*Leukemia and Lymphoma*, 2003 Vol. 44 (3), pp. 505–508

Taylor & Francis
healthsciences

# Analysis of the Polymorphism [gIVS12-6T > C] in the *hMSH2* Gene in Lymphoma and Leukemia

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*(Received 15 August 2002)*

Given the importance of mismatch repairing genes in keeping the genetic stability in cells, any alterations in their structure or function could generate instability in the genome and predispose the development of oncogenic processes. *hMSH2* is the principal gene involved in the post-replicating DNA mismatch repair system. In this study, exon 13 of the *hMSH2* gene was analyzed in different neoplasias, leukemias and lymphomas. The aim of our work was to determine the association between the presence of polymorphisms in this region with the development of alterations in the hematological system. The 227 samples including lymphoma, leukemia and myelodysplasic syndromes, where analyzed by PCR-SSCP followed by automated sequencing. A single nucleotide polymorphism was found in 30 individuals. This polymorphism is a T to C substitution at the −6 intronic splice acceptor site of exon 13 of *hMSH2* gene [gIVS12-6T > C]. In the lymphoma group the polymorphism frequency found was 0.09, with statistical significant differences ($p < 0.01$) when compared to the control group. On the other hand, the frequency of the leukemia group was the same of that of the control group (0.05). These findings agree with previous research results of other investigation groups. The results suggest a probable association of the polymorphism with the development of lymphomas but not with leukemia.

*Keywords*: *hMSH2* Gene; Polymorphism; Lymphoma; Leukemia

## INTRODUCTION

Four genes are part of the mismatch repair system (MMR): *hMSH2*, *hMLH1*, *PMS1* and *PMS2* [1]. Mutations in any of this genes are responsible for heritable non-polyposis colon cancer (HNPCC) [2]. In addition to chromosomal translocations responsible of the rearrangement of possible oncogenes, one of the characteristics of this kind of tumors is microsatellite instability (MIN), that has also been observed in other type of tumors, such as neoplasms of the hematological and lymphoid systems [3–6]. There is also a high incidence of lymphoma in HPNCC patients, which may suggest a relationship between HPNCC and the development of lymphoma. An association between mismatch repair genes and lymphoma was provided by studies done in Msh2 − / − mice, which were susceptible to lymphoid tumors with high MIN [7]. This data suggested a direct link between MSH2 deficiency and the pathogenesis of cancer. Afterwards, the direct evidence for MSH2 abnormalities in human lymphomas was established by the sequence analysis of exon 13 of *hMSH2*, which revealed coding region mutations [8].

The target region used for this study, exon 13, has been previously described as the most polymorphic within the *hMSH2* gene [9]. Polymorphisms in this region have been reported to be responsible for the absence of protein MSH2 in patients with leukemia [10]; additionally, sequence analysis in lymphoma patients showed that alterations in the coding region could be one of the reasons for lymphoma progress [7].

Our previous research, which involved 22 non-Hodgkin lymphomas (NHL), found a polymorphism with a frequency of 0.114 compared to the 0.05 frequency for the general Ecuadorian population; these results suggested the importance of the presence of this polymorphism in the hematological system [11]. Thus, the major interest of the present study was to determine

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ISSN 1042-8194 print/ISSN 1029-2403 online 
DOI: 10.1080/1042819021000047038

TABLE I Distribution of the allele [gIVS12-6T > C]

| Group | Number of individuals | | | | Polymorphism Frequency |
|---|---|---|---|---|---|
| | Sample size | T/T | T/C | C/C | |
| Control | 50 | 46 | 3 | 1 | 0.05 |
| Lymphoma | 87 | 70 | 17 | 0 | 0.09** |
| Leukemia | 90 | 81 | 8 | 1 | 0.05 |
| MDS | 4 | 4 | 0 | 0 | 0.00 |

**Significant differences when compared to the control group ($p < 0.01$).

the relationship between a polymorphism in the exon 13 of the *hMSH2* gene and a broad spectrum of hematological disorders, including: lymphomas, leukemias (CML and ALL) and myelodysplasic syndromes, analyzing a larger sample.

## MATERIALS AND METHODS

Exon 13 of the *hMSH2* gene was analyzed in bone marrow samples obtained from 227 patients. Eighty-seven of these belonged to patients with histopathological diagnostic of lymphomas, non-Hodgkin and Burkitt, 90 were obtained from patients with several types of leukemia (CML and ALL) and some other hematopoietic disorders. The control group included 50 samples of healthy, unrelated patients. The samples used in our previous study [11] were included in this report.

DNA extraction from bone marrow samples was performed as previously described [12]. For single strand conformational polymorphism (SSCP) analysis, exon 13 of *hMSH2* was amplified by polymerase chain reaction (PCR) using primers which include intronic regions flanking exon 13 according to previously reported procedures [11–13]. The amplification reaction was carried in a final volume of 25 μl, containing 100 ng of genomic DNA as template, 1 Unit of *Taq* polymerase (Promega Corp., Madison, WI), 10 mM of dNTPs, 0.8 μM of each sense and antisense primer, 1X reaction buffer and 2.5 mM $MgCl_2$ (Promega Corp., Madison, WI). PCR conditions consisted of an initial denaturation for 5 min at 91°C; followed by 35 cycles: 30 s at 91°C, 1 min at 51°C, 1 min and 30 s at 72°C; and a final extension for 8 min at 72°C. For SSCP, PCR products were denatured at 91°C for 5 min and snap-cooled prior to electrophoresis on a 12% non-denaturing, 49:1 acrylamide: bisacrylamide gel containing 10% glycerol. Samples with abnormal profiles were submitted for sequencing. Sequencing was carried out on an Abi Prism 377 Automated Sequencer.

Statistical analysis of lymphoma and leukemia group compared with control group was performed using Chi-square ($\chi^2$) test for proportions. The relationship between the different subgroups: sex, age and lymphoma or leukemia type was evaluated using Fisher's non-parametric test.

## RESULTS

Using SSCP we were able to detect 30 samples with mobility shifts from a total of 227. Direct sequencing of this samples revealed the presence of a single nucleotide polymorphism: a T to C substitution at the −6 intronic splice acceptor site of exon 13 [gIVS12-6T > C]. Within the lymphoma group, 17 patients carried this allele, and 9 within the leukemia group. The control group presented four individuals with the polymorphism. The allelic frequency of the polymorphism for the different groups is shown in Table I. The statistical analysis showed significant differences between the control group and the lymphoma group, whereas the leukemia group showed no differences. Only two homozygotic individuals were identified for the polymorphism (C/C), one in the control group and the other in the leukemia group. In the lymphoma group, 10 men and 7 women carried the polymorphism, while 6 men and 3 women in the leukemia group. In the analysis made by lymphoma type (non-Hodgkin and Burkitt), 16 of the 17 patients with the polymorphism were non-Hodgkin Lymphoma-diagnosed and only one was a patient with Burkitt lymphoma. In leukemia patients, four had acute lymphoblastic leukemia, three chronic myeloid leukemia and one acute myeloid leukemia (Table II). The statistical analysis showed no relationship between neither sex nor the type of lymphoma or leukemia.

## DISCUSSION

The development of genetic disease, e.g. cancer, should involve more than one factor to break out a malignant process. Polymorphisms in genes that perform the maintenance of cellular genetic stability, like MMR

TABLE II Polymorphism distribution by lymphoma and leukemia type

| | Lymphoma | | Leukemia | | |
|---|---|---|---|---|---|
| | NHL | BL | ALL | CML | MDS/Other |
| T | 68 | 2 | 43 | 23 | 4/12 |
| C | 16 | 1 | 4 | 3 | 0/1 |
| Total | 87 | | 90 | | |

Abbreviations: NHL, Non-Hodgkin Lymphoma; BL, Burkitt lymphoma; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplasic syndromes.

genes, may contribute to the risk of developing this kind of disorders [14]. The polymorphism [gIVS12-6T > C] found in this study might modify the normal function of the *hMSH2* gene; this modification could contribute to lymphoma progress due to the high frequency found in this group (0.09). The frequency found in the leukemia group (0.05) suggests no relationship between the polymorphism and the disease.

The allelic frequency of the polymorphism found in the group of patients with lymphoma is 0.09, showing statistical significant differences ($p < 0.01$) when compared to the control group. On the other hand, the frequency in then leukemia group was the same of that of the control group (0.05). The frequency found in the control group (0.05) is the same as that previously reported in a Caucasian population [15,16]. Although this polymorphism [gIVS12-6T > C] is found in normal population, its frequency of almost two fold in lymphoma patients may suggest some kind of role for the allele in this disorder. It has been suggested that this polymorphism may predispose to cancer on the setting of ulcerative colitis at an earlier age and with a longer period of prevalence [9].

The polymorphism [gIVS12-6T > C] is a single nucleotide polymorphism (SNP). The genome map describes 1.42 million SNPs, most of them do not affect the normal function of the gene where they occur, but may add phenotypic variation that could influence the individual features that are related to the risk to acquire sickness and the answer to environment [17].

Other studies carried on lymphomas and leukemia have not found any modification in *hMSH2*, or some other MMR genes, but the presence of MIN was a common feature in all of the studied lymphoma cellular lines [6,18]. MIN is closely related with point mutations in MMR genes, however, the inactivation of MMR genes is not due to deletions or other chromosomic alterations, otherwise characteristic of lymphoma or leukemia, even though, the loss of heterozygocity of any of the four MMR genes (*hMSH2*, *hMLH1*, *PMS1*, *PMS2*) produces instability in some hematopoietic tumors [19].

The presence of MIN is uncommon in leukemia [20–22]. According to our findings and previous research studies, there is no relationship between the polymorphism found [gIVS12-6T > C] and the development of leukemia; on the other hand, a recently described homozygous novel mutation in *hMSH2* gene might be associated with leukemia development [23], unlike lymphomas, where something different occurs [4,6].

As mentioned above, HNPCC patients develop an excessive number of lymphoid tumors [24], mainly caused by alterations in MMR genes. Nonetheless, many arguing opinions have surrounded this issue; Rosty *et al.* [25] stated that lymphomas are almost never encountered in patients with HNPCC. Our findings, on the other hand, support the results found by Lowsky *et al.* [7], who confirmed the theory that mutations or polymorphisms in *hMSH2* a MMR gene, have a relationship with the development of lymphomas. Since the analysis they made showed an association involving the [gIVS12-6T > C] polymorphism and lymphomas in a small sample, this work studied a large number of lymphomas in order to confirm this association.

Considering the multifactorial origin of lymphomas and leukemias, the loss of *MSH2* could be involved in the pathogenesis of the hematological dysplasia associated with genomic instability, however, this can not be taken as the only causal agent of the neoplasia. The defects in MMR genes would not be a trait of hematopoietic disorders, the relationship between the polymorphism found and the development of lymphoid tumors is not definitive, the polymorphism may not be pathogenic by itself but could be part of the mutator phenotype that finally results in the progression of a neoplasia or it is linked to a locus nearby, which would be responsible for the disease [26,27].

The results showed that this mutator phenotype is associated with lymphoma development but it is not part of the mutator phenotype involved with leukemia. The presence of [gIVS12-6T > C] in a high frequency in patients with lymphoma might give us an insight into a new pathway of oncogenesis.

### *Acknowledgements*

This study was supported by funds from Inter-American Development Bank-FUNDACYT-PUCE grant 111. We thank Dr Angel Pestaña of the Instituto de Investigaciones Biomédicas (CSIC-Spain) for the support provided for the automatic sequencing of the samples.

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