APOPTOSIS DEREGULATION EXPRESSION IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS IN A PORTUGUESE POPULATION

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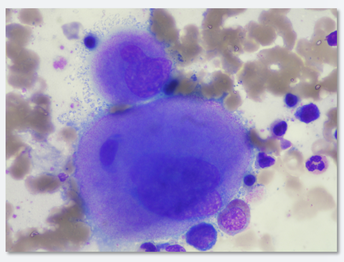

1. Background

Although somatic mutations in the Janus kinase 2 gene (*JAK2*) occur in many Philadelphia-chromosome negative chronic myeloproliferative neoplasms (PN-MPNs), disease evolution, distinct phenotypes and the continuous clinical evidence of an increasing number of cases, with younger patients affected, have been pointing to a growing involvement of environmental factors in the pathogenesis of these diseases.

Several single nucleotide polymorphisms (SNPs), influencing DNA repair capacity and apoptotic status, confer genetic predisposition to disease and determine therapeutic response. Moreover, despite the development of more efficient drugs in the last years, some patients with PN-MPNs still evolve to myelodisplasia, myelofibrosis and acute leukaemia, conditions more difficult to treat, with an incidence of 10% following certain types of chemotherapy and 2-3% without treatment with cytotoxic agents.

Genetic polymorphisms encoding apoptotic proteins are candidates for association with PN-MPNs, since apoptosis is a high regulated process in carcinogenesis.

2. Objectives

We intend to evaluate the role of apoptosis SNPs in PN-MPNs susceptibility.

3. Methods

We performed a case-control study in 133 Caucasian Portuguese PN-MPNs patients and 281 matched controls. All SNPs included in this study: rs1045485 and rs1035142 (*CASP8*), rs1052576, rs2308950, rs1132312 and rs1052571 (*CASP9*), rs2227309 and rs2227310 (*CASP7*) and rs13006529 (*CASP10*) were genotyped using real-time PCR (RT-PCR 7300 Applied Biosystem), through TaqMan® SNP genotyping assays (Life Technology), according to manufacturer instruactions. Differences in genotype frequency, smoking status, age class, gender, therapeutic and pathology distributions between patients and controls were evaluated using SPSS 22.0 (SPSS Inc.).

4. Results

When considered individually, none of the studied apoptosis polymorphisms is associated with PN-MPNs risk. No significant difference was found between the case and control groups concerning age distribution, gender, smoking habits or genotype frequencies (Table 1). However, alcohol consumption is significantly increased in patients, when compared to control group (Table 1). Patients distribution by diagnosis was 80 (60.2%) with ET, 39 (29.3%) with PV and 14 (10.5%) with PMF. After stratification by pathology diagnosis our results showed (Table 2) a significant increased risk for patients diagnosed with ET when present at least one variant allele (T) for *CASP9*_rs1132312 (C653T) polymorphism (heterozygous individuals OR 2.300 CI95% [1.180 – 4.484], P_{value}=0.014; combination of heterozygous with homozygous for variant allele OR 2.203 CI95% [1.163 – 4.176], P_{value}=0.015). The same effect was found, after stratification by gender in women (OR 4.370 CI95% [1.608 – 11.873], P_{value}=0.004) and also in those patients who present *JAK2* mutation (OR 2.886 CI95% [1.303 – 6.393], P_{value}=0.009) (Table 2).

Analyzing the results as haplogroup analysis, we only could establish a positively haplogroup for *CASP9* gene correlated with a decrease risk for PN-MPNs (Table 3).

Concerning the number of SNPs of different genes under study, and grouping the effector caspases genes as a hole, we stablish a new haplogroup (Table 4).

5. Conclusions

Our results revealed that for ET patients and when they are women and *JAK2* positive, there is a significant increased risk when carrying at least one variant allele for *CASP9*_rs1132312 (C653T) polymorphism.

Haplogroup association studies didn't allowed us to stablish a global haplogroup, but the correlation for SNPs of *CASP9* gene showed a decreased risk for two haplogroups (GCC and GTT); a similar effect was obtained when effector caspases were grouped.

Although larger studies are required to confirm these results and to provide conclusive evidence of association between these and other apoptosis variants and PN-MPNs susceptibility, these new data may contribute to a best knowledge of the pathophysiology of these disorders and, in the future, to a more rational and efficient choice of therapeutic strategies to be adopted in PN-MPNs treatment.

6. References

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Table 1 – General characteristics for the PN-MPNs cases (*n*=133) and control population (*n*=281).

Characteristics	Cases, *n* (%)	Controls, *n* (%)	*P* value
Gender			
Male	61 (45.9)	133 (47.3)	0.8
Female	72 (54.1)	148 (52.7)	
Age [a, b]			0.6
30-49	16 (12.0)	43 (15.3)	
50-69	50 (37.6)	107 (38.1)	
≥70	67 (50.4)	131 (46.6)	
Smoking habits			0.6
Never	104 (78.2)	213 (76.1)	
Current	29 (21.8)	67 (23.9)	
Alcohol habits			**<0.0001**
Never	103 (77.4)	191 (68.2)	
Social	20 (15.0)	25 (8.9)	
Regular	10 (7.5)	64 (22.9)	
CASP7 (Lys249Arg)			0.8
G/G	77 (57.9)	154 (55.2)	
G/A	50 (37.6)	109 (39.1)	
A/A	6 (4.5)	16 (5.7)	
CASP7 (Asp255Glu)			0.9
C/C	73 (55.3)	154 (55.8)	
C/G	52 (39.4)	106 (38.4)	
G/G	7 (5.3)	16 (5.8)	
CASP8 (Asp270His)			0.8
G/G	101 (76.5)	220 (78.9)	
G/C	26 (19.7)	51 (18.3)	
C/C	5 (3.8)	8 (2.9)	
CASP8 (3'UTR)			0.8
G/G	50 (37.6)	97 (34.8)	
G/T	61 (45.9)	137 (49.1)	
T/T	22 (16.5)	45 (16.1)	
CASP9 (Arg173His)			0.2
G/G	129 (97.0)	276 (98.9)	
G/A	3 (2.3)	3 (1.1)	
A/A	1 (0.8)	0 (0.0)	
CASP9 (Phe136Phe)			0.1
C/C	31 (23.3)	87 (31.2)	
C/T	74 (55.6)	128 (45.9)	
T/T	28 (21.1)	64 (22.9)	
CASP9 (Ala28Val)			0.2
C/C	70 (25.1)	87 (31.2)	
C/T	129 (46.2)	128 (45.9)	
T/T	80 (28.7)	64 (22.9)	
CASP10 (Ila522Leu)			0.6
A/A	36 (29.8)	82 (29.4)	
A/T	58 (47.9)	123 (44.1)	
T/T	27 (22.3)	74 (26.5)	

[a] Age of diagnosis for cases
[b] Age of control population at the time of diagnosis for the matched case

Table 2 – ORs (95% CI) for *CASP9* (Phe136Phe) polymorphism and PN-MPNs association.

	n	*CASP9* (Phe136Phe)	OR crude (95% CI)	OR adjusted (95% CI)[a]
All cases	133	C/C[b]	1 (Reference)	1 (Reference)
		C/T	1.6 (1.0-2.7)	1.7 (1.0-2.8)
		T/T	1.2 (0.7-2.2)	1.3 (0.7-2.4)
		C/T + T/T	1.5 (0.9-2.4)	1.5 (1.0-2.5)
Pathology stratification				
ET		C/C[b]	1 (Reference)	1 (Reference)
	80	C/T	**2.2 (1.2-4.3)***	**2.3 (1.2-4.5)***
(*P* value **0.05**)		T/T	1.9 (0.9-4.1)	2.0 (0.9-4.3)
		C/T + T/T	**2.1 (1.1-4.0)***	**2.2 (1.2-4.2)***
ET, females		C/C[b]	1 (Reference)	1 (Reference)
	48	C/T	**4.4 (1.6-12.1)***	**4.7 (1.7-13.0)***
(*P* value **<0.01**)		T/T	**3.9 (1.3-12.2)***	**3.8 (1.2-11.9)***
		C/T + T/T	**4.3 (1.6-11.4)***	**4.4 (1.6-11.9)***
ET, *JAK2* positive		C/C[b]	1 (Reference)	1 (Reference)
(*P* value **0.02**)	58	C/T	**3.1 (1.4-6.9)***	**3.1 (1.4-7.1)***
		T/T	2.4 (0.9-6.0)	2.4 (1.0-6.2)
		C/T + T/T	**2.8 (1.3-6.2)***	**2.9 (1.3-6.4)***

[a] ORs were adjusted for age (30-49, 50-69 and ≥70), smoking status (never and current)
[b] The individuals with C/C genotype were considered as reference class
* *P* Crude and *P* Adjusted < 0.02

Table 3 – Haplogroup association response for SNPs of *CASP9* gene.

Haplogroup association response				
	CASP9		OR crude	*P* Value
rs2308950	rs1132312	rs1052571	(95% CI)	
G	C	T	1 (Reference)	
G	T	C	1.15 (0.79-1.68)	0.46
G	**C**	**C**	**0.24 (0.11-0.52)**	**$3e^{-4}$**
G	**T**	**T**	**0.41 (0.25-0.70)**	**0.001**

Table 4 – Haplogroup association response for SNPs present in all effector caspases studied.

Haplogroup association response							
CASP8		*CASP9*			*CASP10*	OR crude	*P*
rs1045485	rs1035142	rs2308950	rs1132312	rs1052571	rs13006529	(95% CI)	Value
G	G	G	C	T	A	1 (Reference)	
G	**G**	**G**	**T**	**T**	**A**	**0.18 (0.03-0.96)**	**0.046**
G	**T**	**G**	**C**	**C**	**T**	**0.08 (0.01-0.82)**	**0.034**