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CXCL12 rs1801157 Polymorphism in Patients with Breast Cancer, Hodgkin's Lymphoma, and Non-Hodgkin's Lymphoma

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Chemokines and their receptors regulate the trafficking of immune cells during their development, inflammation, and tissue repair. The single-nucleotide polymorphism (SNP) rs1801157 (previously known as CXCL12-A/ stromal cell-derived factor-1 (SDF1)-3′A) in CXCL12/SDF1 gene was assessed in breast cancer, Hodgkin's lymphoma (HL), and non-Hodgkin's lymphoma (NHL), since the chemokine CXCL12, previously known as SDF1, and its receptor CXCR4 regulate leukocyte trafficking and many essential biological processes, including tumor growth, angiogenesis, and metastasis of different types of tumors. Genotyping was performed by PCR-RFLP (polymerase chain reaction followed by restriction fragment length polymorphism) using a restriction enzyme *HpaII* cleavage. No significant difference was observed in genotype distribution between breast cancer patients (GG: 57.3%; GA: 39.8%; AA: 2.9%) and healthy female controls (GG: 62.9%; GA: 33%; AA: 4.1%) nor between HL patients (GG: 61.1%; GA:27.8%; AA: 11.1%) and healthy controls (GG: 65.6%; GA: 28.9%; AA: 5.5%), whereas a significant difference was observed in genotype distribution between NHL patients (GG: 51.4%; GA: 47.1%; AA: 1.5%) and healthy controls (GG: 65.6%; GA: 28.9%; AA: 5.5%). Further studies will be necessary to elucidate the cancer chemokine network. However, this study suggests that CXCL12 rs1801157 polymorphism may have important implications in the pathogenesis of NHL. J. Clin. Lab. Anal. 23:387–393, 2009. © 2009 Wiley-Liss, Inc.

Key words: CXCL12 rs1801157 polymorphism; breast cancer; Hodgkin's lymphoma; non-Hodgkin's lymphoma; chemokines

INTRODUCTION

In Brazil, estimates for the year 2009 indicate that 466,730 new cases of cancer will occur (1). Cancer was responsible for 7.6 million of a total of 58 million deaths worldwide, representing 13% of all deaths. Of the total number of deaths from cancer that occurred in 2005, more than 70% occurred in middle- or low-income countries (2).

Chemokines, a family of small pro-inflammatory cytokines, and their receptors regulate various immune responses to infection, inflammation, and tissue repair (3), and they also control the trafficking of immune cells during their development (4). Attention has been focused on the chemokine molecules and receptors expressed on cancer cells because cancer cell migration and metastasis show similarities to leukocyte trafficking (5).

The chemokine CXCL12, also known as stromal cell-derived factor-1 (SDF1), is a small protein constitutively expressed in various organs (6) and it was first cloned from a bone marrow-derived stromal cell line (7) and

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was later identified as a pre-B-cell growth stimulating factor (8). CXCL12 is secreted by marrow stromal and endothelial cells (8), heart (9) and skeletal muscle (10), liver (11), brain (12), kidney parenchymal cells (13), and osteoblasts (14), but is mainly produced by osteoblasts, fibroblasts, and endothelial cells in the bone marrow (15). It regulates leukocyte trafficking and many essential biological processes, including cardiac and neuronal development, stem cell motility, neovascularization, and tumorigenesis (16–20).

CXCL12 may contain a single nucleotide polymorphism (SNP) in the 3′-untranslated region, known as rs1801157 (CXCL12-A/SDF1-3′A) (21). Many studies suggest that the rs1801157 polymorphism in CXCL12 gene increases the amount of CXCL12 available to bind CXCR4 (22). The rs1801157 allele frequency has been assessed in different populations and pathological conditions to evaluate the polymorphism implications in disease susceptibilities, clinicopathological features and clinical outcomes in different conditions. rs1801157 has been implicated in the aggressiveness of the autoimmune process, leading to type 1 diabetes (23), in a possible late-stage protective effect on HIV-1 disease progression in the Brazilian population (24), presents important implications for the pathogenesis of chronic myelogenous leukemia (25), is associated with advanced stages of oral cancer, especially in alcohol abusers (26) and may be considered as a factor increasing the susceptibility of Iranian patients to lung cancer (27).

CXCL12 and its receptor, CXCR4, are reported to have important roles in tumor growth, angiogenesis, and metastasis of different types of tumors (28) because CXCL12 is variably expressed in a number of normal cells and cancer tissues (29).

Based on the suggested role of CXCL12 in the pathogenesis of cancer, the objective of this study was to assess the influence of a SNP of CXCL12 gene (rs1801157) on breast cancer, Hodgkin's lymphoma (HL), and NHL susceptibility.

PATIENTS AND METHODS

Patients and Sample Selection

Following approval from the Human Ethics Committee of the Londrina State University, Institute of Cancer from Londrina, peripheral blood was drawn in sterile syringe containing heparin, as anticoagulant, from 187 healthy subjects, 103 breast cancer patients, 36 HL, and 70 NHL patients. A term of free informed consent was signed by all sample donors and doctors involved prior to blood collection.

All breast cancer, HL, and NHL patients were attended in the University Hospital of Londrina and Institute of Cancer from Londrina, Paraná State, Brazil. Samples of healthy subjects were obtained from Londrina Blood Center, Londrina State University, Brazil.

DNA Extraction

Genomic DNA was isolated from peripheral blood cells using the technique described by Kirby (30). DNA was extracted from whole blood in the presence of 0.2 M NaCl and 0.25% SDS, for 4 hr at 37°C. After precipitation with ethanol, the pellet was dried and resuspended in 50 μL of *milli Q* water.

Polymerase Chain Reaction (PCR)–SDF

DNA (200 ng) was analyzed using PCR with specific primers for SDF 3′UTR-F1 (forward 5′-CAGTCAACCTGGGCAAAGCC-3′) and SDF 3′UTR-R2 (reverse 5′-CCTGAGAGTCCTTTTGCGGG-3′) (GenBank accession number L36033). Samples were amplified using the kit buffer plus 1.25 units of *Taq* polymerase (Invitrogen™, Carlsbad, California). PCR conditions were: 5 min denaturation at 94°C, 35 cycles of 1 min at 94°C, 1 min at 60°C and 1 min at 72°C, and 10 min elongation at 72°C in a thermocycler (PCR-Sprint Hybaid-Guelph, Ontario, Canada). Amplicons of 293 base pairs were analyzed by electrophoresis in a 2% agarose gel and visualized using UV fluorescence after staining with ethidium bromide.

CXCL12 rs1801157 Genotyping

PCR products were subjected to restriction digestion by incubating with *HpaII* (Invitrogen™, Carlsbad) for 3 hr at 37°C. The restriction digestion products were analyzed by electrophoresis on 10% acrylamide gel and detected by a nonradioisotopic technique using a commercially available silver staining method. CXCL12 GG genotype yielded 100 and 193 base pair products, while AA genotype yielded a 293 base pair product.

Sequencing

After purification, some PCR products were sequenced with MegaBACE1000-Pharmacia using DYEnamic™ ET Dye Terminator Kit, Pharmacia. Sequence analysis of SDF1 was performed comparing with data in NCBI-NIH (Blastn), available in http://www.ncbi.nlm.nih.gov/blast.

Statistical Analysis

RS1801157 allele frequency was calculated as: $[1 \times (h+2H)]/2N$, where h represents the heterozygous genotype, H the homozygous genotype, and N the sample size for each population. SNP alleles frequencies were tested against Hardy–Weinberg Equilibrium by

comparing observed with expected genotype frequencies using a χ^2 test. Genotype data were analyzed by the χ^2 test with the level of significance set at $P<0.05$. In order to account for the multiple nonindependent statistical tests, Fisher's exact test was performed in SAS 8.2 for all groups and genotypes at the same time with the level of significance set at $P<0.05$.

RESULTS

The frequency of CXCL12 rs1801157 genotypes was assessed in 209 patients (103 breast cancer patients, 36 HL, and 70 NHL patients) and compared to 187 healthy subjects (97 healthy female subjects and 90 healthy subjects).

There were two control groups in this study, the first (control group 1) consisting of 97 healthy women and the other (control group 2) consisting of 90 healthy subjects, men and women, since breast cancer is common among women and Hodgkin and non-Hodgkin lymphoma can affect both men and women.

The age range among women from control group 1 and breast cancer patients was 25–76 years old. Considering breast cancer development, it was observed that women aged between 45 and 54 years were more assaulted.

Regarding the HL patients and NHL patients, it was observed that men were more assaulted than women, 72.2% in HL and 52.3% in NHL. HL presented higher prevalence among patients aged over 21 years (77.8%), while for non-Hodgkin's lymphoma, this prevalence was observed in patients over 30 years (77.2%).

To assess CXCL12 rs1801157 polymorphism in breast cancer, HL, and NHL patients, genotyping was performed in the three case and two control samples. To analyze primers specificity, three samples were randomly chosen, sequenced, and compared with data in NCBI-NIH. The analysis demonstrated that the amplified fragment was compatible with sequence in dbSNP rs1801157.

The analysis of CXCL12 rs1801157 genotype frequencies in both control groups had similar genotype and allele frequencies and both were in Hardy–Weinberg Equilibrium (Table 1). Breast cancer patients and controls did not show a statistically significant difference. Genotype frequency distributions were in Hardy–Weinberg equilibrium in both groups. As a result, 59 (57.3%) patients and 61 (62.9%) controls were detected with the GG genotype, 41 (39.8%) patients and 32 (33%) controls had the GA genotype, whereas 3 (2.9%) patients and 4 (4.1%) controls had the AA genotype. Consequently, there was no difference in AA and GA genotypes frequencies between patients and healthy subjects (Table 2).

There was no significant difference in genotype distribution between HL and healthy controls or in allelic frequencies (Table 3). On the other hand, a significant difference was observed in genotype distribution between NHL patients and healthy controls. The control group and the HL group did not show a significant deviation from the Hardy–Weinberg

TABLE 1. Genotypic and Allelic Frequencies for the rs1801157 allele-A and allele-G of SDF1/CXCL12 in Control Subjects

Healthy control subjects	Number of samples	Genotype[a]			Allelic frequency[b]	
		GG	GA	AA	allele-G	allele-A
Group 1 (only women)	97*	61 (62.89%)	32 (32.99%)	4 (4.12%)	0.79	0.21
Group 2 (men and women)	90**	59 (65.56%)	26 (28.89%)	5 (5.56%)	0.80	0.20

*χ^2 in HWE = 0.0058. 1 degree of freedom. $P = 0.9971$. **χ^2 in HWE = 0.8506. 1 degree of freedom. $P = 0.6535$.
[a]Group 1 × Group 2. $\chi^2 = 0.504$. (2 degrees of freedom; $P = 0.7773$).
[b]Group 1 × Group 2. $\chi^2 = 0.075$. (1 degrees of freedom; $P = 0.9841$).

TABLE 2. Genotypic and Allelic Frequencies for the rs1801157 allele-A and allele-G of SDF1/CXCL12 in Breast Cancer Patients

Study subjects	Number of samples	Genotype[a]			Allelic frequency[b]	
		GG	GA	AA	allele-G	allele-A
Control group 1	97*	61 (62.89%)	32 (32.99%)	4 (4.12%)	0.79	0.21
Breast cancer patients	103**	59 (57.28%)	41 (39.81%)	3 (2.91%)	0.77	0.23

*χ^2 in HWE = 0.0058. 1 degree of freedom. $P = 0.9971$. **χ^2 in HWE = 1.746. 1 degree of freedom. $P = 0.4177$.
[a]Breast cancer patients × Control group 1. $\chi^2 = 1.106$ (2 degrees of freedom; $P = 0.5750$).
[b]Breast cancer patients × Control group 1. $\chi^2 = 0.2833$ (1 degree of freedom; $P = 0.6810$).

TABLE 3. Genotypic and Allelic Frequencies for the rs1801157 allele-A and allele-G Alleles of SDF1/CXCL12 in NHL and HL Patients

Study subjects	Number of samples	Genotype[a,b]			Allelic frequency[c,d]	
		GG	GA	AA	allele-G	allele-A
Control group 2	90*	59 (65.56%)	26 (28.89%)	5 (5.56%)	0.8	0.2
Non-Hodgkin's lymphoma patients	70**	36 (51.43%)	33 (47.14%)	1 (1.43%)	0.75	0.25
Hodgkin's lymphoma patients	36***	22 (61.11%)	10 (27.78%)	4 (11.11%)	0.75	0.25

*χ^2in HWE = 0.8506. 1 degree of freedom. $P = 0.6535$. **χ^2 in HWE = 4.6285. 1 degree of freedom. $P = 0.0988$. ***χ^2 in HWE = 2.4197. 1 degree of freedom. $P = 0.2982$.
[a]Non-Hodgkin's lymphoma patients × Control group 2. $\chi^2 = 6.6698$ (2 degrees of freedom; $P = 0.0356$).
[b]Hodgkin's lymphoma patients × Control group 2. $\chi^2 = 1.2012$ (2 degrees of freedom; $P = 0.5485$).
[c]Non-Hodgkin's lymphoma patients × Control group 2. $\chi^2 = 1.1403$ (1 degree of freedom; $P = 0.3512$).
[d]Hodgkin's lymphoma patients × Control group 2. $\chi^2 = 0.7636$ (1 degree of freedom; $P = 0.4815$).

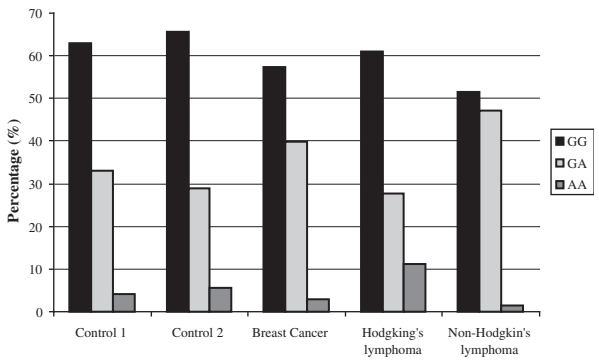

Fig. 1. Genotypic distribution of SDF1/CXCL12 in Breast Cancer, HL, NHL patients and healthy individuals. *Fisher's exact test for all groups and variables $P = 0.0882$.

equilibrium, but the NHL patient group was not in agreement with this distribution.

The genotypic frequency distribution for all analyzed conditions is presented in Figure 1, where it is possible to observe that there was no significant difference in multiple testing using the Fisher exact test and all groups and genotypes.

DISCUSSION

Breast cancer is the second most common type of cancer worldwide and the most common among women. Each year, breast cancer constitutes around 22% of all new cases of cancer in women (1). Age continues to be a major risk factor for breast cancer. Incidence rates of this cancer increase rapidly up to 50 years of age, increasing more slowly thereafter (1).

In this study, a mean age of 52.9 years was observed that is in accordance to Moura-Gallo et al. (31), Moraes et al. (32), and Marinho et al. (33) whom described a mean age of 57, 54, and 55.9 years among breast cancer patients, respectively.

HL can occur in any age range; however, it is more common from 15 to 40 years of age, with higher incidence among patients aged 25–30 years (1). In this study, there was overall male predominance (72.2%) among HL patients and a higher prevalence among patients aged over 21 years (77.8%) male predominance was also reported by Mauch et al. (34). Vassalo et al. (35) observed a male/female ratio of 1.5, and an age distribution showing a maximum of cases in the third decade (26.9%). HL has a bimodal incidence curve; it occurs most frequently in two separate age groups, the first being young adulthood (aged 15–35) and the second being in those over 55 years old although these peaks may vary slightly with nationality (34).

Regarding NHL, there was a slight male predominance (52.3%), and a higher prevalence in patients over 30 years (77.3%). From 2001 to 2005, the median age at diagnosis for non-Hodgkin lymphoma was 67 years of age in the USA (36). Pracchia et al. (37) reported a median age of 34 years (18–53) among NHL patients, and a median age of 58 years was observed (17–85 years) by Costa et al. (38). In Sweden and Denmark, the mean age of the 597 non-Hodgkin's lymphoma cases was 60 years (SD = 11 years), with a median of 62 years (39).

Chemokines and their receptors are a large family of molecules that control the trafficking of immune cells during their development and in response to inflammation (4). It has been established that cancer cells exploit signaling through chemokine receptors for several key steps in initiation and progression of primary and metastatic cancer (3). During the past 6 years there has been a chemokine revolution in cancer and all scientists and clinicians in oncology-related fields are now aware of their crucial role in tumor initiation, promotion, and progression (40,41).

In particular, the chemokine CXCL12 regulates many essential biological processes, including stem cell motility, neovascularization, and tumorigenesis (18–20). CXCL12 is expressed in a number of normal and cancer tissues (29) and binds to CXCR4 (42,43) and to the recently identified receptor CXCR7/RDC1 (44),

originally cloned from a dog cDNA library (Receptor Dog cDNA) (45) as a putative G-protein coupled receptor (GPCR) for the vasoactive intestinal peptide hormone (VIP) (46).

CXCL12 gene contains a common polymorphism, termed rs1801157, in an evolutionarily conserved segment of the 3′ untranslated region (UTR) (25). This variant allele may have an important regulatory role by increasing the production of CXCL12 (21). CXCL12 is expressed constitutively in a variety of tissues, including the lungs, liver, lymph nodes, bone marrow, and adrenal glands (6,47).

Tumor cells, including breast cancers, were found to express high levels of the CXCR4 receptor, whereas human organs targeted by metastatic breast tumor cells, in turn, expressed high levels of CXCL12 (6).

Since CXCL12 mRNA and protein expression may be regulated by common polymorphism rs1801157, and CXCL12–CXCR4 interaction has a prominent role in tumorigenesis, the association of gene variant rs1801157 with breast cancer, HL, and NHL was verified in this study.

Control groups presented similar genotypic distribution and were in Hardy–Weinberg Equilibrium. Control group 1 consisting of only women presented 62.9% GG, 33% GA, and 4.1% AA, and in the group 2 consisting of men and women GG was observed in 65.6% individuals, 28.9% GA, 5.5% AA, which are in accordance to Razmkhah et al. (28) who observed 55.8% GG, 37% GA, and 7.2% AA among health individuals and to Watanabe et al. (48) who reported 65% GG, 30% GA, and 5% AA among healthy Brazilian blood donors.

No statistically significant difference was observed when breast cancer patients were compared to control group 1, although Razmkhah et al. (28) and Zafiropoulos et al. (49) reported that CXCL12 rs1801157 polymorphism was associated with an increased susceptibility to breast cancer development.

Inflammation is accompanied by the generation of free radicals, stimulation of cytokines, chemokines, growth, and angiogenic factors (50). The CXCL12-CXCR4 axis is closely correlated with angiogenesis of tumors (51).

Interruption of the interaction between CXCL12 and its receptor may inhibit the metastatic process. Therefore, the CXCR4 receptor could be an important therapeutic target for cancer treatment (52).

As reported before for breast cancer (53), the CXCL12-CXCR4 axis has an important biological role in mediating tumor metastasis of NHL (54). The majority of lymphoma cases arise in lymph nodes, but primary extranodal disease accounts for 20–30% cases (55). Non-Hodgkin lymphomas (NHL) are among the few neoplasms whose incidence and mortality have been rising in Europe and North America over the last few decades (56,57).

CXCL12 mRNA is abundantly expressed in stromal cells isolated from the lymph nodes of patients with malignant lymphoma (58). Cavassin et al. (59) reported that Brazilian Lymphoma patients are more likely to carry the rs1801157 allele-A than patients with lymphoid leukemias, but Cavassin et al. (59) considered both NHL and HL patients as lymphoma patients.

In this study variant alleles were assessed separately among NHL, HL patients, and healthy individuals. It was observed that CXCL12 rs1801157 genotype distribution in NHL patients was statistically significant when compared to healthy individuals, while no significant difference was observed between HL and healthy controls. This is in agreement with Rabkin et al. (60) who reported that both homozygous and heterozygous rs1801157 variants were associated with an increased risk of NHL in HIV-1-infected individuals. This could be explained by increased CXCL12 mRNA levels in circulating mononuclear cells from AIDS-related NHL pediatric patients (61).

NHL derives from the neoplastic transformation of lymphocytes at different stages of differentiation and may show systemic, nodal, and extranodal localization as well as metastasis in different sites, NHL's express functional chemokine receptors, which dictate tissue localization and perhaps metastatic potential (4).

Lymphoma cells from the lymph nodes of NHL patients express high levels of CXCR4 (54). CXCL12 has been found to enhance migration of follicular NHL cells (62) what can be explained by high levels of CXCL12 mRNA expressed by stromal cells of bone marrow involved by NHL (54).

The high expression of CXCL12 and CXCR4 in serum and bone marrow cells can be used as detective factors for hematologic malignant tumor since a correlation exists between the high expression of CXCR4 and the infiltration of hematologic malignant cells (63).

Since the CXCL12–CXCR4 axis has a uniquely important biological role in mediating many types of tumor metastasis, different types of chemokine antagonists have been tested in vitro and in vivo and several peptide or small molecule inhibitors have reached Phase I/II clinical trials for different diseases (4).

The comprehensive study of chemokines and receptors in primary tumors, metastatic lesions, and corresponding normal tissues will be crucial to further understanding of the cancer chemokine network. This study suggests that CXCL12 rs1801157 polymorphism may have important implications in the pathogenesis of NHL, in spite of its limitations.

The weakness of this study is the sample size, which is quite small for a genetic association study standards. Nevertheless, the absence of an association with cancer risk does not exclude the possibility that rs1801157 may influence cancer progression. Most studies have addressed the effects of some SNPs, categorizing them as "not associated," and thus concluded that they are not important in cancer or disease risks. It is possible that some SNPs without main effects, or with main effects too small to detect, may interact with others and confer a changed risk for cancers, as demonstrated by Lin et al. (64). Especially concerning the influence of CXCL12 genotype rs1801157 on cancer progression, as long as the functional importance of rs1801157 in the expression of the CXCL12 transcript and protein are still unclear. Therefore, further studies are needed to clarify the role of CXCL12 in breast cancer, HL, and non-Hodgkin's lymphoma.

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