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UNIVERSIDADE NOVA DE LISBOA

IDENTIFICATION OF GENETIC DETERMINANTS ASSOCIATED WITH SUSCEPTIBILITY AND THERAPEUTIC EFFICACY IN PHILADELPHIA-NEGATIVE MYELOPROLIFERATIVE NEOPLASMS

CENTRO HOSPITALAR DE LISBOA OCIDENTAL, E.P.E.
HOSPITAL DE SÃO FRANCISCO XAVIER

Ana P. Azevedo[1,2,3], Susana N. Silva[1], Alice Reichert[4], Fernando Lima[4], Esmeraldina Júnior[3], José Rueff[1] and Jorge F. Gaspar[1]

[1]Center of Toxicogenomics and Human Health and [2]Department of Celular and Molecular Medicine, NOVA Medical School / Universidade Nova de Lisboa (UNL), Lisbon
[3]Department of Clinical Pathology, Hospital de S. Francisco Xavier, Centro Hospitalar de Lisboa Ocidental (CHLO), Lisbon
[4]Department of Clinical Haematology, Hospital de S. Francisco Xavier, Centro Hospitalar de Lisboa Ocidental (CHLO), Lisbon

Centre for Toxicogenomics and Human Health
NOVA Medical School / Faculdade de Ciências Médicas
Universidade Nova de Lisboa
(anpazevedo@gmail.com)

Background

The Philadelphia-chromosome negative myeloproliferative neoplasms (PN-MPN) (Fig 1), which include Polycythemia vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF), are associated with several somatic mutations, alone or in association with *JAK2* V617F. Several Single Nucleotide Polymorphisms (SNPs) have been identified, in other malignant disorders, that may influence DNA repair capacity and apoptosis mechanisms which, in turn, increase genetic predisposition to disease and determine therapeutic response. Moreover, in PN-MPNs, despite the development of more efficient drugs in the last years, some patients with PN-MPNs still evolve to myelodysplasia, myelofibrosis and acute leukaemia, conditions more difficult to treat.

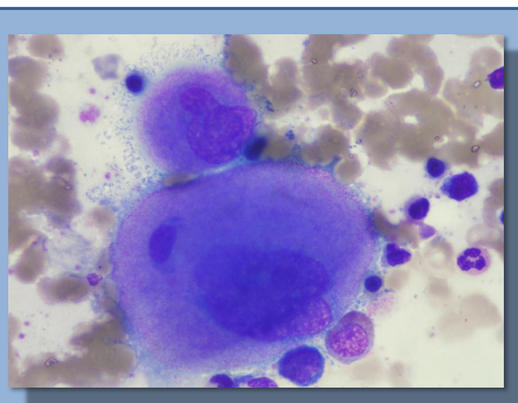

Fig. 1 – Megakaryocytes in essential thrombocythaemia, bone marrow aspirate smear (x100).

Methods

Concerning PN-MPNs susceptibility, evaluate the role of base excision repair and apoptotic genes.
Case-control study in 121 Caucasian Portuguese patients (73 with ET, 35 with PV and 13 with PMF) and 280 matched controls.
Most of the patients were diagnosed and are followed by some of the elements of this working group.

Apoptosis - rs2227309 and rs2227310 (*CASP7*), rs1045485 and rs1035142 (*CASP8*), rs2308950, rs1820204 and rs1052571 (*CASP9*) and rs13006529 (*CASP10*)

BER - rs1799782 and rs25487 (*XRCC1*), rs1052133 (*OGG1*), rs1136410 (*PARP1*), rs13428 and rs1050112 (*PARP4*), rs1130409 (*APEX1*) and rs3219489 (*MUTYH*)
All SNPs under study were genotyped using real-time PCR (RT-PCR 7300 Applied Biosystem), through TaqMan® SNP genotyping assays (Life Technology), according to manufacturer instructions.
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.).

Results

Alcohol consumption is associated with PN-MPNs risk (Table1).

Apoptosis – When considering ET, a consistent increase in overall PN-MPNs risk was observed for **rs1820104 (*CASP9*)** (Table1 and 2).

BER – When considering men with PV, a consistent increase in overall PN-MPNs risk was observed for the presence of at least one variant allele carriers for **rs3219489 (*MUTYH*)** (Table 1 and 2).
When considering women with ET, a protective effect in overall PN-MPNs risk was observed for the presence of at least one variant allele carriers for **rs25487 (*XRCC1_399*)** (Table 1 and 2).
PARP4_13 is in linkage disequilibrium with *PARP4_01*.

No significant difference was found between the case and control groups concerning age distribution, gender, smoking habits or genotype frequencies (Table 1). No significant change in crude or adjusted OR was observed for any of the other genotypes considered.

Table 1 – General characteristics for the PN-MPNs cases (*n*=121) and control population (*n*=280).

Characteristics	Cases, *n* (%)	Controls, *n* (%)	*P* value
Gender			0.8
Male	55 (45.8)	132 (47.1)	
Female	65 (54.2)	148 (52.9)	
Age [a, b]			0.9
30-49	16 (13.2)	43 (15.4)	
50-69	47 (38.8)	106 (37.9)	
≥70	58 (47.9)	131 (46.8)	
Smoking habits			0.9
Never	93 (76.9)	212 (76.0)	
Current	28 (23.1)	67 (24.0)	
Alcohol habits			**<0.0001**
Never	92 (76.0)	190 (68.1)	
Social	20 (16.5)	25 (9.0)	
Regular	9 (7.4)	64 (22.9)	
APOPTOSIS			
***CASP9* (Phe136Leu; rs1820204)**			0.2
Leu/Leu	28 (23.1)	87 (31.3)	
Leu/Phe	66 (54.5)	128 (46.0)	
Phe/Phe	27 (22.3)	63 (22.7)	
BER			
***MUTYH* (Gln335His; rs3219489)**			0.7
His/His	61 (50.4)	142 (51.3)	
Gln/His	47 (38.8)	112 (40.4)	
Gln/Gln	13 (10.7)	23 (8.3)	
***XRCC1_399* (Gln399Arg ; rs25487)**			0.8
Arg/Arg	52 (43.0)	113 (40.8)	
Arg/Gln	54 (44.6)	134 (48.4)	
Gln/Gln	15 (12.4)	30 (10.8)	

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

Conclusions

It seems that **alcohol** consumption increases the risk for PN-MPNs development.
Apoptosis – In ET, our results reveal a significant involvement of **rs1820104 (*CASP9*)** polymorphism on the individually susceptibility towards PN-MPNs.
There are studies that show modifications in the expression of molecules that participate in the regulation of apoptosis, indicating that this mechanism is involved in the pathophysiology of these diseases.
BER – Our results suggest that polymorphisms such as **rs3219489 (*MUTYH*)** and **rs25487 (*XRCC1_399*)** may influence PN-MPNs susceptibility, when considering pathology and gender stratification.

JAK2V617F was not statistically analyzed yet, due to the lack of information about its results.

Larger studies are required to confirm these results and to provide conclusive evidence of association between these and other variants and PN-MPNs and therapeutic response.
Identification of the main molecules that are altered in MPNs allows the development of drugs more directly targeted to the pathophysiology of the disease, with high efficacy, fewer adverse effects, contributing to compliance of the patients with treatments.

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

Pathology stratification	*n*	*SNP*	OR crude (95% CI)	*P* value
APOPTOSIS				
ET	73	***CASP9* (Phe136Leu; rs1820204)**		
		Leu/Leu[a]	1 (Reference)	
		Leu/Phe	2.3 (1.2-4.7)	0.018
		Phe/Phe	2.3 (1.0-5.0)	0.038
BER				
Male with PV	19	***MUTYH* (Gln335His; rs3219489)**		
		His/His[a]	1 (Reference)	
		His/Gln or Gln/Gln	3.1 (1.0-9.0)	0.041
Female with ET	40	***XRCC1_399* (Gln399Arg; rs25487)**		
		Arg/Arg[a]	1 (Reference)	
		Arg/Gln or Gln/Gln	0.5 (0.2-1.0)	0.043

[a] The genotype considered as reference class

Future projects

We also intend to study 90 samples from PN-MPN patients treated with hydroxyurea, that are under clinical follow up, to assess the association of selected critical DNA repair genes (e.g. base excision repair, homologous recombination and non-homologous end joining pathways) with therapeutic efficacy and prognosis.
In the future, these new data may contribute to a more rational and efficient choice of therapeutic strategies to be adopted in the treatment of PN-NMPs.

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