indication of any possible contribution of the XPD Lys751Gln to the pathogenesis of PMF. There was no difference at diagnosis between the 65 patients with homozygous C/C genotype and those

Table 1. Genotype and allele frequencies of the Lys751Gln SNP of XPD in 456 patients with primary myelofibrosis (PMF) and the healthy control populations.

	N.	Lys751Gln *XPD* genotype				Allele frequency	
		A/A	A/C	C/C	A/C + C/C	A	C
PMF patients	456	155 (34%)	236 (51.7%)	65 (14.2%)	301 (66%)	59.8%	40.1%
Local controls	138	55 (38.8%)	65 (47.1%)	18 (13%)	83 (60.1%)	63.4%	36.6%

** The difference between the A/C plus C/C genotype frequency in patients with PMF with respect to local controls was not statistically different (P=0.2). **There was no statistical difference in C allele frequency between PMF patients and local controls (OR: 0.86; 95% CI: 0.48-1.52; P=0.4).*

Table 2. Progression to AML transformation according to the XPD SNP (Lys751Gln) genotype and JAK2 V617F mutation.

	Global (n=456)	Observed frequency XPD A/A +A/C (n=391)	XPD C/C (n=65)	OR (95%CI)
*JAK2*V617F mutated	43/262* (16.4%)	33/220 (15%)	10/42 (23.8%)	0.54 (0.24-1.22); P=0.14
*JAK2*V617F –non mutated	41/185* (22.2%)	34/162 (21%)	7/23 (30.4%)	0.62 (0.21-1.62); P=0.32
Total	84/456 (18.4%)	67/391 (17.1%)	17/65 (26.1%)	0.57 (0.31-1.05); P=0.07

** Of the 456 patients studied, 447 had JAK2V617F genotype.*

with the A/A or A/C genotype regarding age, sex, hemoglobin (Hb), white blood cell (WBC) count, platelet count, spleen index, lactic dehydrogenase plasma levels, and $CD34^+$ circulating hematopoietic progenitor cells at their diagnosis (*data not shown*).

Logistical regression was used to test whether the XPD variant might affect occurrence of AML transformation. No association between this SNP and risk of leukemic transformation was found (Table 2). To account for the influence of time on disease outcomes, we used the Kaplan-Meier method to analyze the risk of severe anemia (Hb < 10 g/dL), large splenomegaly (spleen extending > 10 cm from left costal margin), thrombocytopenia (platelets < 150 x 10^9/L), leukopenia (WBC count < 4 x 10^9/L), death from any cause, and AML transformation[15] in patients with different *XPD* Lys751Gln genotypes. There was no significant difference in any of the analyzed outcomes between the three SNP genotypes.

Patients' characteristics did not allow us to study the synergism between *XPD* polymorphism and exposure to cytoreductive agents. In fact, only 10% of patients did not receive cytoreduction during their disease course.

The SNP studied in this report interests one of four control genes involved in DNA repair mechanisms and leukemia transformation.[8] The XPD protein has an important role in the nucleotide excision repair pathway and is also involved in transcription initiation and control of cell cycle.[16] The variant had been previously associated with outcome after chemotherapy for acute leukemia and also found to predict for an increased risk of developing leukemia after chemotherapy.[17] In this study, we did not find any association between *XPD* variant and hematologic and clinical outcomes in PMF. In particular, we did not find any predisposition toward AML transformation attributable to the polymorphism. This latter finding is at variance with that reported by Hernandez-Boluda in PV and ET,[8] and reinforces the hypothesis that the pathogenesis of AML in PMF is due to complex oncogenic events that differ from those affecting AML transformation in PV and ET disorders.[18]

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