



KIR genotype distribution among patients with chronic lymphocytic leukemia: Is there a role for *KIR 2DS4* and *KIR 2DS5* genes?



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ARTICLE INFO

Article history:

Received 19 December 2015

Received in revised form 28 December 2015

Accepted 28 December 2015

Available online 23 February 2016

Keywords:

CLL

KIR

Genotype

Lebanon

ABSTRACT

Introduction: The function of natural killer (NK) cells is regulated by different antigen receptors including killer immunoglobulin-like receptors (KIR). In addition to their important role in fighting infection, natural killer cells produce cytotoxicity against some cancer cells. Studies demonstrated that NK cells have a reduced function in chronic lymphocytic leukemia (CLL).

Aim: The aim of this study is to investigate KIR expression of NK cells in CLL patients to check for any association between KIR genotypes and this disease.

Methods: KIR genotype was analyzed for 120 healthy Lebanese patients and 56 CLL patients using the KIR Genotyping SSP kit.

Results: Among the 56 CLL patients, the AA, AB, and BB genotypes frequencies were, respectively, 38%, 46%, and 16% with an A:B ratio of 1.55:1. As for the controls, the AA, AB, and BB genotypes frequencies were, respectively, 39.17%, 50%, and 10.83% with an A:B ratio of 1.79:1. *KIR 2DS4*001/002* and *KIR 2DS5* were found to be significantly more prevalent among CLL patients as compared to controls.

Conclusion: This is the first study that reports such an interesting prevalence of KIR genes in CLL necessitating further clinical and translational research pertaining to the pathophysiology of this disease.

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1. Introduction

Natural killer cells (NK) play an important role in protecting the human body from any pathogen or unusual transformation. In addition to their major action in the innate immune response, NK cells take part in initiating the adaptive immune response. The function of NK cells is regulated by different antigen receptors including: killer immunoglobulin-like receptors (KIR), Ly49 receptors, and CD94/NKG2 receptors. Among these families of receptors, KIRs have the major effect on human NK cells (Boyton and Altmann, 2007).

KIRs are not only expressed by NK cells but also found in subpopulations of T cells. These receptors differ in the number of extracellular immunoglobulin-like domains (2D, 3D), and in the length of the cytoplasmic tail (short or long; S or L). Most short-tailed KIRs are activating receptors and most long-tailed KIRs are inhibitory receptors with few exceptions; for example, *KIR2DL4* can be both inhibitory and

activating (McQueen et al., 2007). KIR genes are located on chromosome 19 in the leukocyte receptor complex region and they differ between various ethnic groups depending on the type and number of present genes (Hsu et al., 2002; Yawata et al., 2002a; Yawata et al., 2002b; Uhrberg et al., 2002).

In addition to their important role in fighting infection, natural killer cells produce cytotoxicity against some cancer cells (Imai et al., 2005). Studies demonstrated that NK cells have a reduced function in chronic lymphocytic leukemia (CLL), although they are known to be the first line of defense against malignancy (Kay et al., 2002). B-CLL patients have a high frequency of autoantibodies and hypogammaglobulinaemia (Junevik et al., 2007) and B-CLL is characterized by the presence of clonal B cells in the peripheral blood, bone marrow, lymph nodes, and spleen (Jaffe et al., 2001). These B cells have low levels of IgM, IgD, and CD79b on the membrane and express CD19, CD5, and CD23 (Chiorazzi et al., 2005).

Monoclonal antibodies like Rituximab (anti-CD20) and Campath-1H (anti-CD52) are used for the treatment of CLL patients with possible mechanisms for including antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated lysis of the abnormal leukemic cells (Byrd et al., 2001; Keating et al., 2002). It is important to note here that, in ADCC, cytotoxic natural killer (NK) and T cells enhance the efficacy of monoclonal antibody therapy (Whiteside et al., 1997; Kim et al., 2000). Previous studies have shown NK cells cytotoxic activity to

Abbreviations: KIR, killer cell immunoglobulin-like receptors; NK, natural killer; PCR, polymerase chain reaction; SSP, sequence-specific primers; CLL, chronic lymphocytic leukemia; ADCC, antibody-dependent cell-mediated cytotoxicity; Ig, immunoglobulin; HLA, human leukocyte antigens; MHC, major histocompatibility complex; UV, ultraviolet.

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Table 1
KIR genetic profile among 56 CLL patients.

Profile	N	%	# of genes	2DL1	2DL2	2DL3	2DL4	2DL5A	2DL5B	2DS1	2DS2	2DS3	2DS4*001/002	2DS4*003/007	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP*001/002/004	3DP1*003
AA	1	1.8	6																			
AA	1	1.8	6																			
AA	2	3.5	7																			
AA	5	9	7																			
AA	3	5.4	8																			
AA	1	1.8	8																			
BB	1	1.8	8																			
BB	1	1.8	8																			
AA	1	1.8	9																			
AB	2	3.5	9																			
AB	2	3.5	9																			
AA	1	1.8	10																			
AB	1	1.8	10																			
AB	2	3.5	10																			
AB	1	1.8	10																			
BB	1	1.8	10																			
AA	1	1.8	11																			
AA	1	1.8	11																			
AA	2	3.5	11																			
AB	1	1.8	11																			
AB	2	3.5	11																			
BB	1	1.8	11																			
BB	1	1.8	11																			
AA	1	1.8	12																			
AA	1	1.8	12																			
AB	2	3.5	12																			
AB	1	1.8	12																			
AB	1	1.8	12																			
BB	1	1.8	12																			
BB	1	1.8	12																			
AB	1	1.8	13																			
AB	1	1.8	13																			
AB	2	3.5	13																			
BB	1	1.8	13																			
AB	1	1.8	14																			
AB	1	1.8	14																			
AB	1	1.8	14																			
BB	1	1.8	14																			
AB	3	5.4	15																			
AB	1	1.8	15																			
Total	56	100																				

Table 2
KIR genetic profile among the 120 healthy Lebanese controls.

Profile	N	%	No. of Loci	2DL1	2DL2	2DL3	2DL4	2DL5A	2DL5B	3DL1	3DL2	3DL3	2DS1	2DS2	2DS3	2DS4*001/002	2DS4*003	2DS5	3DS1
AA	31	25.83	7																
AB	17	14.17	9																
AB	17	14.17	11																
AA	10	8.33	11																
AB	8	6.67	11																
AB	6	5.00	14																
AB	5	4.17	15																
BB	4	3.33	13																
BB	3	2.50	10																
AB	3	2.50	12																
AA	2	1.67	11																
AA	2	1.67	9																
BB	1	0.83	12																
BB	1	0.83	12																
AA	1	0.83	8																
AB	1	0.83	12																
AB	1	0.83	11																
AB	1	0.83	13																
AB	1	0.83	13																
BB	1	0.83	13																
BB	1	0.83	7																
BB	1	0.83	11																
BB	1	0.83	10																
AA	1	0.83	6																
Total	120	100																	

be defective in B-CLL, and for unknown reasons (Ziegler HW et al., 1981; Katrinakis et al., 1996).

In Lebanon, various studies were conducted to investigate any possible correlation between KIR genotypes and certain conditions such as tuberculosis (Mahfouz et al., 2011), follicular lymphoma (Khalaf et al., 2013), and familial Mediterranean fever (Mahfouz et al., 2009). This study is the first in Lebanon and the Mediterranean region to look at the KIR genotypic profiling among CLL patients as compared to healthy individuals.

2. Materials and methods

2.1. Study population

This Institutional Review Board (IRB)-approved study was performed at the American University of Beirut Medical Center (AUBMC), a major tertiary care Lebanese center. KIR genotype was analyzed for 56 CLL patients and 120 healthy controls using the KIR Genotyping SSP kit. The 120 healthy controls were deduced from the major study by Mahfouz et al. (2006), describing the KIR prevalence in the healthy Lebanese population mainly from donors of a bone marrow transplantation center representing all ethnicities and districts in the country.

Table 3
Frequency distribution of AA, AB, and BB genotypes among controls and CLL patients.

Genotype	Controls		CLL patients	
	No.	%	No.	%
AA	47	39.17	21	38
AB	60	50.00	26	46
BB	13	10.83	9	16
Total	120	100	56	100

Haplotype	Controls		CLL patients	
	No.	%	No.	%
A	154		68	
B	86		44	
A:B ratio	1.79:1		1.55:1	

2.2. DNA extraction and KIR genotyping

PEL-FREEZ kits (Pel-freez/Dynal, Norway) were used for DNA extraction from 2 to 3 ml of collected peripheral blood. The DNA material was properly labeled and stored at -80°C . Based on our Institutional Review Board (IRB) committee rules and study approval protocols, confidentiality was strictly observed for all analyzed samples.

Primer mixes were purchased from PEL-FREEZ/DYNAL company (Oslo, Norway) as part of the KIR Genotyping SSP kit, which is a PCR-based method designed to detect the absence or presence of the following 16 gene loci of KIR (variants also tested): 2DL1, 2DL2, 2DL3, 2DL4, 2DL5, 2DS1, 2DS2, 2DS3, 2DS4, 2DS5, 3DL1, 3DL2, 3DL3, 3DS1, and 3DP1. Two variants for KIR2DL5 were typed KIR2DL5A*001 and KIR2DL5B*002/003/004 and two variants for KIR2DS4 were tested and reported as KIR2DS4*001/002 and KIR2DS4*003-006. In addition, two variants for the pseudogene KIR3DP1 were tested: KIR3DP1*001/002/004 and KIR3DP1*003.

KIR genotyping was performed as recommended by the manufacturer and all steps were strictly followed. Briefly, 25 μl of DNA were added to 150 μl of PCR buffer and 2.4 μl of Taq DNA polymerase and dispensed as aliquots of 8 μl into a supplied 96-well plate for a total reaction volume of 23 μl in each well (reaction + paraffin oil). The thermocycling steps include an initial heating step at 95°C for 1 min, followed by 30 cycles of 94°C for 20 s, 63°C for 20 s, and 72°C for 90 s. A final holding step was performed at 4°C . Electrophoresis of the 2% agarose gel was done in ethidium bromide and visualization performed under UV light transillumination.

2.3. Statistical analysis

We utilized direct counting for the observed phenotype frequencies of KIR. SPSS 15.0 was used to conduct statistical analysis. Genetic expression was expressed as number and frequency. Chi-square was used to test for association between group (case vs control) and genetic expression. A p -value less than 0.05 was considered statistically significant.

Table 4

Comparison and statistically significant difference between CLL patients and healthy controls.

	Negative N = 120	CLL N = 56	p-value
2DL1	119 (99.2%)	54 (96.4%)	0.24
2DL2	73 (60.8%)	33 (58.9%)	0.47
2DL3	106 (88.3%)	45 (80.4%)	0.12
2DL4	120 (100%)	56 (100%)	–
2DL5A	38 (31.7%)	20 (35.7%)	0.36
2DL5B	45 (37.5%)	22 (39.3%)	0.47
2DS1	49 (40.8%)	23 (41.1%)	0.55
2DS2	73 (60.8%)	35 (62.5%)	0.48
2DS3	45 (37.5%)	26 (46.4%)	0.17
2DS4*001/002	20 (16.7%)	18 (32.1%)	0.02
2DS4*003/007	99 (82.5%)	42 (75.0%)	0.17
2DS5	34 (28.3%)	24 (42.9%)	0.04
3DL1	115 (95.8%)	51 (91.1%)	0.29
3DL2	120 (100%)	56 (100%)	–
3DL3	120 (100%)	56 (100%)	–
3DS1	45 (37.5%)	26 (46.4%)	0.26
2DP1	116 (96.7%)	54 (96.4%)	1
3DP*001/002/004	33 (27.5%)	15 (26.8%)	0.54
3DP1*003	120 (100%)	55 (98.2%)	0.32

Bold numbers correspond to the significant p-values.

A p-value <0.05 was considered significant.

3. Results

KIR genotypic profile distribution among the 56 Lebanese patients with CLL is shown in Table 1 while Table 2 shows the distribution of the different KIR genotypes among the 120 healthy Lebanese controls.

In both the patients and controls, the content of KIR genes ranged from 6 to 15 and as per Table 3, the AA, AB, and BB genotypes frequencies among the CLL patients were, respectively, 38%, 46%, and 16% with an A:B ratio of 1.55:1. As for the healthy controls, the AA, AB, and BB genotypes frequencies were, respectively, 39.17%, 50%, and 10.83% with an A:B ratio of 1.79:1.

Table 4 shows the distribution of the different KIR genes among the 120 healthy controls and the 56 CLL patients. *KIR 2DL4*, *KIR 3DL2*, and *KIR 3DL3* were present in all individuals. *KIR 2DS4*001/002* and *KIR 2DS5* were found to be significantly (with a p-value of 0.02 and 0.04, respectively) more prevalent among CLL patients as compared to controls.

4. Discussion

The international literature is adding up more information related to the relationship between KIR genes and different diseases and clinical entities. Based on the gene content, two groups of KIR haplotypes, A and B, have been identified in humans. Haplotype A encodes inhibitory receptors and consists of nine genes (3DL3, 2DL3, 2DP1, 2DL1, 3DP1, 2DL4, 3DL1, one activating (2DS4), 3DL2, and 2DL5) whereas haplotype B carries a variety of gene combinations and encodes more activating receptors as compared to haplotype A (3DL3, 2DS2, 2DL2, 2DL5B (inhibitory) 2DS3, 2DP1, 2DL1, 3DP1, 2DL4, 3DS1, 2DL5A (inhibitory), 2DS5, 2DS1, and 3DL2) (McQueen et al., 2007; Bashirova et al., 2011).

Our study data are consistent with the presence of the two major KIR haplotypes, group A and group B with predominance of the group A haplotype among the chronic lymphocytic leukemia patients similar to its prevalence among healthy individuals. In addition, and among the former, the frequency distribution of AA, AB, and BB genotypes was 38%, 46%, and 16% respectively, reflecting the low occurrence of BB homozygous state among the CLL patients, which is also the case in healthy individuals. All our genotypes included between 6 and 15 genes with an average number of 11 KIR loci per patient (excluding the pseudogenes *KIR2DP1* and *KIR3DP1*), which is comparable to the 11 KIR loci in the general healthy population (Mahfouz et al., 2006).

The interesting observation of the significant increase of *KIR2DS4* and *KIR2DS5* genes, both activating, among chronic lymphocytic leukemia patients is worth further clinical and translational research in order to better understand the pathogenesis of this expression. Our study is the first to report the KIR genes' prevalence among CLL patients and compared it to a large healthy control population in the Mediterranean region. Future research studies will be needed to follow on the significance of these findings especially at the prognosticating level. This is important since the current study did not include any clinical information pertaining to the CLL cases and is purely a prevalence report with exciting information related to two KIR genes.

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