DNA repair genes polymorphisms and genetic susceptibility to Philadelphia-negative myeloproliferative neoplasms in a Portuguese population: The role of base excision repair genes polymorphisms

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Abstract. The role of base excision repair (BER) genes in Philadelphia-negative (PN)-myeloproliferative neoplasms (MPNs) susceptibility was evaluated by genotyping eight polymorphisms [apurinic/apyrimidinic endodeoxyribonuclease 1, mutY DNA glycosylase, earlier mutY homolog (*E. coli*) (MUTYH), 8-oxoguanine DNA glycosylase 1, poly (ADP-ribose) polymerase (PARP) 1, PARP4 and X-ray repair cross-complementing 1 (XRCC1)] in a case-control study involving 133 Caucasian Portuguese patients. The results did not reveal a correlation between individual BER polymorphisms and PN-MPNs when considered as a whole. However, stratification for essential thrombocythaemia revealed i) borderline effect/tendency to increased risk when carrying at least one variant allele for XRCC1_399 single-nucleotide polymorphism (SNP); ii) decreased risk for Janus kinase 2-positive patients carrying at least one variant allele for XRCC1_399 SNP; and iii) decreased risk in females carrying at least one variant allele for MUTYH SNP. Combination of alleles demonstrated an increased risk to PN-MPNs for one specific haplogroup. These findings may provide evidence for gene variants in susceptibility to MPNs. Indeed, common variants in DNA repair genes may hamper the capacity to repair DNA, thus increasing cancer susceptibility.

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Introduction

Among myeloproliferative neoplasms (MPNs), and besides chronic myelogenous leukemia, the World Health Organization (WHO) classification includes Philadelphia-negative (PN)-MPNs, namely, polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF) (1,2).

Major genetic insights into the pathogenesis of PN-MPNs include the identification of somatic point gain-of-function mutations in the Janus kinase 2 (*JAK2*) gene (V617F mutation in exon 14 first in 2005, then in exon 12) (3-7) and the myeloproliferative leukemia virus oncogene (most frequently W515), in addition to recently identified calreticulin mutations (8-11), with implications in the understanding of these diseases, their diagnosis and management. The corresponding frequencies of these mutations are ~95, 0 and 0% in PV; 60, 3 and 20% in ET; and 60, 7 and 25% in PMF, respectively (12,13).

Those mutations, however, could not fully explain the phenotypic heterogeneity of PN-MPNs. Furthermore, genetic defects still await identification in ~40% of ET and PMF cases (12-14). Single-nucleotide polymorphisms (SNPs) at various loci and additional somatic genetic effects may be important for PN-MPNs phenotype definition and for prognosis evaluation, although less specific than known variations (12-16). Not all mutations involved in cancer initiation may lead to cancer. This can occur due to different variants within the same gene or between variants in different genes. The latter must be considered, as the number of passenger mutations in a tumour may modulate the effect of driver mutations, thus acting as putative modifier genes (17). Additionally, epimutations that can silence tumour-suppressor genes must be taken into account (17), which highlights the concept that, probably more important than the genes, are their levels of expression.

MPNs have high morbidity with thrombohaemorrhagic complications and risk of progression to acute myeloid

leukemia (AML), in occasions preceded by a phase of myelo-fibrosis or myelodysplastic syndromes (MDS) (18). Life expectancy in patients with PV or ET is reduced compared with that in the general population (19,20). The 'true' rate of transformation is not accurate due to selection bias in clinical trials and underreporting in population-based studies (21). The occurrence of leukemia in ET and PV is associated with a bad prognosis, and has been reported to occur in 5-10% of patients 10 years following the initial diagnosis (1,22). It is well known that high doses of alkylating agents and combined cytoreductive treatments undoubtedly increase the risk of malignant transformation (23). However, there is also an intrinsic propensity in MPNs to progress to AML/MDS, in an extent that is not fully known (23,24). It cannot be ruled out that mutational burden, polymorphic variants of several genes, ambient/dietary exposure and immune system characteristics could be predisposing factors to susceptibility to these disorders (14,25-28).

DNA damage to haematopoietic precursor cells would appear to be essential for the development of leukaemia, notwithstanding DNA repair systems act to repair the DNA damage, thus maintaining genetic integrity (29,30).

Several polymorphisms in DNA repair genes have been identified that may affect protein function and thus DNA damage repair, leading to susceptibility to malignancy, in spite of their low genetic penetrance (25,29-32). Previous reports have identified base excision repair (BER) genes polymorphisms associated with breast and thyroid cancer risk (33-35) among other malignancies, and a nucleotide excision repair gene polymorphism displayed strong association with leukaemic transformation and development of non-myeloid malignancies in patients with ET and PV (24).

The BER pathway typically repairs a small region (1-13 nucleotides) around the damaged base, involving apurinic/apyrimidinic endodeoxyribonuclease 1 (APEX1), 8-oxoguanine DNA glycosylase 1 (OGG1), poly (ADP-ribose) polymerase (PARP) 1 or X-ray repair cross-complementing 1 (XRCC1) (36).

Several SNPs in genes of the BER pathway [*APEX1*, mutY DNA glycosylase, earlier mutY homolog (*E. coli*) (*MUTYH*), *OGG1*, *PARP1*, *PARP4* and *XRCC1*] have been identified and studied for their association with the risk of leukaemia and disease outcome (25,30,37).

A wider characterization of molecular genetic features in PN-MPNs may contribute to a better understanding of the pathogenesis of these diseases and provide new specific diagnostic, prognostic and therapeutic tools (14).

Since data on the role of BER gene variants in PN-MPNs are absent in the literature, the present study describes a hospital-based, case-control study in a Caucasian Portuguese population in order to help assessing a possible role of BER genes on the individual susceptibility to PN-MPNs.

Materials and methods

Study subjects. The present case-control study involved 133 Caucasian Portuguese PN-MPNs patients (80 with ET, 39 with PV and 14 with PMF) in addition to 281 age- and gender-matched controls selected within the Portuguese population, who were recruited between January 2009

and July 2016, followed and treated at the Departments of Clinical Haematology and Clinical Pathology, Hospital of São Francisco Xavier, West Lisbon Hospital Centre (Lisbon, Portugal), a public general hospital that provides healthcare to the western population of Lisbon. Diagnosis criteria for all patients were those updated by the WHO (38). For all cases, at least two control individuals (n=281) without neoplastic pathology, and matched for age (±2 years), gender and ethnicity were recruited, who had no personal or family history of PN-MPNs, no previous or current malignant disease and no history of blood transfusions. All patients and controls in the study were Portuguese, with Portuguese ascendants. For each patient and control, information was recorded concerning demographic characteristics, family history of cancer, lifestyle habits (e.g. tobacco smoking and alcohol consumption) and exposure to ionizing radiation. Concerning tobacco smoking habits, former smokers were considered as non-smokers if they had stopped smoking either 2 years prior to PN-MPN diagnosis in the case of patients or 2 years prior to the inclusion date in the study in the case of controls. The recorded information was coded to assure anonymity of the participants, and written informed consent was obtained from all those involved, prior to blood withdrawal, in agreement with the Declaration of Helsinki. The present study was also conducted with approval by the institutional ethics' boards of the involved institutions (NOVA Medical School and Hospital de São Francisco Xavier, Centro Hospitalar de Lisboa Ocidental). General characteristics for PN-MPNs patients and control populations are summarized in Tables I and II.

DNA extraction. DNA was obtained from cells of peripheral blood samples through a commercially available kit (QIAamp® DNA Mini kit; Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. All DNA samples collected were stored at -20˚C until analysis.

SNP selection. The appropriate SNPs analyzed in the present study were selected concerning their relevance in the DNA repair pathway (33,34). All SNPs had a minor allele frequency of >0.1 in Caucasian populations (Table III).

Genotyping. The polymorphisms rs1130409 (*APEX1*), rs3219489 (*MUTYH*), rs1052133 (*OGG1*), rs1136410 (*PARP1*), rs13428 and rs1050112 (*PARP4*), and rs1799782 and rs25487 (*XRCC1*) were genotyped using real-time PCR reaction in a 7300 Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with TaqMan® SNP Genotyping Assays (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol and to previous reports from our group and using primers from the Taqman kit (33,39-42), with a minor modification where the final volume of the reaction was adjusted to 10 μl. RT-PCR genotype determinations were conducted in 20% of samples in independent experiments (60 randomly selected individuals for the control group and 30 for the case group), and all the inconclusive samples were reanalyzed. Table III summarizes the information on the SNP genotyping assay.

JAK2 V617F mutational status was determined using RT-PCR in a 7300 Real-Time PCR system with TaqMan®

Table I. General characteristics of the Philadelphia-negative myeloproliferative neoplasm cases (n=133) and control population (n=281).

Characteristics	Cases, n (%)	Controls, n (%)	P-value[c]
Gender			0.780
Male	61 (45.9)	133 (47.3)	
Female	72 (54.1)	148 (52.7)	
Age, years[a,b]			0.622
30-49	16 (12.0)	43 (15.3)	
50-69	50 (37.6)	107 (38.1)	
≥70	67 (50.4)	131 (46.6)	
Tobacco smoking habits			0.633
Never	104 (78.2)	213 (76.1)	
Current	29 (21.8)	67 (23.9)	
Alcohol consumption habits			<0.0001
Never	103 (77.4)	191 (68.2)	
Social	20 (15.0)	25 (8.9)	
Regular	10 (7.5)	64 (22.9)	
JAK2 V617F mutation			0.020
Yes	99 (75.0)		
ET	58 (73.4)		
PV	34 (87.2)		
PMF	7 (50.0)		
No	33 (25.0)		

[a]Age at diagnosis for cases. [b]Age of control population at the time of diagnosis for the matched case. [c]P-value determined by χ^2 test. ET, essential thrombocythaemia; PV, polycythaemia vera; PMF, primary myelofibrosis.

Table II. Gender distribution for the Philadelphia-negative myeloproliferative neoplasm cases (n=133).

Diagnosis	Patients, n	Male, n (%)	Female, n (%)
ET	80	32 (40.0)	48 (60.0)
PV	39	20 (51.3)	19 (48.7)
PMF	14	9 (64.3)	5 (35.7)

ET, essential thrombocythaemia; PV, polycythaemia vera; PMF, primary myelofibrosis.

SNP Genotyping Assays according to the manufacturer's protocol.

Statistical analysis. The analysis of Hardy-Weinberg frequencies for all alleles in the enrolled populations was carried out using exact probability tests available in SNPstat website software (http://bioinfo.iconcologia.net/SNPstats) (43). Differences in genotype frequency, tobacco smoking/alcohol consumption status, age class and gender distributions between PN-MPNs cancer patients and controls were evaluated by the χ^2 test. The crude and adjusted odds ratios (ORs) corresponding to 95% confidence intervals (CIs) were calculated by unconditional multiple logistic regression and statistical analysis performed with SPSS version 22.0 (IBM SPSS, Armonk, NY, USA). The adjusted OR was determined correcting the results for gender, age at diagnosis, and tobacco smoking and alcohol consumption habits.

Since the present study is not a conclusive final study, but an exploratory one, on the role of BER polymorphisms in PN-MPNs, and the data obtained should be considered as proof of concept on that possible role, the Bonferroni adjustment was deemed as not necessary, as it is too conservative.

Results

Characterization of populations. The present study included 133 PN-MPNs patients and 281 age- and gender-matched controls. According to the diagnosis criteria, the patients' distribution was as follows: 80 patients (60.2%) with ET, 39 (29.3%) with PV and 14 (10.5%) with PMF (Table II). The baseline characteristics (gender, age, alcohol consumption and tobacco smoking habits) of the case and control populations are listed in Tables I and II.

The case group included 72 (54.1%) females and 61 (45.9%) males, with an overall mean age of 68 years, in agreement with the gender distribution usually observed in this type of pathology (1,44). No significant differences were observed between the control and patient groups concerning age distribution or tobacco smoking habits (Table I). However,

Table III. Selected single-nucleotide polymorphisms and detailed information on the corresponding base and amino acid exchanges as well as MAF.

Gene	Codon	Exchange, base (amino acid)	MAF (%)[a]
APEX1	148	T→G (Asp/Glu)	44.0
MUTYH	335	G→C (Gln/His)	31.9
OGG1	326	C→G (Ser/Cys)	29.9
PARP1	762	T→C (Val/Ala)	24.4
PARP4	1,280	G→C (Gly/Arg)	45.8
	1,328	C→A (Pro/Thr)	45.8
XRCC1	194	C→T (Arg/Trp)	13.1
	399	G→A (Arg/Gln)	26.6

[a]According to http://www.ncbi.nlm.nih.gov/snp/. MAF, minor allele frequency; APEX1, apurinic/apyrimidinic endonuclease 1; MUTYH, mutY DNA glycosylase, earlier mutY homolog (*E. coli*); OGG1, 8-oxoguanine DNA glycosylase 1; PARP, poly (ADP-ribose) polymerase; XRCC1, X-ray repair cross-complementing 1.

alcohol consumption was significantly increased in patients compared with that in controls (P<0.0001) (Table I).

SNPs genotyping. Of the eight SNPs included in the study, only seven were completely genotyped (Table III), since both SNPs of the *PARP4* gene were in linkage disequilibrium (r^2>0.80), thus behaving as tag SNPs.

The genotype frequencies determined for all polymorphisms under study are shown in Table IV. When considered individually, no correlation between polymorphisms of the BER pathway genes and individual susceptibility to PN-MPNs as a whole could be identified. All the SNPs studied were in agreement with the Hardy-Weinberg law (P>0.05, exact probability test), with the exception of *PARP1* Val762Ala (P=0.029, exact probability test).

The genotypic frequencies obtained in the control populations are similar to those reported previously in other Caucasian populations (33,34,40,45).

As shown in Table IV, no significant differences in genotypic frequencies were observed for any of the seven polymorphisms between cases and controls as a whole (P>0.05, χ^2 test). However, when the population was stratified for pathology status, gender and presence of *JAK2* mutation, a tendency for decrease risk was noticed for *MUTYH* Gln335His and *XRCC1* Gln399Arg polymorphisms (Table V).

Upon stratification for pathology status, the results revealed that, for ET, the presence of at least one variant allele for the *XRCC1* Gln399Arg gene polymorphism displayed a borderline effect on the population (crude OR, 0.623; 95% CI, 0.378-1.028; P=0.069) (Table V).

The results demonstrated a decreased risk in the female group with ET diagnosis and with at least one variant allele for *MUTYH* Gln335His gene polymorphism (OR, 0.478; 95% CI, 0.238-0.962) upon adjustment for potential risk factors (Table V).

The relevance of JAK2 mutation in PN-MPNs is well known (3-5,7). Therefore, the population was also stratified according to the presence of *JAK2* mutation in patients, showing that the presence of at least one variant allele for *XRCC1* Gln399Arg gene polymorphism constitutes a decreased risk for ET patients (OR, 0.500; 95% CI, 0.278-0.896) (Table V).

Overall, the results indicate that only XRCC1 Gln399Arg and MUTYH Gln335His gene polymorphisms appeared to be associated with PN-MPNs risk. For the remaining polymorphisms under study, no significant changes in crude or adjusted OR were observed (Tables IV and V).

Haplogroup association. A key point that should be explored in studies such as the present one is the effect of the combination of all genotypes, since the real situation is the effect of the variants altogether. The results provided by the SNPstat software yielded 60 different combinations, the most frequent of which are shown in Table VI. Analyzing the results as haplogroup association response, an increased risk to develop a MPN was obtained for one specific combination (OR, 3.91; 95% CI, 1.02-14.95) (Table VII).

Discussion

DNA repair deficiencies and genetic or epigenetic changes may decrease the efficiency of DNA repair, thus contributing to individual susceptibility to DNA damaging agents and to cancer risk (46-48). However, to the best of our knowledge, no clinical association studies have been performed to evaluate the role of BER pathway polymorphisms on PN-MPNs susceptibility.

The present study revealed a higher incidence of *JAK2* V617F mutation in ET patients and a lower incidence in PV patients, compared with that reported in the literature (12,13). This finding is probably due to the small population studied and to the fact that ET was the most frequent diagnosis among the patients included in the current case group.

The present study was intended to ascertain the possible role of the genetic polymorphisms *APEX1* Asp148Glu, *MUTYH* Gln335His, *OGG1* Ser326Cys, *PARP1* Val762Ala, *PARP4* Gly1280Arg, and *XRCC*1 Arg194Trp and Arg399Gln on the individual susceptibility for PN-MPNs. The genotypic frequencies of the different SNPs in the control population are similar to those reported in other Caucasian populations (33,34,40).

The MUTYH protein acts as a BER glycosylase and is mainly involved in the repair of oxidative DNA lesions (34,40,46,49-56). MUTYH dysfunction may therefore be of special relevance in human tumourigenesis, since it is the only mechanism for repairing 8-oxo-dG/adenine mismatches (49). Indeed, two specific germline mutations in this gene, Tyr165Cys and Gly382Asp, have been associated with a colorectal adenoma and carcinoma predisposition syndrome that is now referred to as MUTYH-associated polyposis (49). However, various others mutations and SNPs have been described to date, for which a specific phenotypic consequence is unknown (49). An example of one common SNP in the *MUTYH* gene is the nonsynonymous Gln335His variation in codon 12 (49,50). The enzyme encoded by this variant has been demonstrated to have partially impaired glycosylase activity *in vitro*, and could therefore contribute to cancer susceptibility, being much more

Table IV. Genotype distribution and myeloproliferative risk for the APEX1 Asp148Glu, MUTYH Gln335His, OGG1 Ser326Cys, PARP1 Val762Ala, PARP4 Gly1280Arg, and XRCC1 Gln399Arg and Arg194Trp polymorphisms in the myeloproliferative neoplasms case (n=133) and control (n=281) populations.

Genetic polymorphism	Controls, n (%)	Cases, n (%)	P-value[a]	OR crude (95% CI)	OR adjusted (95% CI)[b]
APEX1 (Asp148Glu)			0.952		
Asp/Asp	73 (26.4)	37 (27.8)		1.000 (Reference)	1.000 (Reference)
Asp/Glu	136 (49.1)	64 (48.1)		0.928 (0.566-1.523)	0.963 (0.580-1.599)
Glu/Glu	68 (24.5)	32 (24.1)		0.928 (0.521-1.653)	0.923 (0.512-1.663)
Asp/Glu+Glu/Glu	204 (73.6)	96 (72.2)		0.928 (0.584-1.477)	0.949 (0.591-1.526)
MUTYH (Gln335His)			0.877		
His/His	142 (51.3)	68 (51.1)		1.000 (Reference)	1.000 (Reference)
His/Gln	112 (40.4)	52 (39.1)		0.970 (0.626-1.502)	0.902 (0.550-1.413)
Gln/Gln	23 (8.3)	13 (9.8)		1.180 (0.654-2.471)	1.075 (0.506-2.283)
His/Gln+Gln/Gln	135 (48.7)	65 (48.9)		1.005 (0.665-1.521)	0.932 (0.609-1.425)
OGG1 (Ser326Cys)			0.545		
Ser/Ser	182 (65.7)	83 (62.6)		1.000 (Reference)	1.000 (Reference)
Ser/Cys	83 (30.0)	41 (30.8)		1.083 (0.687-1.708)	1.075 (0.672-1.720)
Cys/Cys	12 (4.3)	9 (6.8)		1.645 (0.667-4.055)	1.603 (0.631-4.072)
Ser/Cys+Cys/Cys	95 (34.3)	50 (37.6)		1.154 (0.751-1.774)	1.144 (0.735-1.781)
PARP1 (Val762Ala)			0.769		
Val/Val	214 (77.0)	104 (78.2)		1.000 (Reference)	1.000 (Reference)
Val/Ala	63 (22.7)	29 (21.8)		0.947 (0.575-1.559)	1.019 (0.607-1.712)
Ala/Ala	1 (0.4)	0 (0.0)		ND	ND
Val/Ala+Ala/Ala	64 (23.0)	29 (21.8)		0.932 (0.567-1.533)	1.000 (0.596-1.677)
PARP4 (Gly1280Arg)			0.593		
Gly/Gly	105 (37.9)	54 (40.6)		1.000 (Reference)	1.000 (Reference)
Gly/Arg	141 (50.9)	61 (45.9)		0.841 (0.539-1.313)	0.806 (0.510-1.273)
Arg/Arg	31 (11.2)	18 (13.5)		1.129 (0.579-2.200)	1.000 (0.501-1.997)
Gly/Arg+Arg/Arg	172 (62.9)	79 (59.4)		0.893 (0.585-1.363)	0.841 (0.543-1.301)
XRCC1 (Arg194Trp)			0.263		
Arg/Arg	236 (85.5)	121 (91.0)		1.000 (Reference)	1.000 (Reference)
Arg/Trp	39 (14.1)	12 (9.0)		0.600 (0.303-1.188)	0.650 (0.323-1.307)
Trp/Trp	1 (0.4)	0 (0.0)		ND	ND
Arg/Trp+Trp/Trp	40 (14.5)	12 (9.0)		0.585 (0.296-1.156)	0.633 (0.315-1.270)
XRCC1 (Gln399Arg)			0.318		
Arg/Arg	113 (40.8)	61 (45.9)		1.000 (Reference)	1.000 (Reference)
Arg/Gln	134 (48.4)	54 (40.6)		0.747 (0.479-1.163)	0.762 (0.483-1.204)
Gln/Gln	30 (10.8)	18 (13.5)		1.111 (0.573-2.155)	1.044 (0.531-2.052)
Arg/Gln+Gln/Gln	164 (59.2)	72 (54.1)		0.813 (0.536-1.234)	0.818 (0.532-1.255)

[a]P-value determined by χ^2 test. [b]ORs were adjusted for age (30-49, 50-69 and >70 years), tobacco smoking status (never or former and current) and alcohol consumption (never, social and regular drinkers). APEX1, apurinic/apyrimidinic endonuclease 1; MUTYH, mutY DNA glycosylase, earlier mutY homolog (*E. coli*); OGG1, 8-oxoguanine DNA glycosylase 1; PARP, poly (ADP-ribose) polymerase; XRCC1, X-ray repair cross-complementing 1; OR, odds ratio; CI, confidence interval; ND, none determined.

frequently detected in Japanese and Chinese populations than in European populations (34,52). The Gln335His variant allele has been suggested to be associated with increased risk of colorectal cancer (50,52,57) and less consistently with lung cancer (53). For the latter, however, existing evidence is conflicting or significant only when taking into account gene-gene interactions (53). Notably, an almost significant decrease in breast cancer risk (OR, 0.80; 95% CI, 0.59-1.07) was described previously by our

group in *MUTYH* Gln335His heterozygotes (40). Furthermore, gene-gene interactions among BER polymorphisms were observed in ever tobacco smokers in a bladder cancer susceptibility study (54). Those previous results suggest that genetic variation in BER genes may contribute to cancer risk through gene-gene and gene-environmental interactions.

To the best of our knowledge, there are no clinical association studies in which the role of the *MUTYH* Gln335His

Table V. ORs (95% CI) for MUTYH (Gln335His) and XRCC1_399 (Gln399Arg) polymorphisms and Philadelphia-negative myeloproliferative neoplasms association.

Pathology stratification	Patients, n	SNP	P-value[a]	OR crude (95% CI)	OR adjusted (95% CI)[b]
ET	80	*XRCC1_399* (Gln399Arg; rs25487)	0.166		
		Arg/Arg		1.000 (Reference)	1.000 (Reference)
		Arg/Gln		0.602 (0.354-1.025)[i]	0.611 (0.355-1.053)[i]
		Gln/Gln		0.717 (0.305-1.690)	0.667 (0.279-1.595)
		Arg/Gln+Gln/Gln		0.623 (0.378-1.028)[i]	0.622 (0.373-1.038)[i]
ET, females	48	*MUTYH* (Gln335His; rs3219489)	0.015		
		His/His		1.000 (Reference)	1.000 (Reference)
		His/Gln		0.342 (0.152-0.773)[c]	0.325 (0.142-0.744)[d]
		Gln/Gln		1.331 (0.480-3.692)	1.229 (0.435-3.475)
		His/Gln+Gln/Gln		0.507 (0.256-1.003)[i]	0.478 (0.238-0.962)[j]
ET, JAK2	58	*XRCC1_399* (Gln399Arg; rs25487)	0.044		
		Arg/Arg		1.000 (Reference)	1.000 (Reference)
		Arg/Gln		0.471 (0.255-0.871)[e]	0.490 (0.263-0.915)[f]
		Gln/Gln		0.554 (0.199-1.538)	0.539 (0.192-1.514)
		Arg/Gln+Gln/Gln		0.486 (0.274-0.864)[g]	0.500 (0.278-0.896)[h]

[a]P-value determined by χ^2 test. [b]ORs were adjusted for age (30-49, 50-69 and >70 years), tobacco smoking status (never or former and current) and alcohol consumption (never, social and regular drinkers). [c]P_{crude}=0.010; [d]$P_{adjusted}$=0.008 (P-values are adjusted by unconditional multiplicative logistic regression). [e]P_{crude}=0.016; [f]$P_{adjusted}$=0.025 (P-values are adjusted by unconditional multiplicative logistic regression). [g]P_{crude}=0.014; [h]$P_{adjusted}$=0.020 (P-values are adjusted by unconditional multiplicative logistic regression). [i]Represents results that almost reached the significance threshold, demonstrating a borderline effect. [j]$P_{adjusted}$=0.039 (P-values are adjusted by unconditional multiplicative logistic regression). SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval; MUTYH, mutY DNA glycosylase, earlier mutY homolog (*E. coli*); XRCC1, X-ray repair cross-complementing 1; ET, essential thrombocythaemia; JAK2, Janus kinase 2.

Table VI. Haplogroup frequencies for the single-nucleotide polymorphisms under study.

			Haplogroup estimation					Haplogroup frequencies	
APEX D148E	*MUTYH* Q335H	*OGG1* S326C	*PARP1* V762A	*PARP4* G1,280R	*XRCC1*_194 R194W	*XRCC1*_399 R399Q	Global frequency	Controls	Cases
D	H	S	V	G	R	R	0.116	0.167	0.068
E	H	S	V	G	R	R	0.084	0.060	0.130
E	H	S	V	G	R	Q	0.069	0.065	0.062
E	H	S	V	R	R	R	0.050	0.058	0.044
D	H	S	V	R	R	Q	0.045	0.036	0.060
D	H	S	V	R	R	R	0.045	0.030	0.060
E	H	C	V	G	R	R	0.033	0.025	0.042
E	Q	S	V	R	R	R	0.032	0.028	0.053

APEX1, apurinic/apyrimidinic endonuclease 1; MUTYH, mutY DNA glycosylase, earlier mutY homolog (*E. coli*); OGG1, 8-oxoguanine DNA glycosylase 1; PARP, poly (ADP-ribose) polymerase; XRCC1, X-ray repair cross-complementing 1.

polymorphism had been evaluated on PN-MPNs susceptibility. The results reported in the present study suggest that, when considering females with ET, a consistent decrease in overall PN-MPNs risk was observed when at least one variant allele carrying *MUTYH* Gln335His is present (Table V).

The XRCC1 nuclear protein serves an important role in assisting and enabling the repair of single-strand breaks by interacting and recruiting to the DNA lesion sites multiple enzymatic components of repair reactions (58). Arg194Trp and Arg399Gln polymorphisms are among the most extensively

Table VII. Haplogroup association response for the single-nucleotide polymorphisms under study.

APEX D148E	MUTYH Q335H	OGG1 S326C	PARP1 V762A	PARP4 G1280R	XRCC1_194 R194W	XRCC1_399 R399Q	OR (95% CI)	P-value
D	H	S	V	G	R	R	1.00 (Reference)	
E	H	S	V	G	R	R	3.17 (0.99-10.17)	0.05
E	H	S	V	G	R	Q	0.92 (0.34-2.53)	0.88
E	H	S	V	R	R	R	1.55 (0.50-4.76)	0.45
D	H	S	V	R	R	Q	2.13 (0.60-7.53)	0.24
D	H	S	V	R	R	R	1.90 (0.41-8.79)	0.41
E	H	C	V	G	R	R	3.41 (0.87-13.36)	0.08
E	Q	S	V	R	R	R	3.91 (1.02-14.95)	0.05[a]

APEX1, apurinic/apyrimidinic endonuclease 1; MUTYH, mutY DNA glycosylase, earlier mutY homolog (*E. coli*); OGG1, 8-oxoguanine DNA glycosylase 1; PARP, poly (ADP-ribose) polymerase; XRCC1, X-ray repair cross-complementing 1; OR, odds ratio; CI, confidence interval. [a]$P<0.047$.

studied SNPs in the *XRCC1* gene (25,29,30,33-35,37,46,51, 53-55,59-63). These two SNPs have been shown to alter the functional activity of the resulting protein *in vitro* and to interfere with cancer susceptibility. The Arg194Trp variant allele has been associated with decreased risk of certain cancers, particularly among tobacco smokers (59). Conversely, the Arg399Gln variant allele has been suggested to be associated with decreased DNA repair capacity and higher sensitivity to genotoxicants compared with the Arg194Trp variant allele (64). However, previous epidemiological results have been inconsistent and dependent on the cancer type (51,65,66). Several interactions such as gene-environment (e.g. alcohol consumption or menopausal age) and gene-gene (e.g. other DNA repair or chemical metabolizing enzymes) have also been reported for both SNPs (46). However, several well-powered studies and meta-analyses have not confirmed these supposed effects (46,60). Generally, the results reported to date suggest a modest impact of both *XRCC1* gene polymorphisms on protein activity and cancer susceptibility. However, certain studies have suggested that they may represent a risk factor for haematological malignancies such as leukaemia, according to previous studies on *XRCC1* polymorphisms in association with AML, acute lymphoblastic leukaemia, chronic lymphocytic leukaemia and lymphoma (25,29,30). The present results support a borderline effect for ET patients and a protective effect in overall PN-MPNs risk when considering ET patients presenting JAK2 mutation, as observed under the presence of at least one variant allele carrying *XRCC1* Arg399Gln (Table V). Although the Arg194Trp allele did not show any association with the risk of developing PN-MPNs, it should be noted that the Arg194Trp variant allele may be associated with higher DNA repair capacity, lower sensitivity to gentoxicants and decreased risk of other cancers, when compared with other variants. In addition, other factors may influence the biological effect of the Arg194Trp polymorphism, such as disease development stage, specific environmental factors and even different genetic background among populations.

In order to clarify the role of *XRCC1* polymorphisms in PN-MPNs susceptibility, larger studies and/or a meta-analysis are required.

Regarding the *OGG1* Ser326Cys polymorphism, the current results did not reveal a significant contribution on individual susceptibility towards PN-MPNs (Tables III and IV). The *OGG1* Ser326Cys polymorphism has been demonstrated to impair protein function (46), and therefore, it has been widely evaluated in different case-control studies (67). Significant results were observed in lung (59,68), head and neck (69,70), colorectal (51,71) and gallbladder cancer (65,72). However, several previous meta-analyses (68,70,73-76) did not reveal any significant association between this *OGG1* polymorphism and other cancers risk.

Human APEX1 is a multifunctional enzyme that holds complementary key roles in cancer. Notably, this enzyme is a crucial component of the BER pathway due to its ability to process AP sites and other 3'DNA termini that may result, for example, from exposure to ionizing radiation or direct attack by free radicals (59,77,78). Among the 18 identified SNPs for the *APEX1* gene, the most studied one is the T>G transition at codon 148 of exon 5, which leads to a change in amino acid from Asp to Glu (56). Its potential role on cancer was evaluated in four meta-analyses on breast cancer susceptibility (55,79-81), two meta-analyses on prostate cancer (82,83) and several studies on other types of cancer (84-87). However, the results published remain inconclusive. Concerning the present results, the association of *APEX1* Asp148Glu with PN-MPNs risk did not exhibit any association.

PARP1 is an abundant nuclear protein that can bind to DNA and promote the poly (ADP-ribosylation) of a variety of proteins. PARP1 acts on single- and double-stranded DNA breaks by recruiting DNA repair factors (88). It has a major signaling role in DNA damage detection and repair, acting as a molecular nick sensor to initiate the recruitment of XRCC1 and the assembly of the single-strand break repair machinery (89). *PARP1* Val762Ala is one of the most common nonsynonymous SNPs studied in this gene, resulting in an amino acid

substitution within the COOH-terminal catalytic domain of the enzyme (90,91). This variant has been associated with reduced enzymatic activity (61) and limited capacity for interaction with *XRCC1* (62). This may result in decreased BER capacity, thus increasing cancer predisposition in *PARP1* Val762Ala carriers. Indeed, this variant form has been associated in various well-powered clinical association studies with increased cancer susceptibility, namely to lung (61) and gastrointestinal tract (62,92) cancer, while Adel Fahmideh *et al* (74) described a decreased risk for glioma associated with this SNP. Studies regarding other types of cancer such as breast cancer (55,63) failed to demonstrate an association between *PARP1* Val762Ala and cancer susceptibility. The present results do not suggest any association between *PARP1* Val762Ala polymorphism and PN-MPNs risk (Table IV).

Concerning the analysis of haplogroups' association response, an increased risk to PN-MPNs could be observed for one specific combination (Table VII). This result should, however, be taken with care when evaluating this parameter risk for PN-MPNs due to its low frequency (3.2%) in the studied population, even though it may represent a tendency on how SNPs in BER genes influence PN-MPNs.

Overall, the present results reveal that the *XRCC1* Gln399Arg and *MUTYH* Gln335His gene polymorphisms appear to be associated with PN-MPNs risk. For all other polymorphisms under study, no significant change was observed (Tables IV and V).

Although certain published studies consider tobacco smoking as a contributing factor for PN-MPNs (93,94), the present study did not reveal an important association, probably due to the small number of tobacco smokers included.

Additional studies involving larger populations should be conducted to further clarify the potential value of the different BER genotypes as predictive biomarkers of susceptibility to PN-MPNs and to study gene-environment and gene-gene interactions. In addition, stratified analysis according to histological subtype and disease stage should be conducted.

Acknowledgements

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