Meta Gene 4 (2015) 57–63

Contents lists available at ScienceDirect

Meta Gene

KIR genotype distribution among Lebanese patients with Hodgkin's lymphoma

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ARTICLE INFO

Article history:
Received 31 October 2014
Revised 23 January 2015
Accepted 27 February 2015
Available online 25 March 2015

Keywords:
Hodgkin's
Lymphoma
KIR
Genotype
Lebanon

ABSTRACT

Introduction: In addition to their important role in fighting infection, natural killer cells are cytotoxic to cancer cells. Studies demonstrated that some KIR genes were responsible for the reduction of the risk of Hodgkin's lymphoma (HL) while others were associated with an increased risk of HL.
Aim: The aim of this study is to assess KIR genotypic distribution in Lebanese patients with Hodgkin's lymphoma.
Methods: KIR genotype was analyzed in 41 HL patients and 120 healthy Lebanese individuals using the *KIR Genotyping SSP kit*.
Results: No significant association between HL and any KIR gene was found. Among HL patients, the AA, AB, and BB genotype frequencies were, respectively, 41.46%, 43.9% and 14.63% with an A:B ratio of 1.73:1. As for the controls, the AA, AB, and BB genotype frequencies were, respectively, 39.17%, 50%, and 10.83% with an A:B ratio of 1.79:1.
Conclusion: In this first study from the Mediterranean region, KIR genotype does not seem to be associated with Hodgkin's lymphoma. Further clinical and translational research is needed to rule out the protective or predisposing role of KIR genes in this important clinical entity.

Abbreviations: KIR, Killer Cell Immunoglobulin-like Receptors; NK, Natural killer; HL, Hodgkin's Lymphoma; IRB, Institutional Review Board; DNA, Deoxyribonucleic acid; PCR, Polymerase Chain Reaction; SSP, Sequence Specific Primers; LRC, leukocyte Receptor Complex; LPHL, Lymphocyte predominant Hodgkin's lymphoma; HLA, Human Leukocyte Antigens; MHC, Major Histocompatibility Complex; UV, Ultraviolet.

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http://dx.doi.org/10.1016/j.mgene.2015.02.004

Introduction

Natural killer (NK) cells are important components of the innate immune system and they represent a first line of defense against infections. These cells were also found to play a role in the killing of some tumor cell lines characterized by little or no major histocompatibility complex (MHC) class I molecule expression. The action of NK cells is controlled by inhibitory and activating receptors, as well as the interaction of these receptors with MHC class I antigens on host cells (Mahfouz et al., 2009; Moretta et al., 2008).

Human NK cells possess various receptors that are structurally different including killer cell immunoglobulin-like receptors (KIRs) and the CD94/NKG2 receptor system. In addition to few pseudogenes, 16 different KIR genes are localized within the Leukocyte Receptor Complex (LRC) region on chromosome 19q13.4 and are highly polymorphic (Gardiner, 2007). There are two main groups of KIR haplotypes: group A including three genes encoding for inhibitory KIRs (2DL1, 2DL2 and 3DL1) and one activating KIR (2DS4), and group B including seven genes (2DS1, 2DS2, 2DS3, 2DL2, 2DL5, 3DS1 and 2DS5) and mostly encoding for activating receptors (Rea et al., 2013). In general, NK cells are richer with activating receptors than with inhibitory receptors (Cheent and Khakoo, 2008).

NK cells mediated cytotoxicity and T-cell receptor (TCR) mediated killing are inhibited due to the ligation action between KIR and HLA class I molecules on target cells and this inhibition effect of KIRs is controlled by immunoreceptor tyrosine based inhibition motifs (ITIM) (Dukers et al., 2001).

Hodgkin's lymphoma (HL) is divided into two entities: classical HL that accounts for 95% of HL cases and Lymphocyte predominant Hodgkin's lymphoma (LPHL). In both types, neoplastic cells represent less than 2% of the total tumor load (Diehl et al., 2003). Classical Hodgkin's lymphoma is subdivided into four categories: nodular sclerosing HL, mixed cellularity HL, lymphocyte depleted HL and lymphocyte-rich HL (Küppers, 2009).

Treatment of HL can achieve a cure rate of 80–90%. Treatment protocols involve multi-agent chemotherapy, including ABVD regimen (doxorubicin, bleomycin, vinblastine, and dacarbazine), and/or radiotherapy (Diehl et al., 2003; Küppers, 2009).

According to Kuppers, Epstein–Barr virus (EBV) was found positive in almost 40% of Hodgkin's lymphoma cases in the Western community (Küppers, 2009). EBV is thought to transform B cells in immunosuppressed patients and by doing so, to cause lymphoma. EBV-transformed B cells are resistant to NK cells due to the high expression of HLA class I molecules on these cells. However, EBV-transformed B cells become prone to NK cell killing activity when latent EBV is reactivated (Lanier, 2008). A familial study done in France shows that KIR3DS1, KIR2DL5, KIR2DS5 and KIR2DS1 reduce the risk of Hodgkin's lymphoma while KIR2DS4 is associated with a higher risk of HL (Besson et al., 2007). However, Besson et al. did not find any significant association in a tentative replication case/control study of 68 HL cases.

Here, we studied KIR expression of NK cells in HL patients and healthy controls, to check for any association between certain KIR genotypes and Hodgkin's lymphoma. To our knowledge, this the first study in Lebanon that targets the correlation between KIR genotypes and HL. Previous studies were performed among the Lebanese population to investigate the role of KIR genes in various conditions including tuberculosis (Mahfouz et al., 2011), familial Mediterranean fever (Mahfouz et al., 2009) and Follicular lymphoma (Khalaf et al., 2013).

No association was found between KIR genotyping profile and Follicular lymphoma, however, in the tuberculosis study, more inhibitory KIR genes were found in patients compared to controls. As for the FMF study, KIR3DP1*003 was more frequently detected among patients and the BB genotype was more prevalent in this category.

Materials and methods

Study population

The 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 41 patients with Hodgkin's lymphoma and 120 healthy controls, using the *KIR Genotyping SSP kit*. The HL patients were diagnosed in the department of Pediatrics and Internal Medicine and the healthy controls belong to a pool of healthy Lebanese Bone Marrow Transplantation donors at AUBMC.

Table 1
KIR genotypic profile among the 41 Hodgkin's lymphoma patients.

Profile	N	%	# of genes	2DL1	2DL2	2DL3	2DL4	2DL5A	2DL5B	2DS1	2DS2	2DS3	2DS4*001	2DS4*003	2DS5	3DL1	3DL2	3DL3	3DS1	2DP1	3DP*001	3DP1*003
AA	9	21.95	7	■		■	■							■		■	■	■		■		■
AA	1	2.44	7	■			■						■	■		■	■	■		■		■
AA	2	4.88	8	■		■	■						■	■		■	■	■		■		■
AA	1	2.44	9	■		■	■	■		■					■		■	■	■	■		■
AA	1	2.44	10	■		■	■			■				■	■	■	■	■	■	■		■
AA	1	2.44	10	■		■	■	■		■					■	■	■	■	■	■		■
AA	1	2.44	11	■		■	■	■		■			■		■	■	■	■	■	■		■
AA	1	2.44	11	■		■	■	■			■	■	■			■	■	■	■	■		■
AB	1	2.44	8	■	■	■					■		■			■	■		■	■	■	■
AB	1	2.44	9	■	■	■	■			■	■				■	■		■	■	■		■
AB	1	2.44	9	■	■	■	■				■		■			■	■	■	■	■	■	■
AB	1	2.44	9	■	■	■	■				■			■		■	■	■	■	■		■
AB	1	2.44	10	■	■	■	■				■			■	■	■	■	■	■	■	■	■
AB	1	2.44	11	■	■	■	■		■		■	■	■			■	■	■	■	■		■
AB	1	2.44	11	■			■	■			■	■	■	■		■	■	■	■	■	■	■
AB	1	2.44	12	■	■	■	■		■	■	■			■	■	■	■	■	■	■		■
AB	1	2.44	12	■	■	■	■			■	■		■		■	■	■	■	■	■	■	■
AB	1	2.44	12	■	■	■	■		■		■	■		■	■	■	■	■	■	■		■
AB	1	2.44	12	■	■	■	■		■		■	■	■		■	■	■	■	■	■	■	■
AB	1	2.44	12	■	■	■	■	■		■	■			■	■	■	■	■	■	■	■	■
AB	4	9.75	14	■	■	■	■	■	■	■	■	■		■		■	■	■	■	■		■
AB	2	4.88	15	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■		■
BB	1	2.44	6		■						■			■		■	■	■			■	■
BB	1	2.44	12	■	■		■		■	■	■	■		■	■	■	■	■		■		■
BB	1	2.44	12	■	■		■		■	■	■	■			■	■	■	■	■	■		■
BB	1	2.44	12	■	■		■	■	■	■	■	■			■		■	■	■	■		■
BB	2	4.88	14	■	■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■
	41	100																				

Table 2
KIR genotypic profile among the 120 healthy controls.

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

Table 3
KIR gene frequency among HL patients and healthy controls.

	Negative	HL	p-Value
	N = 120	N = 41	
2DL1	119 (99.2%)	40 (97.6%)	0.45
2DL2	73 (60.8%)	25 (61.0%)	0.57
2DL3	106 (88.3%)	34 (82.9%)	0.26
2DL4	120 (100%)	39 (95.1%)	0.06
2DL5A	38 (31.7%)	15 (36.6%)	0.35
2DL5B	45 (37.5%)	15 (36.6%)	0.54
2DS1	49 (40.8%)	19 (46.3%)	0.33
2DS2	73 (60.8%)	25 (61.0%)	0.57
2DS3	45 (37.5%)	16 (39.0%)	0.50
2DS4*001	20 (16.7%)	11 (26.8%)	0.12
2DS4*003	99 (82.5%)	29 (70.7%)	0.08
2DS5	34 (28.3%)	16 (39.0%)	0.14
3DL1	115 (95.8%)	39 (95.1%)	1.00
3DL2	120 (100%)	40 (97.6%)	0.25
3DL3	120 (100%)	41 (100%)	–
3DS1	45 (37.5%)	20 (48.8%)	0.14
2DP1	116 (96.7%)	40 (97.6%)	1.00
3DP*001	33 (27.5%)	10 (24.4%)	0.43
3DP1*003	120 (100%)	41 (100%)	1.00

p-Value <0.05 was considered significant.

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* (Cat no. 789303) 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*, *2DP1*, 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*. Internal controls are included as part of the assay.

KIR genotyping was performed as recommended by the manufacturer. Briefly, 25 μl of DNA was 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

Table 4
Frequency distribution of KIR genotypes and haplotypes among HL patients and control.

Genotype	Controls		HL patients	
	No.	%	No.	%
AA	51	42.5	17	41.46
AB	60	50	18	43.90
BB	9	7.5	6	14.63
Total	120	100	41	100

Haplotype	Controls	HL patients
A	162	52
B	78	30
A:B ratio	2.08:1	1.73:1

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 on 2% agarose gel was done in ethidium bromide and visualization performed under UV light transillumination.

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. p-Value less than 0.05 was considered statistically significant.

KIR haplotype was inferred from the genotype as follows:

$$\text{A haplotype} = (\text{Number of AA individuals} \times 2) + \text{Number of AB individuals}$$
$$\text{B haplotype} = (\text{Number of BB individuals} \times 2) + \text{Number of AB individuals.}$$

Results

KIR genotypic profile distribution among the 41 Hodgkin's lymphoma Lebanese patients is shown in Table 1. The content of KIR genes ranged from 6 to 15 and as per Table 4, the AA, AB, and BB genotype frequencies were, respectively, 41.46%, 43.9% and 14.63% with an A:B ratio of 1.73:1.

Table 2 shows the distribution of different KIR genotypes among the 120 healthy Lebanese controls. The content of KIR genes ranged from 6 to 15 and, as per Table 4, the AA, AB, and BB genotype frequencies were, respectively, 39.17%, 50%, and 10.83% with an A:B ratio of 1.79:1.

Table 3 shows the distribution of different KIR genes among the 120 controls and the 41 HL patients. No significant difference between patients and controls was detected.

In this genetic prevalence study, no clinical information has been collected for the participating patients and controls. Therefore, the results obtained are descriptive in nature and no extrapolation to outcome, survival, or response to treatment could be analyzed in this research.

Discussion

Natural killer (NK) cells are known to induce antiviral and antitumor immunity via production of pro-inflammatory cytokines and based on their KIR gene content, two groups of haplotypes are known in humans: A and B. 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) (Middleton and Gonzelez, 2009). It is the balance between the inhibitory and activating signals that regulates the function of NK cells and predisposes or protects an individual against microbes or diseases, according to the building international literature about KIR genotypic profiling.

The study conducted by Besson et al. is the single international research paper reported in the literature that described the association between Hodgkin's lymphoma and certain KIR genes (Besson et al., 2007). The study found out that five KIR genes were found significantly associated with HL supported by a dominant protective effect of KIR3DS1 and/or KIR2DS1 and applicable to familial cases included in the Besson et al. series. However, when a case–control replication study was performed using 68 HL patients, no significant association was found with any KIR gene. The interesting aspect of Besson's article is its reporting on variable clinical parameters related to the patients in question, specifically the EBV status of the Hodgkin lymphoma patients, which we did not have in our current research because of the genetically prevalent nature of this study.

Our research paper is the second international paper assessing the association between KIR genes and Hodgkin's lymphoma, however, the first in the Mediterranean region and involving a different ethnic population of patients. The concordant results of both international studies may be enough to state that KIR

genes probably do not have any role in the pathogenesis of Hodgkin's lymphoma and we strongly advocate for further larger studies with clinical outcome assessment.

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