

Analysis of the -398C/T polymorphism in the perforin gene in oncohematological patients

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Background: Recently, single nucleotide polymorphisms (SNPs) were identified in the promoter region of the perforin gene (*PRF1*) and it was found that the -398T mutant allele is correlated with lower amounts of protein in circulating CD8⁺ cytotoxic T lymphocytes.

Objective: The aim of this study was to investigate the presence of the -398C/T polymorphism in the perforin gene in oncohematological patients.

Methods: Sixty-two patients with hematological malignancies treated at the teaching hospital of the Universidade Federal do Triângulo Mineiro were invited to participate in this study. The identification of the polymorphism was achieved by amplification using polymerase chain reaction, digestion using the *TaqI* enzyme and electrophoresis in 1% agarose gel.

Results: The heterozygous -398C/T polymorphism was identified in 16.7% patients with acute lymphoblastic leukemia, 40% with multiple myeloma, 50% with essential thrombocythemia, 14.3% with Hodgkin's disease, 7.7% with non-Hodgkin lymphoma and 33.3% with chronic lymphocytic leukemia. The homozygous mutant allele was identified in one mulatto individual (25%) with myelodysplastic syndrome. When Afro-Brazilian and Whites were analyzed together, there was a higher frequency of the -398T allele in patients than in healthy individuals (*p*-value = 0.0291).

Conclusion: One patient was homozygous for the -398T allele. Based on these findings, further studies should be conducted to assess whether the presence of this polymorphism may be a risk factor for the development of hematologic malignancies.

Keywords: Polymorphism, genetic; Polymorphism, single nucleotide; Perforin; Hematologic neoplasms

Introduction

Cytotoxic T lymphocytes (CTLs) and natural killer cells are effector lymphocytes that have similar cytotoxic mechanisms and are required for protection against transformed cells or those infected by viruses. The main mechanism of cytolysis mediated by CTLs is the release of granules containing cytotoxic proteins against the target cell;⁽¹⁾ perforin is the main constituent of these granules.

The importance of perforin was proven when it was found that biallelic mutations in perforin gene (*PRF1*), followed by a complete absence or severe deficiency of the encoded protein, are found in a proportion of patients with hemophagocytic lymphohistiocytosis.^(2,3) This is a serious disease that usually occurs in childhood, has features similar to metabolic disorders and acute lymphoblastic leukemia and is characterized by deficiency in cytolytic activity of CTLs and natural killer cells. Other defects caused by genetic polymorphisms in *PRF1* have been described and correlated with hematologic diseases such as aplastic anemia,⁽⁴⁾ lymphomas in children,⁽⁵⁾ non-Hodgkin lymphoma and Hodgkin's disease.⁽⁶⁾

The influence of polymorphisms in the promoter region of *PRF1* on the progression of HIV infection was evaluated and three new single nucleotide polymorphism (SNPs) were found: 63A/G (-1347A/G), 112A/G (-1298A/G) and 1012C/T (-398C/T). In addition, the -398CT genotype was correlated with less perforin in circulating CD8⁺ CTLs.⁽⁷⁾

As perforin may be related to a mechanism of immune surveillance in tumors,⁽⁸⁻¹⁰⁾ the aim of this study was to investigate the presence of the -398CT polymorphism in the promoter region of *PRF1* in oncohematological patients.

Methods

Patients

Sixty-two patients with hematological malignancies treated at the teaching hospital of the Universidade Federal do Triângulo Mineiro (UFTM) were invited to participate of

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this study. All subjects or their guardians (if under 18 years old) were informed about the nature of the study and signed a consent form.

Clinical and epidemiological data such as gender, ethnic background, type of disease and age at diagnosis were obtained from the medical records service. This study was approved by the Ethics Committee of the UFTM (case # 1094).

Investigation of the polymorphism

Five milliliters of peripheral blood were collected from each individual and DNA samples were extracted from the buffy coat using the DNA Flexi Gene Mini Kit (Quiagen, USA) following the manufacturer's instructions. The PRF1 promoter region that contains the -398C/T polymorphism was obtained by PCR amplification using the following pair of primers: (forward): 5'-CCAAGCACTTCACAACAACC-3' and (reverse): 5'-AAGCGGCTACACAGATGGAT-3' (Invitrogen Life Technologies, Brazil), according to the methodology described by Garcia et al.⁽¹¹⁾ The PCR products were digested with the TaqI restriction enzyme (Invitrogen Life Technologies, Brazil). The digestion products were run in 1% agarose gel and visualized after staining with 1% ethidium bromide. A heterozygous individual for the polymorphism was used as a positive control and an individual, previously identified as homozygous for the wild type allele by direct sequencing, was used as a negative control.

Statistical analysis

Descriptive analysis was used to compare clinical and epidemiological data. Statistical analysis comparing the frequencies of polymorphisms between patients and the general population was performed using the chi-square test, with significance set for a p-value < 0.05.

Results

Epidemiological and clinical data

Of the 62 patients evaluated, 56% were male and 44% were female with a mean age of 44.4 years (range: 1 to 85 years).

The study group was made up of 69% Whites, 21% mulattos and 10% Afro-Brazilians. Forty-three (69.4%) patients had malignancies of lymphoid origin, 18 (29.0%) of myeloid origin, and 1 (1.6%) had histiocytosis. In children and adolescents (0 to 20 years), 63.2% had acute lymphoblastic leukemia, but among adults (21 to 40 years), non-Hodgkin lymphoma and Hodgkin disease represented 55.6% and 33.3% of cases, respectively. In older age groups there were higher incidences of chronic disease and diseases with myeloid origin.

Analysis of the polymorphism

Of the 62 patients studied, 53 were homozygous for the wild allele, eight were heterozygous and one was homozygous for the -398T allele (Figure 1).

On analyzing the distribution of the -398T allele by ethnic background, the SNP was only present in Whites and mulattos (Table 1). There were no significant differences in allele frequencies between these groups (p-value = 0.087).

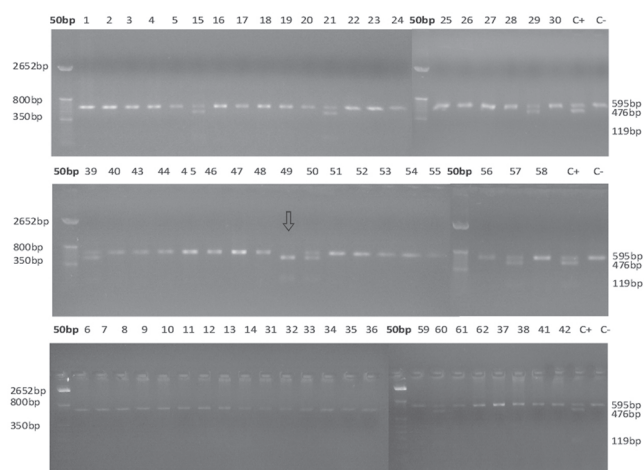


Figure 1 – Digestion of the PCR products with restriction enzyme TaqI to identify the -398C/T polymorphism in the PRF1 promoter in oncohematological patients included in this study. 50bp: molecular weight marker; 1-62: patients; C+: positive control (-398CT individual); C-: negative control (-398CC individual). The arrow indicates the homozygous -398TT

Table 1 - Allelic and genotypic frequencies of the -398C/T single nucleotide polymorphism in the PRF1 gene according to ethnic background

SNP - 398	Patients			Healthy individuals ⁽¹¹⁾	
	White	Mulatto	Afro-Brazilian	White	Afro-Brazilian
C	0.942	0.808	1.000	0.924	1.000
T	0.058	0.192	0	0.076	0
CC	0.884	0.692	1.000	0.849	1.00
CT	0.116	0.231	0	0.151	0
TT	0	0.077	0	0	0

SNP - Single nucleotide polymorphism

Chi-square test for patients: allelic frequency - p-value = 0.087; genotypic frequency - p-value = 0.352 (Whites vs. mulattos), p-value = 1.000 (Whites vs. Afro-Brazilians), p-value = 0.515 (mulattos vs. Afro-Brazilians). All loci evaluated in the PRF1 gene fitted the projected Hardy-Weinberg equilibrium for healthy individuals

In regards to the subtypes of hematological malignancies, two patients with acute lymphoblastic leukemia were heterozygous for the SNP, as were two with multiple myeloma, one with essential thrombocythemia, one with Hodgkin's disease, one with non-Hodgkin lymphoma and one with chronic lymphoblastic leukemia. The homozygous mutant allele was identified in one patient with myelodysplastic syndrome.

Discussion

Recently, the -398T allele was identified at an overall frequency of 0.026 in the Brazilian population (Whites, Afro-Brazilians and Asians).⁽¹¹⁾ This study showed that the distributions of SNPs in this region differ significantly between groups; only individuals who had suffered no influence of other ethnic groups in the two previous generations were included in the research.

McIlroy et al.⁽⁷⁾ did not describe the composition of their sample assessed for the presence of the -398T allele. However, Garcia et al.⁽¹¹⁾ only identified the mutant allele in white individuals. In the present work, the mutant allele was found in Whites and mulattos. As a previous study described the frequency of polymorphisms in well defined ethnic groups, we believe that mulatto patients carrying the mutant allele probably inherited it from a white ancestor.

On evaluating the presence of the -398C/T polymorphism in 62 oncohematological patients treated at the teaching hospital of UFTM, a frequency of 0.16 was found for the mutant allele. There were no significant differences in allele (p-value = 0.087) and genotype frequencies in respect to ethnic background (p-value = 0.352 for Whites vs. mulattos; p-value = 1.000 for Afro-Brazilians vs. Whites; p-value = 0.515 for mulattos vs. Afro-Brazilians).

There was no significant difference between the -398T allele frequency in white patients in this study compared to that found by Garcia et al.⁽¹¹⁾ (p-value = 0.852). The same was true for Afro-Brazilians as no mutant allele was found for this group in either study. However, when Afro-Brazilians and white patients were grouped together and compared with comparable healthy individuals, the frequency of the -398T allele was higher in patients than controls (p-value = 0.0291). To perform a similar analysis in mulattos, a randomly selected control group of healthy individuals needs to be created.

A very important finding of this study was the identification of a patient with the -398TT genotype. In a work of our group assessing healthy subjects, we only found the heterozygous form of this mutation.⁽¹¹⁾ As perforin is less expressed in -398CT individuals,⁽⁷⁾ we believe that the amount of protein in the cells of a -398TT patient is even lower.

Because of the fundamental characteristics of immunoprotection and immunomodulation of perforin, we believe that further research is very important to define whether the presence of this polymorphism in PRF1 in oncohematological patients may be a risk factor for the development of disease. Thus, it is necessary to increase the

number of individuals studied, that is, the number of patients in each subgroup, to reach any firm conclusion.

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