A polymorphism in the XPD gene predisposes to leukemic transformation and new nonmyeloid malignancies in essential thrombocythemia and polycythemia vera

Juan-Carlos Hernández-Boluda,1 Arturo Pereira,2 Francisco Cervantes,3 Alberto Alvarez-Larrán,4 María Collado,1 Esperanza Such,6 M. Jesús Arilla,6 Concepción Boqué,7 Blanca Xicoy,8 Margherita Maffioli,3 Beatriz Bellosillo,9 Isabel Marugán,1 Paula Amat,1 Carles Besses,4 and Vicent Guillerm1

Patients with essential thrombocythemia (ET) and polycythemia vera (PV) have an increased incidence of acute myeloid leukemia and new nonhematologic malignancies compared with the general population. However, information on the factors determining the risk for such complications is limited. In the present study, we investigated whether constitutional genetic variations in DNA repair predispose to leukemic transformation and new nonmyeloid neoplasias in patients with ET and PV. Case-control studies for predisposition to both types of malignancies were nested in a cohort of 422 subjects diagnosed with ET or PV during the period 1973-2010 in several institutions in Spain. A total of 64 incidence cases of leukemia and 50 cases of primary nonmyeloid cancers were accrued. At conditional regression analysis, the Gln/Gln genotype in the XPD codon 751 showed the strongest association with both leukemic transformation (odds ratio [OR] = 4.9; 95% confidence interval [95% CI], 2.0-12) and development of nonmyeloid malignancies (OR = 4.2; 95% CI, 1.5-12). Additional predictive factors were exposure to cytoreductive agents for leukemic transformation (OR = 3.5; 95% CI, 2.0-6.2) and age for nonmyeloid malignancies (OR = 2.0; 95% CI, 1.4-2.8). These findings provide further evidence about the contribution of inherited genetic variations to the pathogenesis and clinical course of myeloproliferative neoplasms. (Blood. 2012;119(22):5221-5228)

Introduction

Essential thrombocythemia (ET) and polycythemia vera (PV) are myeloproliferative neoplasms (MPNs) characterized by an indolent clinical course, a tendency to develop thrombohemorrhagic complications, and a risk of transformation into acute myeloid leukemia (AML) sometimes preceded by a phase of myelofibrosis or myelodysplastic syndrome.1 Leukemic transformation of ET and PV is associated with an ominous prognosis and has been reported to occur in 5%-10% of patients 10 years from diagnosis, with its incidence increasing with disease duration.2-3 A recent study by the French Polycythemia Study Group identified AML as the first cause of death in patients in whom PV was diagnosed before the age of 65 and who were therefore followed up for a long period of time.6

Although the pathogenic mechanisms underlying the progression to AML remain largely unknown, there is considerable evidence supporting the idea that some cytoreductive therapies used in ET and PV can contribute to this complication.3,5,7,8 Nevertheless, a significant number of patients who develop AML have either never been exposed to cytoreductive agents or the cumulative doses that they have received are too low to be considered as leukemogenic.7 This observation emphasizes the hypothesis that individual factors not related to the treatment could play a significant role in the leukemic transformation of ET and PV.

Recently, concern has been raised on the increased risk of new nonmyeloid cancers in MPN patients.9,11 In a nationwide registry-based study from Denmark, the standardized incidence ratio of nonhematologic cancer was 1.2 for ET patients and 1.4 for PV patients.9 A study from Italy reported a 3.4-fold increased risk of developing lymphoid malignancies in patients with ET or PV compared with the general population.11 Interestingly, the risk was significantly higher in the JAK2V617F-mutated patients. Although the increased risk of new malignancies may be related to the cytoreductive drugs used for the treatment of ET and PV, the evidence supporting such a relationship is not conclusive and patient-related factors might also be involved.

Hereditary genetic defects in the cellular mechanisms of DNA repair increase the susceptibility to cancer.12 The nucleotide excision repair (NER) and the base excision repair (BER) pathways play a major role in cell protection against genotoxic damage by repairing DNA lesions such as those induced by UV irradiation or chemical carcinogens.13,14 Inherited variations in DNA-repair
efficiency have been implicated in the predisposition to de novo15 and therapy-related AML,16,17 and increased susceptibility to a variety of nonhematological cancers.18,19 Therefore, it is reasonable to hypothesize that common polymorphisms in genes encoding for proteins of the NER and BER pathways could influence on the likelihood of developing cancer in patients with ET and PV. To test this hypothesis, in the present study, we investigated the association between 5 single nucleotide polymorphisms (SNPs) of 4 critical genes involved in NER and BER mechanisms and the risk of either leukemic transformation or development of new nonmyeloid malignancies in patients with ET and PV.

### Methods

#### Study subjects

Case-control studies for the predisposition to either leukemic transformation or new primary nonmyeloid malignancies were nested within a cohort of white patients diagnosed with ET or PV. The study population consisted of 64 patients who progressed to AML and 358 ET/PV patients who did not progress. Patients were accrued from several Spanish hospitals on the basis of the availability of DNA samples for genotyping, and were followed up for a median time of 8.6 years (range, 1-37). Clinical and hematologic features at diagnosis of ET and PV, as well as treatment-related data and the incidence of new nonmyeloid malignancies, were obtained from the clinical institutions and conducted according to the Declaration of Helsinki. The DNA samples of the MPN patients with leukemic transformation were obtained from the chronic phase of the disease (n = 17), the myelofibrotic phase (n = 4), and the leukemic phase (n = 43). Genotyping analysis of the SNPs was performed by real-time PCR using the TaqMan SN Genotyping on Demand Assays, which are commercially supplied by Applied Biosystems (Life Technologies). Assays were performed according to the manufacturer’s instructions. Briefly, each sample reaction was composed of 2.5 μL of TaqMan Genotyping Master Mix, 0.12 μL of TaqMan probe assay 40×, and 2.5 μL of DNA sample at 5 μg/mL. Thermal cycling and detection was performed in a Fast-Real time PCR system 7900HT from Applied Biosystems (Life Technologies). Thermal cycler conditions were: a first stage of 50°C for 2 minutes, a second stage of 95°C for 10 minutes, and a third stage consisting on 45 cycles of 95°C for 15 seconds, 60°C for 1 minute.

Genotyping of intronic JAK2 SNP (C/G; rs12340895) was performed using the TaqMan SNP genotyping assay referenced as C_31941686_10, supplied by Applied Biosystems (Life Technologies). The JAK2 46/1 haplotype was tagged by the G allele at the Jak2 SNP, as described previously.21 The detection of the JAK2V617F mutation was based on quantitative allele-specific PCR technology with the JAK2 Mutant quant kit (IPSOGEN). A sample was considered JAK2V617F positive when the mutated DNA rate was higher than 0.21. JAK2 exon 12 mutation screening was performed by direct sequencing using standard methods.

#### Statistical analysis

The statistical tools for genotype analysis of SNPs (Hardy-Weinberg equilibrium, allele and genotype distributions, and association tests) were provided by SNPSstats.22 This web-based application generates odds ratios (ORs), 95% confidence intervals (CIs), and P values for multiple inheritance models (codominant, dominant, recessive and log-additive). The Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) were calculated to select the best inheritance model for each specific polymorphism, with the preferred model being the one with the lowest AIC/BIC value. Because SNPSstats calculates unconditional ORs, logistic regression conditional on the matched groups of cases and controls was performed when appropriate.

For each case patient who progressed to AML, 2-5 ET/PV control patients were randomly selected from the subgroup that did not progress and matched to the case by type of MPN (ET or PV) and duration of follow-up. The latter was done by constraining the matching criteria so that dates of diagnosis and last follow-up of the controls selected for a given case anteceded ET/PV diagnosis and postdated AML transformation, respectively, of that given case. This matching criterion guaranteed that controls had the same opportunity as cases to progress to AML and also that every case and his/her controls were followed up roughly during the same calendar years.

The association between candidate SNPs and risk of leukemic transformation, adjusted for other potentially predisposing factors, was assessed by logistic regression conditional on the set of matched cases and controls. Clinicohematologic data at diagnosis of ET/PV and treatment-related covariates investigated for their association with progression to AML were selected on the basis of having been identified as predictors of leukemic transformation in previous studies.2,4,23 These included age (categorized by quintiles), sex, leukocytosis (dichotomized at 10 × 10^9/L or 15 × 10^9/L), cytogenetic abnormalities, JAK2 mutational status, and exposure to hydroxyurea (HU) and/or other cytoreductive agents.

Controls (n = 2-5) for each patient diagnosed with a new primary nonmyeloid malignancy were randomly selected on the basis of having a follow-up time longer than the time elapsed from the diagnosis of ET/PV to

---

### Table 1. Characteristics of patients according to the type of MPN

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ET</th>
<th>PV</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>297</td>
<td>125</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>93/204</td>
<td>55/70</td>
</tr>
<tr>
<td>Age, y*</td>
<td>59 (13-93)</td>
<td>62 (19-89)</td>
</tr>
<tr>
<td>Hb, g/dL</td>
<td>139 (82-174)</td>
<td>179 (136-238)</td>
</tr>
<tr>
<td>Platelets, × 10^9/dL</td>
<td>8.7 (3.9-24)</td>
<td>10.7 (5-29)</td>
</tr>
<tr>
<td>Abnormal karyotype</td>
<td>5/196 (3%)</td>
<td>7/77 (6%)</td>
</tr>
<tr>
<td>JAK2 mutation</td>
<td>149/281 (53%)</td>
<td>103/109 (95%)</td>
</tr>
<tr>
<td>New nonmyeloid malignancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin, nonmelanoma</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Lymphoid</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Gastrointestinal tract</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Prostate</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Kidney and urinary tract</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lung</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>22</td>
<td>32</td>
</tr>
<tr>
<td>Follow-up, y*</td>
<td>8.7 (1-24)</td>
<td>8.4 (1-37)</td>
</tr>
<tr>
<td>Died</td>
<td>47 (16%)</td>
<td>43 (34%)</td>
</tr>
</tbody>
</table>

*Median (range).
1 Four patients developed 2 new nonmyeloid malignancies each. The "other" category includes 1 each of lymphoma, thyroid gland, endometrium, and ovarian cancer.

---

**Genotyping**

An analysis of 5 SNPs located in 4 DNA-repair genes involved in the NER (ERCC2) [also known as XPD], ERCC5 [XPG], and XPC] and BER (XRCCI) pathways was performed. The selected SNPs have high heterozygosity and are located on codifying regions involving an amino acid change. The 5 candidate SNPs were: XPD Lys751Gln (rs13181), ERCC5 Asp1104His (rs17655), XPC Ala499Val (rs2228000), XPC Lys939Gln (rs2228001), and XRCCI Arg399Gln (rs25487; Table 2).

The DNA samples of the MPN patients with leukemic transformation were obtained from the chronic phase of the disease (n = 17), the myelofibrotic phase (n = 4), and the leukemic phase (n = 43). Genotyping analysis of the SNPs was performed by real-time PCR using the TaqMan SN Genotyping on Demand Assays, which are commercially supplied by Applied Biosystems (Life Technologies). Assays were performed according to the manufacturer’s instructions. Briefly, each sample reaction was composed of 2.5 μL of TaqMan Genotyping Master Mix, 0.12 μL of TaqMan probe assay 40×, and 2.5 μL of DNA sample at 5 μg/mL. Thermal cycling and detection was performed in a Fast-Real time PCR system 7900HT from Applied Biosystems (Life Technologies). Thermal cycler conditions were: a first stage of 50°C for 2 minutes, a second stage of 95°C for 10 minutes, and a third stage consisting on 45 cycles of 95°C for 15 seconds, 60°C for 1 minute.

Genotyping of intronic JAK2 SNP (C/G; rs12340895) was performed using the TaqMan SNP genotyping assay referenced as C_31941686_10, supplied by Applied Biosystems (Life Technologies). The JAK2 46/1 haplotype was tagged by the G allele at the Jak2 SNP, as described previously.21 The detection of the JAK2V617F mutation was based on quantitative allele-specific PCR technology with the JAK2 Mutant quant kit (IPSOGEN). A sample was considered JAK2V617F positive when the mutated DNA rate was higher than 0.21. JAK2 exon 12 mutation screening was performed by direct sequencing using standard methods.

---

**Statistical analysis**

The statistical tools for genotype analysis of SNPs (Hardy-Weinberg equilibrium, allele and genotype distributions, and association tests) were provided by SNPSstats.22 This web-based application generates odds ratios (ORs), 95% confidence intervals (CIs), and P values for multiple inheritance models (codominant, dominant, recessive and log-additive). The Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) were calculated to select the best inheritance model for each specific polymorphism, with the preferred model being the one with the lowest AIC/BIC value. Because SNPSstats calculates unconditional ORs, logistic regression conditional on the matched groups of cases and controls was performed when appropriate.

For each case patient who progressed to AML, 2-5 ET/PV control patients were randomly selected from the subgroup that did not progress and matched to the case by type of MPN (ET or PV) and duration of follow-up. The latter was done by constraining the matching criteria so that dates of diagnosis and last follow-up of the controls selected for a given case anteceded ET/PV diagnosis and postdated AML transformation, respectively, of that given case. This matching criterion guaranteed that controls had the same opportunity as cases to progress to AML and also that every case and his/her controls were followed up roughly during the same calendar years.

The association between candidate SNPs and risk of leukemic transformation, adjusted for other potentially predisposing factors, was assessed by logistic regression conditional on the set of matched cases and controls. Clinicohematologic data at diagnosis of ET/PV and treatment-related covariates investigated for their association with progression to AML were selected on the basis of having been identified as predictors of leukemic transformation in previous studies.2,4,23 These included age (categorized by quintiles), sex, leukocytosis (dichotomized at 10 × 10^9/L or 15 × 10^9/L), cytogenetic abnormalities, JAK2 mutational status, and exposure to hydroxyurea (HU) and/or other cytoreductive agents.

Controls (n = 2-5) for each patient diagnosed with a new primary nonmyeloid malignancy were randomly selected on the basis of having a follow-up time longer than the time elapsed from the diagnosis of ET/PV to
the new primary cancer in the case patient. Matching was based on dates as
detailed in the preceding paragraphs and ensured that controls had the
opportunity of developing a new primary cancer at the time when the
incidence case occurred. The association between candidate SNPs and risk
of new primary cancer was then adjusted through conditional logistic
regression for age, sex, type of MPN (ET/PV), JAK2 mutational status,
JAK2 SNP genotype (rs12340895), and previous exposure to cytoreductive
therapies.

Potential synergisms among risk factors (eg, SNPs and exposure to
cytoreductive agents) were investigated by including the corresponding
interaction terms in the regression models. To safeguard against associa-
tions occurring by chance because of multiple simultaneous tests, the cutoff
values for the z test were Bonferroni adjusted by dividing 0.05 by the
number of covariates included in each regression model. The descriptive
comparisons of variables between cases and controls was done by univariate
conditional logistic regression. Statistical analyses were performed using
the SPSS Version 15.0 software package and the Stata Version 11.1 software
(www.stata.com).

Results

Analysis of factors associated with the risk of progression to
AML

The case-control study included the 64 patients with ET or PV who
evolved into AML and 271 who did not. The characteristics of the
case and control patients are listed in Table 3. Median follow-up
time to diagnosis of AML in case patients or to death or last
follow-up in control patients was 9.3 years (range, 1.2-29) and
12.3 years (range, 2.5-37), respectively. As can be seen in Table
3, case patients were older than control patients, had higher WBC
counts at presentation, more frequently displayed a
leukocytosis. Neverthe-
less, they accumulated a significantly lower dose of HU than
control patients, mainly because of the shorter treatment duration
on this drug.

Among the presenting clinicohematologic features, only age,
categorized by quintiles, was independently associated with an
increased risk of AML (OR = 1.4; 95% CI, 1.1-1.7; P = .008
adjusted for sex and leukocytosis). Because there were relatively
few patients who received radioactive phosphorus (32P), busulfan,
melphalan, or cytotoxic agents other than HU, which precluded to
accurately ascertain the individual contribution of each agent to the
risk of AML, exposure to cytoreductive therapies was graded as
“no exposure,” “HU only,” and “other agents alone or in combina-
tion” (Table 3). For the purposes of the present study, patients who
received IFN or anagrelide as the only cytoreductive treatment
were included into the “no exposure” group because these drugs are
widely regarded as nonleukemogenic. Taking the “no exposure”
category as the baseline risk, the OR for the association with AML
increased by 3.2 (95% CI, 1.7-5.8; P < .001) through the other
2 categories, reflecting the increasing frequency of AML across the
3 levels of exposure to cytoreductive agents (Table 4). Age lost its
predictive value for leukemic transformation when it was adjusted
for the categories of exposure to cytoreductive agents. Further
investigation of this finding unveiled a strong association between
either covariates, with exposure to cytoreductive agents being more
frequent in the older age groups (data not shown).

All candidate SNPs in DNA-repair genes (XPDLys751Gln,
ERCC5Asp1104His, XPCR4Ala499Val, XPCR5Glu939Gln, andXRCC1
Arg399Gln) were successfully genotyped in more than 95% of the
study samples. Genotypic distribution of the 5 SNPs was found to
be in Hardy-Weinberg equilibrium. The only DNA-repair gene
polymorphism independently associated with leukemic risk was
XPDLys751Gln (Table 3). Although the inheritance model with the
lowest AIC/BIC at the unmatched analysis was the log additive,
and the conditional logistic regression analysis, the best fit was
obtained by the recessive model. Therefore, patients homozygous
for the C minor allele (Gln/Gln genotype) in
had a signifi-
cantly higher risk of AML (OR = 4.4; 95% CI, 1.8-10.9; P = .001
adjusted for the other candidate SNPs) than carriers of the A
wild-type allele (Lys/Lys and Lys/Gln genotypes). Interaction
between the XPDLSNP and other SNPs did not modify the risk of
progression to AML significantly. No definite baseline clinicoha-
ematologic profile was noted in patients carrying the minor allele of
XPDL in homozygosis. Moreover, the median time from the
diagnosis of ET/PV to leukemic transformation did not differ
between the different XPDL genotypes.

JAK2 SNP (rs12340895) was also tested with regard to its
putative influence on the risk of progression to AML and no
association between this SNP and the risk of leukemic transforma-
tion was found.

At the multivariate analysis, both the XPDL genotype and the
category of exposure to cytoreductive agents emerged as independ-
ent predictors of the progression to AML (OR = 4.9; 95% CI,
2.0-12; P = .001, and OR = 3.5; 95% CI, 2.0-6.2; P < .001,
respectively). No significant interaction was observed between the
covariates, and the higher risk driven by the Gln/Gln genotype in
XPDL SNP grew in parallel across the 3 categories of increasing
exposure to cytoreductive agents (Table 4).
Finally, a similar case-control analysis including the 29 patients who progressed to myelofibrosis and 139 control patients who did not was performed. Neither the clinicohematologic features at diagnosis of ET/PV nor the SNP genotypes predicted for the risk of progression to myelofibrosis.

Characteristics of patients who progressed to AML

Median patient age at the time of leukemic transformation was 71 years (range, 31-86). Acute leukemia occurred abruptly in 47 patients (73%), whereas a preceding diagnosis of myelodysplasia or myelofibrosis was made in 5 and 12 instances, respectively. All patients diagnosed with myelodysplasia developed AML soon after (median period, 5 months; range, 2-7). In contrast, the interval between diagnosis of myelofibrosis and leukemic transformation was more variable, ranging from 3-99 months (median, 12).

Information on the karyotype at diagnosis of AML was available in 44 patients. Among them, only 6 (14%) had no cytogenetic abnormalities, whereas 28 (64%) exhibited a complex karyotype (defined as 3 or more unrelated abnormalities). The most common individual cytogenetic aberrations involved total or partial deletions of

Table 4. Progression to AML according to the XPD SNP (Lys751Gln) and category of exposure to cytoreductive agents: observed frequencies and ORs derived from the regression model

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No exposure</th>
<th>HU only</th>
<th>Other agents alone or in combination</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global (n = 335)</td>
<td>1/61 (1.6%)</td>
<td>0/48 (0%)</td>
<td>1/2 (8%)</td>
<td>2/74 (3%)</td>
</tr>
<tr>
<td>XPD AA/AC (n = 292)</td>
<td>2/62 (3.2%)</td>
<td>1/44 (2.3%)</td>
<td>1/2 (8%)</td>
<td>4/78 (5.1%)</td>
</tr>
<tr>
<td>XPD CC (n = 41)</td>
<td>1/2 (5%)</td>
<td>1/2 (5%)</td>
<td>1/2 (8%)</td>
<td>3/16 (18.8%)</td>
</tr>
</tbody>
</table>

OR (95% CI)

- No exposure: 1.0 (0.1-10.0)
- HU: 5.9 (1.4-23.7)
- Other agents: 3.7 (0.9-14.9)

Successful genotyping for the XPD SNP was achieved in 333 of 335 cases. 

chi^2 = 21, P < .001.

chi^2 = 14, P = .001.

chi^2 = 10, P = .005.
Table 5. Risk factors investigated for their association with new nonmyeloid malignancy in ET and PV: distribution in case patients who developed a nonmyeloid cancer and control patients who did not

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n = 50)</th>
<th>Controls (n = 221)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET/PV</td>
<td>30 (60%)/20 (40%)</td>
<td>150 (68%)/71 (32%)</td>
<td>.3</td>
</tr>
<tr>
<td>Median age, y (range)</td>
<td>64 (21-81)</td>
<td>53 (13-84)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>21 (42%)/29 (58%)</td>
<td>75 (34%)/146 (66%)</td>
<td>.3</td>
</tr>
<tr>
<td>JAK2 mutation</td>
<td>34/45 (76%)</td>
<td>107/202 (53%)</td>
<td>.008</td>
</tr>
<tr>
<td>JAK2 genotype (rs12340895)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>21 (44%)</td>
<td>86 (40%)</td>
<td>.3</td>
</tr>
<tr>
<td>CG</td>
<td>15 (31%)</td>
<td>106 (50%)</td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>12 (25%)</td>
<td>22 (10%)</td>
<td></td>
</tr>
<tr>
<td>GG/CC + CG (R)</td>
<td>12/49 (24%)</td>
<td>22/214 (10%)</td>
<td>.01</td>
</tr>
<tr>
<td>Exposure to cytoreductive agents</td>
<td>38 (76%)</td>
<td>164 (74%)</td>
<td>.1</td>
</tr>
<tr>
<td>DNA repair SNPs*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERCC2 (XPD) Lys751Gln: CC/AA + AC (R)</td>
<td>11 (22%)/38 (78%)</td>
<td>20 (9%)/200 (91%)</td>
<td>.007</td>
</tr>
<tr>
<td>ERCC5 Asp1104His: GC + CC/GG (D)</td>
<td>19 (40%)/29 (60%)</td>
<td>103 (50%)/105 (50%)</td>
<td>.2</td>
</tr>
<tr>
<td>XPC Ala499Val: TT/CC + CT (R)</td>
<td>5 (10%)/44 (90%)</td>
<td>17 (8%)/197 (92%)</td>
<td>.6</td>
</tr>
<tr>
<td>XPC Lys699Gln: AC + CC/AA (D)</td>
<td>29 (60%)/19 (40%)</td>
<td>141 (66%)/74 (34%)</td>
<td>.5</td>
</tr>
<tr>
<td>XRCC1 Arg399Gln: GA + AA/GG (D)</td>
<td>24 (49%)/25 (51%)</td>
<td>134 (61%)/85 (39%)</td>
<td>.1</td>
</tr>
</tbody>
</table>

*Results from the best inheritance model for each specific polymorphism. Genetic models: R indicates recessive; and D, dominant.

Discussion

In the present study, a polymorphism in the XPD gene (Lys751Gln) was identified for the first time as an independent risk factor for leukemic transformation in ET and PV. Specifically, homozygous carriers for the minor allele (Gln/Gln) of XPD SNP had a nearly 5-fold higher risk of progression to 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 nonmyeloid malignancy during follow-up, regardless of the type of treatment given to the patients. These findings provide further evidence supporting a pathogenetic role for inherited genetic factors in determining the clinical features of the MPNs.

There is little information on the biologic factors predisposing to AML in ET/PV. Because AML is a relatively rare event that often appears late in the course of ET and PV and can be influenced by therapeutic exposure, studies must include large numbers of patients with long follow-up times to allow for the predisposing factor to become apparent. To overcome this limitation, we conducted a nested case-control study in which case patients with ET or PV who developed AML over a 35-year period were compared with control patients who did not progress to AML despite having been monitored for at least as long as the cases. Such a study design has been proposed as an efficient methodology to ascertain genetic predisposition to second neoplasms and its interaction with therapeutic exposures, and has been applied...
recently to the investigation of clinical and therapy-related factors predicting AML transformation in MPNs.7

Patient age, leukocytosis at diagnosis, and exposure to cytoreductive treatments have been identified previously as risk factors for progression to AML in both ET2,4,8 and PV.3,23,29 Nevertheless, the relative contribution of each of these factors is difficult to ascertain because they are often closely interrelated. For example, most elderly patients had been exposed to cytoreductive agents in our series, so this latter covariate abrogated the predictive value of age. Patients with leukocytosis at presentation are likely to start cytoreductive treatment earlier, and therefore the predictive value of each factor is difficult to separate. Persistent leukocytosis, however, has been identified recently as a risk factor for leukemic transformation in PV patients on HU treatment, probably reflecting a higher myeloproliferative potential.30

In the present study, we found a significant association between the level of exposure to cytoreductive agents and the risk of AML transformation, with the OR increasing by 3-fold from the group of patients never exposed to cytoreductive agents to the group who received HU alone and to those treated with agents other than HU (mainly 32P, busulfan, and melphalan). Previous studies have reported an increased risk of AML in MPN patients treated with 32P,5,7 pipobroman,3,6 and alkylating agents,3,5,7 especially when several cytoreductive drugs are used in combination or sequentially.3,5,7,8,31,32 In contrast, the leukemogenic potential of long-term HU therapy in ET and PV remains controversial. Although there is compelling experimental evidence that HU reduces DNA repair, its mutagenic and carcinogenic potential seems to be low when assessed by in vitro assays.33,34 In the clinical setting, some studies have failed to find an association between exposure to HU alone and AML transformation,2,3,7 whereas in others the risk of AML associated with this drug was higher than expected.5,8 Regarding our present data, patients who progressed to AML accumulated a significantly lower dose of HU than control patients, mainly because they were exposed to the drug for a shorter time. This observation is more consistent with a patient selection bias driven by the indication for starting on HU than with a drug-related, cumulative leukemogenic effect. Alternatively, it could be hypothesized that patients carrying the Gln/Gln XPD genotype in the XPD SNP would be more susceptible to low cumulative doses of HU because of reduced NER function.

Consistent with the above hypothesis, it has been postulated that perhaps only a subset of MPN patients, such as those susceptible to the effects of HU on DNA repair, might be predisposed to leukemic transformation when exposed to this agent.33 We found an increased risk of AML in patients carrying the Gln/Gln XPD genotype after adjustment for other predisposing factors. Although we could not demonstrate a synergistic effect between the Gln/Gln XPD variant and the degree of cytoreductive exposure on the risk of developing AML, the possibility of such interaction cannot be totally ruled out. First, this genetic variant has been associated previously with chemotherapy-induced AML in patients with solid neoplasms treated with alkylating agents.16 Second, our study may have lacked enough statistical power to unveil a subtle yet clinically relevant interaction between the XPD gene polymorphism and exposure to specific chemotherapy agents, mainly alkylators, because of the limited number of patients who received these drugs.

With regard to the increased risk of new primary nonmyeloid cancer in patients with MPNs,9-11 a possible pathogenic role has been attributed to the cytoreductive agents used in the management of these diseases.31,32,35 Therefore, studies conducted in the late 1990s reported an excess risk of carcinoma in PV patients treated with 32P and HU,32 as well as in ET patients who were sequentially exposed to HU and busulfan.8 In addition, long-term use of HU has been associated with the development of nonmelanoma skin cancer in sun-exposed areas,35 which suggests a role for this drug in the defective nucleotide excision repair of DNA damage induced by UV radiation.36 However, no association was observed between cytoreductive exposure and subsequent nonmyeloid cancer in the present study. Although our study may have been underpowered to unfold a weak association, it is worth mentioning that the available evidence supporting a causal role for cytoreductive therapies in the development of nonmyeloid cancer in ET and PV is weak. Indeed, in the abovementioned reports the associations were either scarcely significant31,32 or based on case-reports39 and the more recent, population-based studies did not investigate the potential causative role of cytoreductive treatments.9,11 In contrast, we found in the present study that older age and the Gln/Gln XPD SNP were significant risk factors for the occurrence of new primary nonmyeloid cancer, reinforcing the pathogenic role of this SNP in determining the clinical evolution of ET and PV. We also found that the JAK2 mutation retained a weak association with the risk of nonmyeloid cancer after adjustment for age and the XPD genotype. This finding is consistent with recent data showing an increased rate of lymphoid malignancies among MPN patients with the JAK2V617F mutation,11 as well as a higher incidence of cancer in the general population carrying this mutation.37 Because our study was not population-based, we were unable to ascertain whether the predisposition to nonmyeloid cancer driven by the Gln/Gln variant of XPD gene, and to some extent by the JAK2V617F mutation, is stronger in patients with ET or PV than in the general population.

Both biologic plausibility and epidemiologic data give support to the XPD genotype (Lys751Gln) as a predisposing factor for leukemic transformation and cancer development in patients with MPNs. XPD gene encodes for a DNA helicase involved in at least 3 crucial cellular mechanisms, DNA repair by NER, transcription initiation, and cell-cycle regulation.38 In this process, ATP binding and hydrolysis are critical for the function of the NER helicases because they cause conformational changes that drive the directional movement of the helicase on DNA.39 XPD SNP rs13181 results in a lysine-to-glutamine transition at position 751, which is predicted to induce a major change in the interaction domain between the XPD protein and its helicase activator, the p44 protein.40 Although such change does not seem to affect the transcriptional activity of XPD,41 it is important for nucleotide excision repair.42 It is biologically plausible that individuals carrying the minor allele (Gln) in XPD SNP rs13181 could be more sensitive to DNA damage and therefore more prone to cancer. Indeed, such susceptibility to malignant transformation might be more pronounced in the setting of the MPNs because of their inherent tendency toward leukemic transformation and the frequent use of cytoreductive agents to manage patients with these disorders. A recent meta-analysis of 56 case-control studies concluded that the minor allele of such SNP is associated with cancer susceptibility regardless of environmental factors.15 Moreover, the Gln/Gln XPD genotype has also been associated with an increased risk of chemotherapy-induced AML,16 and a higher frequency of AML with adverse cytogenetic features has been noted among carriers of the Gln allele.43

In summary, the present study shows that an SNP in the XPD gene predisposes individuals with ET and PV to develop AML and new nonmyeloid malignancies. These results, if confirmed in other series, could allow the identification of high-risk patients who
would benefit from close surveillance and individualized therapeutic approaches.

Acknowledgments

The authors thank the following investigators for sending DNA samples and clinical information for their study: Dr. Israel Buño (Hospital Gregorio Marañón, Madrid, Spain), Dr. Joaquín Martínez-López (Hospital 12 de Octubre, Madrid, Spain), Dr. Javier López (Hospital Ramón y Cajal, Madrid, Spain), and Dr. José Román-Gómez (Hospital Reina Sofia, Córdoba, Spain), Dr. Francisco Ferrer-Marín (Hospital Morales Meseguer, Murcia, Spain), Dr. María Teresa Gómez-Casares (Hospital Dr. Negrin, Las Palmas, Spain), and Dr. Josefa Marco (Hospital General, Castellón, Spain).

This work was supported by the grants 09/02324 and 10/00236 from the Fondo de Investigaciones Sanitarias, Spanish Ministry of Health, P.A. is currently supported by a research grant from the Fundación Científica de la Asociación Española contra el Cáncer.

References


29. Burkitt MJ, Raatad A. Nitric oxide generation from


39. Fuss JO, Tainer JA. XPB and XPD helicases in TFIIF orchestrate DNA duplex opening and damage verification to coordinate repair with transcription and cell cycle via CAK kinase. DNA Repair (Amst). 2011;10(7):697-713.


A polymorphism in the *XPD* gene predisposes to leukemic transformation and new nonmyeloid malignancies in essential thrombocythemia and polycythemia vera

Juan-Carlos Hernández-Boluda, Arturo Pereira, Francisco Cervantes, Alberto Alvarez-Larrán, María Collado, Esperanza Such, M. Jesús Arilla, Concepción Boqué, Blanca Xicoy, Margherita Maffioli, Beatriz Bellosillo, Isabel Marugán, Paula Amat, Carles Besses and Vicent Guillem

Updated information and services can be found at:
[http://www.bloodjournal.org/content/119/22/5221.full.html](http://www.bloodjournal.org/content/119/22/5221.full.html)

Articles on similar topics can be found in the following Blood collections
- Myeloid Neoplasia (1642 articles)

Information about reproducing this article in parts or in its entirety may be found online at:
[http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests](http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests)

Information about ordering reprints may be found online at:
[http://www.bloodjournal.org/site/misc/rights.xhtml#reprints](http://www.bloodjournal.org/site/misc/rights.xhtml#reprints)

Information about subscriptions and ASH membership may be found online at:
[http://www.bloodjournal.org/site/subscriptions/index.xhtml](http://www.bloodjournal.org/site/subscriptions/index.xhtml)