Human Immunology 80 (2019) 842–847

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Haplotype block 1 variant (HB-1v) of the NKG2 family of receptors

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ARTICLE INFO

Keywords:
NKG2
Receptors
Frequency
Haplotype

ABSTRACT

The natural killer group 2 (NKG2) family of receptors, encoded within the NK complex gene region (NKC), modulate the cytotoxic activity of NK cells. Two haplotype blocks throughout the NKC, hb-1 and hb-2 have been associated with different levels of overall natural cytotoxicity. Here, we evaluated allelic and genotype frequencies at rs1049174, rs2617160, rs2617170, rs2617171, rs1983526 (hb-1 haplotype), and rs2255336 and rs2246809 (hb-2 haplotype) in 928 subjects examined from Mexico City. The most frequent alleles and genotypes were as follows: C, CG to rs1049174; G, GG to rs2255336; T, AT to rs2617160; G, GG to rs2246809; C, CT to rs2617170; G, CG to rs2617171; and G, CG to rs1983526. Linkage disequilibrium analysis revealed that rs1049174, rs2617160, rs2617170, and rs2617171 constituted the haplotype block-1 variant (hb-1v) ($r^2 \geq 0.89$). Two predominant haplotypes of hb-1v were identified based on the allele content and included CTCG and GATC. This study is the first to evaluate the allelic and genotype frequency distribution of rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526 in the population of Mexico City.

1. Introduction

Natural Killer (NK) cells are lymphocytes of the innate immune system with cytotoxic activity and cytokine-producing function [1,2]. NK cells participate in immune surveillance and respond in the absence of prior stimulation against virus-infected cells and tumor cells [3,4]. NK cells discriminate between target cells and other healthy "self" cells through a balance of activating and inhibitory receptors that are expressed on their surfaces. Thus, NK activation relies on the integration of activating and inhibitory pathways following interaction with a target cell [5].

Natural killer group 2 (NKG2) is a family of receptors comprising five members (NKG2A, NKG2C, NKG2D, NKG2E and NKG2F). These receptors are type II transmembrane proteins with a C-type lectin-like extracellular domain and are expressed on NK cells. NKG2D receptor is also expressed on NKT cells, γδ T cells, CD8+ T cells and a small subset of effector or memory CD4+ T cells [6–8]. Each receptor exhibits a different function. NKG2A is a unique inhibitory receptor of this family, whereas NKG2C and NKG2D are activating receptors [5]. It was recently reported that NKG2E may function as an intracellular protein, whereas the function of NKG2F receptor remains unclear [9–11]. The ligands of NKG2 receptors are different. NKG2A, NKG2C and NKG2E receptors bind to the nonclassical major histocompatibility complex (MHC) protein HLA-E [12], which presents the peptides derived from the conserved region of the leader sequences of MHC class I molecules. The interaction between HLA-E and NKG2 receptors facilitates the function of NK cells to monitor the expression of MHC class I molecules on target cells and control the capacity to process and present antigen [5]. Although NKG2A and NKG2C receptors have a common ligand, both receptors transmit opposing signals via different intracellular domains [13]. NKG2A contains two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) in its long cytoplasmic tail. In contrast, NKG2C has a short cytoplasmic tail with a lysine residue in the transmembrane domain that contributes to the association with an adapter molecule (DAP12), containing immunoreceptor tyrosine-based activation motif (ITAM). The NKG2D receptor also has a short cytoplasmic tail that is associated with DAP10, which has a YINM motif essential for signaling [5,8,13]. The NKG2D receptor binds to the MHC class I chain-related like molecules A and B (MICA/B) and UL16-binding proteins (ULBPs). The expression of NKG2D ligands on target cells is frequently attributed to the cellular stress caused by events such as oncogenic transformation or viral infection [7,8,14,15].

The members of the family of NKG2 receptors are encoded by genes on human chromosome 12p13 within a region of approximately 2.5 Mb,

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https://doi.org/10.1016/j.humimm.2019.07.276
Received 24 September 2018; Received in revised form 5 July 2019; Accepted 8 July 2019
Available online 15 July 2019

Table 1
Primers for SNP sequencing assays.

SNP	Chr 12: position	Gene	Genic Location	Primer sequence (5′ → 3′)	Product size (bp)
rs1049174	10372766	*KLRK1*	Exon 8	F: CACAAGCCCAGAGTGGATGG R: TTGGGTGGAGGACCCATTA	236
rs2255336	10379727	*KLRK1*	Exon 4	F: TCTAGGGATGACTGGGGAACT R: TGTTGCAATCTACTTCTCTGTTGT	358
rs2617160	10392998	*KLRC4-KLRK1*	Intergenic	F: AGGAAGCTGTGCCAGAGAAAA R: GCATCTATGGCCACACCACC	326
rs2246809	10404445	*KLRC4-KLRK1*	Intergenic	F: TGGAATGATACATGTTTTCTCTGC R: ACAAACAGAAATCTGAGTAACCTCT	405
rs2617170	10408358	*KLRC4*	Intron 2	F: TTGGAAGCGCCTTGAAACATT	689
rs2617171	10408680	*KLRC4*	Exon 3	R: AAAGGACATGCCCTCATATAATCT	
rs1983526	10455414	*KLRC1*	−729 bp	F: ACAGACCTTGAAGTGGACCC R: CATGATCCTTTTACTAGGGCTTCT	125

SNP: single nucleotide polymorphism; Chr: chromosome; F: forward primer sequence; R: reverse primer sequence; bp: base pair.

termed as the natural killer complex (NKC). This cluster is flanked by *KLRD1* (CD94) gene on the telomeric side and *Ly49* gene on the centromeric side. From the telomeric to centromeric region, the NKG2 cluster of 270 kb comprises the following genes: *KLRK1* (NKG2D), *KLRC4* (NKG2F), *KLRC3* (NKG2E), *KLRC2* (NKG2C), and *KLRC1* (NKG2A) [6,16,17]. To investigate whether the differences between the individuals with respect to natural immunological host defense predict the future development of cancer, Imai *et al.* performed an 11-year follow-up study in a group of 3625 healthy individuals. These authors found that the individuals with low cytotoxic activity had a higher risk of developing cancer than those with high cytotoxic activity [18]. Furthermore, cytotoxic activity assays and polymorphism studies revealed the association of NK cell cytotoxic activity with seven single nucleotide polymorphisms (SNPs) located within the NKC gene region, including rs1049174 (*KLRK1*), rs2255336 (*KLRK1*), rs2617160 (*KLRC4-KLRK1*), rs2246809 (*KLRC4-KLRK1*), rs2617170 (*KLRC4*), rs2617171 (*KLRC4*) and rs1983526 (*KLRC1*) [18,19]. These SNPs are in linkage disequilibrium (LD) and generate two haplotype blocks. The haplotype block 1 (hb-1) is constructed from the telomere to the centromere by rs1049174, rs2617160, rs2617170, rs2617171 and rs1983526, whereas the haplotype block 2 (hb-2) is generated by rs2255336 and rs2246809. The two blocks were subsequently studied and classified in four major haplotype alleles, based on the cytotoxic activity of NK cells and the allele content. In this context, CTCCC haplotype (LNK1, low activity) and GATGG haplotype (HNK1, high activity) were found to belong to hb-1. Both haplotypes are located in rs1049174, rs2617160, rs2617170, rs2617171, and rs1983526. The GG haplotype (LNK2, low activity) and AA haplotype (HNK2, high activity), on the other hand, were shown to belong to hb-2 and were located in rs2255336 and rs2246809, respectively [19,20]. To determine whether these SNPs can generate different haplotypes, we studied the allele and genotype frequency distributions of rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526 as well as haplotype generation through LD analysis.

2. Materials and methods

2.1. Study subjects

The seven SNPs of NKG2 were examined in a total of 928 unrelated healthy adults. The individuals were selected from blood donors of a general population that attended blood banks of the Instituto Mexicano del Seguro Social (IMSS), Mexico City. All included subjects were born and living in Mexico City for the last three generations, thereby avoiding variations in the admixture of the Mexican population according to the geographical area. All participants were over 30 years old, without any symptoms of disease, cancer history, genetic or autoimmune disease and treatment of antibiotics or medications. The individuals were informed about the nature of the study, and the participating individuals were asked to sign a consent letter. The study was approved by the ethics committee from the National Council for Research on Health, IMSS, Mexico City.

2.2. DNA samples

A blood sample anti-coagulated with ethylenediaminetetraacetic acid (EDTA)-K2 was drawn from each participant. The genomic DNA was isolated from peripheral blood leukocytes using the salting-out method [21].

2.3. Genotyping

The genotyping of rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526 was performed by real-time polymerase chain reaction (PCR) using Allelic Discrimination TaqMan Assays Applied Biosystems™ (Thermo Fisher Scientific; Waltham, MA, USA). Genotyping was performed according the instructions recommended by the manufacturer with the following available assays: C_9345347-10 (rs1049174), C_22274447-10 (rs2255336), C_1841959-10 (rs2617160), C_1842497-10 (rs2246809), C_1842316-10 (rs2617170), C_26984346-10 (rs2617171), and C_11919464-10 (rs1983526).

2.4. Sequencing

Samples with homozygous genotypes were subjected to direct sequencing using specific primers for each SNP (Table 1). PCR was performed in a final volume of 25 µL containing 125 ng of genomic DNA, 1.5 mM of magnesium chloride ($MgCl_2$), 0.5 pmol of each primer (IDT; San Jose, California, USA), 1 mM dNTPs, 1 × PCR buffer, and 0.1 U Platinum™ Taq DNA Polymerase (Thermo Fisher Scientific; Waltham, MA, USA). The sequences of the forward and reverse primers used for each SNP are listed in Table 1. PCR was performed in a thermal-cycler Applied Biosystems Veriti Thermal Cycler (Thermo Fisher Scientific; Waltham, MA, USA). The PCR reaction conditions were as follows: an initial denaturation at 95 °C for 5 min was followed by 33 cycles at 95 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 30 s, with a final elongation cycle at 72 °C for 7 min. The annealing of rs1983526 was carried out at 61 °C. The PCR products were purified by ExoSAP-IT™ PCR Product Cleanup (Thermo Fisher Scientific; Waltham, MA, USA) in accordance with the instructions recommended by the manufacturer. The purified PCR products were sequenced using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific; Waltham, MA, USA) according the instructions recommended by the manufacturer using Applied Biosystems 3730 × L DNA Analyzer (Thermo Fisher Scientific; Waltham, MA, USA).

2.5. Statistical analysis

The median and interquartile range (IQR) were calculated to describe the age of individuals. The deviation from Hardy-Weinberg

equilibrium (HWE) in all SNPs studied was examined with a goodness-of-fit X^2 test with Haploview software version 4.0 [22]. The allelic and genotype frequencies were estimated by direct counting, whereas the haplotype frequencies were performed using Haploview software. The allelic and genotype frequencies differences were analyzed using X^2 test. Statistical significance was set at $p < 0.05$. LD analysis was performed using Haploview software and haplotype generation was considered at $r^2 \geq 0.80$ [23].

3. Results

3.1. Characteristics of the population

The studied population comprised 53.8% women (499/928) and 46.2% men (429/928). The median age was 36 years, and the interquartile range was 29–45 years. The frequency distribution for NKG2 SNPs was in accordance with H-W equilibrium ($p > 0.05$).

3.2. Allele and genotype frequencies

The more frequent alleles of rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526 were C, G, T, G, C, G and G, respectively (Table 2). We compared these allele frequencies with the frequencies reported in other populations, such as those with Mexican ancestry from Los Angeles California, African, European, South Asian, and Japanese populations (Table 3) [19,24]. In our population, the frequencies of C (rs1049174), T (rs2617160), C (rs2617170), and G (rs2617171) alleles were lower than the previously reported frequencies for the subjects with Mexican ancestry from Los Angeles, California ($p < 0.05$). The frequencies of the SNPs rs2255336, rs2246809, and rs1983526 were similar in both populations [24]. In African population, the frequencies of C (rs1049174), G (rs2255336), T (rs2617160), G (rs2246809), C (rs2617170), and G (rs2617171) alleles were lower, and the frequency of G (rs1983526) allele was higher than the frequencies reported for the subjects from Mexico City ($p < 0.05$) [24]. In European population, C (rs1049174), T (rs2617160), G (rs2617171) and G (rs1983526) alleles were more frequent, and G (rs2255336) and G (rs2246809) alleles were less frequent than in our population ($p < 0.05$) [24]. The frequency of C (rs2617170) was similar between European and Mexico City populations. In the South Asian population, C (rs1049174), T (rs2617160), G (rs2246809), C (rs2617170), and G (rs2617171) alleles were less frequent, and G (rs1983526) allele was more frequent than in the Mexico City population ($p < 0.05$) [24]. The frequency of G (rs2255336) was similar between South Asian and Mexico City populations. The allele frequency results were different in all SNPs studied as compared with the Japanese population [19]. The frequencies of six SNPs C (rs1049174), G (rs2255336), T (rs2617160), G (rs2246809), C (rs2617170), and G (rs2617171) alleles were lower and G (rs1983526) allele was higher in the Japanese population than in our population ($p < 0.05$) (Table 3). Of note, the most striking differences in allele and genotype distribution between Mexicans and Japanese were at rs2617171 (included in hb-1 haplotype block), rs2255336 and rs2246809 (hb-2 haplotype block) ($p < 0.0001$).

The results of our genotype analysis showed that the heterozygotes CG (rs1049174), AT (rs2617160), CT (rs2617170), CG (rs2617171), and CG (rs1983526) and the homozygotes GG (rs2255336) and GG (rs2246809) were the most frequent genotypes (Table 2). The genotype frequencies were compared between different populations [24] and the genotype distribution was found to be different in each population (Table 3).

Table 2
Allele and genotype frequencies in Mexico City population.

SNP	Chr 12: position	Allele	2n = 1856 AF (n)	Genotype	n = 928 GF (n)
rs1049174	10372766	C	0.628 (1166)	CC	0.390 (362)
		G	0.372 (690)	CG	0.476 (442)
				GG	0.134 (124)
rs2255336	10379727	G	0.929 (1724)	GG	0.863 (801)
		A	0.071 (132)	AG	0.131 (122)
				AA	0.005 (5)
rs2617160	10392998	T	0.634 (1176)	TT	0.391 (363)
		A	0.366 (680)	AT	0.485 (450)
				AA	0.124 (115)
rs2246809	10404445	G	0.930 (1727)	GG	0.865 (803)
		A	0.070 (129)	AG	0.130 (121)
				AA	0.004 (4)
rs2617170	10408358	C	0.627 (1164)	CC	0.380 (353)
		T	0.373 (692)	CT	0.494 (458)
				TT	0.126 (117)
rs2617171	10408680	G	0.623 (1156)	GG	0.376 (349)
		C	0.377 (700)	CG	0.494 (458)
				CC	0.130 (121)
rs1983526	10455414	G	0.505 (937)	GG	0.250 (232)
		C	0.495 (919)	CG	0.510 (473)
				CC	0.240 (223)

n: individuals; SNP: single nucleotide polymorphism; Chr: chromosome; AF: allele frequency; GF: Genotype frequency.

3.3. Haplotype analysis

We performed LD analysis to determine whether the seven SNPs (rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526) could generate the previously reported hb-1 and hb-2 haplotypes [19]. The SNPs constituted a haplotype at $r^2 \geq 0.80$ between each pair. LD analysis revealed one haplotype formed by rs1049174, rs2617160, rs2617170, and rs2617171 ($r^2 \geq 0.89$). However, rs1983526 showed no LD (≤ 0.51) with either of the SNPs studied; hence, rs1983526 was excluded from this haplotype, as it was reported for hb-1 haplotype [19]. In fact, rs1983526 failed to generate any haplotype. Thus, the haplotype generated was considered a variant and was named as haplotype block-1 variant (hb-1v) as per a previously reported nomenclature (Fig. 1) [19]. Regarding rs2255336 and rs2246809, the allele and genotype frequencies between both SNPs were similar (Table 2). However, the minor-allele frequencies for both of them were low (0.071 and 0.070, respectively). Of note, all individuals (4, 0.4%) who carry the rs2246809 AA genotype also carry the rs2255336 AA genotype. Though initial LD estimation showed low LD between each other ($r^2 = 0.78$) and no LD with any other SNP studied, r^2 is not appropriate for LD assessment when minor-allele frequencies are low. We thus calculated D′ value and found strong LD (D′ = 0.89) between these two SNPs.

3.4. Haplotypes from haplotype block-1 variant

We found two predominant haplotype alleles of hb-1v; the dominant CTCG and the recessive GATC (rs1049174, rs2617160, rs2617170, and rs2617171, respectively in both haplotypes). Moreover, the LD ($r^2 \geq 0.89$) among CTCG and GATC was supported by sequencing. As was mentioned above, the hb-1v was considered a variant of hb-1, previously reported [19]. In this context, the difference between the haplotype alleles of hb-1v and hb-1 was based on differences in the genotype frequency distribution of rs2617171 between Japanese and Mexico City populations (Table 3) [19].

The CTCG haplotype showed a frequency of 0.610, whereas the GATC haplotype revealed a frequency of 0.356 (Table 4). The most frequent haplotype was heterozygote CTCG/GATC, followed by homozygote CTCG/CTCG and homozygote GATC/GATC (Table 4).

We studied the different combinations of these seven SNPs to determine the most frequent combination, although not all the SNPs were included in LD. The CGTGCGC alleles of rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526 were the most frequent (0.472), followed by the GGAGTCG alleles of the same SNPs (0.282).

Table 3
Comparison with allele and genotype frequencies for other populations.

SNP	Allele Genotype	Population					
		Mexico City n = 928	Mexican ancestry[a] n = 64	African n = 661	European n = 503	South Asian n = 489	Japanese n = 408
		Frequency (n)					
rs1049174	C	0.628 (1166)	0.734 (94)*	0.246 (325)*	0.690 (694)*	0.553 (541)*	0.581 (474)*
	CC	0.390 (362)	0.562 (36)*	0.054 (36)*	0.501 (252)*	0.311 (152)*	0.348 (142)
	CG	0.476 (442)	0.344 (22)*	0.383 (253)*	0.378 (190)*	0.485 (237)	0.466 (190)
	GG	0.134 (124)	0.094 (6)	0.563 (372)*	0.121 (61)	0.204 (100)*	0.186 (76)*
rs2255336	G	0.929 (1724)	0.883 (113)	0.553 (731)*	0.817 (822)*	0.910 (890)	0.772 (630)*
	GG	0.863 (801)	0.781 (50)	0.315 (208)*	0.674 (339)*	0.830 (406)	0.603 (246)*
	AG	0.131 (122)	0.203 (13)	0.477 (315)*	0.286 (144)*	0.160 (78)	0.338 (138)*
	AA	0.005 (5)	0.016 (1)	0.209 (138)*	0.040 (20)*	0.010 (5)	0.059 (24)*
rs2617160	T	0.634 (1176)	0.734 (94)*	0.523 (692)*	0.689 (693)*	0.548 (536)*	0.569 (464)*
	TT	0.391 (363)	0.562 (36)*	0.272 (180)*	0.497 (250)*	0.303 (148)*	0.331 (135)*
	AT	0.485 (450)	0.344 (22)*	0.502 (332)	0.384 (193)*	0.491 (240)	0.475 (194)
	AA	0.124 (115)	0.094 (6)	0.225 (149)*	0.119 (60)	0.207 (101)*	0.194 (79)*
rs2246809	G	0.930 (1727)	0.891 (114)	0.737 (974)*	0.819 (824)*	0.908 (888)*	0.771 (629)*
	GG	0.865 (803)	0.797 (51)	0.551 (364)*	0.676 (340)*	0.826 (404)*	0.598 (244)*
	AG	0.130 (121)	0.188 (12)	0.372 (246)*	0.286 (144)*	0.164 (80)	0.346 (141)*
	AA	0.004 (4)	0.016 (1)	0.077 (51)*	0.038 (19)*	0.010 (5)	0.056 (23)*
rs2617170	C	0.627 (1164)	0.727 (93)*	0.433 (572)*	0.663 (667)	0.544 (532)*	0.562 (459)*
	CC	0.380 (353)	0.547 (35)*	0.174 (115)*	0.455 (229)*	0.303 (148)*	0.324 (132)*
	CT	0.494 (458)	0.359 (23)*	0.517 (342)	0.416 (209)*	0.483 (236)	0.478 (195)
	TT	0.126 (117)	0.094 (6)	0.309 (204)*	0.129 (65)	0.215 (105)*	0.198 (81)*
rs2617171	G	0.623 (1156)	0.719 (92)*	0.240 (317)*	0.663 (667)*	0.536 (524)*	0.436 (356)*
	GG	0.376 (349)	0.531 (34)*	0.067 (44)*	0.455 (229)*	0.294 (144)*	0.196 (80)*
	CG	0.494 (458)	0.375 (24)	0.346 (229)*	0.416 (209)*	0.483 (236)	0.480 (196)
	CC	0.130 (121)	0.094 (6)	0.587 (388)*	0.129 (65)	0.223 (109)*	0.324 (132)*
rs1983526	G	0.505 (937)	0.492 (63)	0.859 (1135)*	0.588 (592)*	0.597 (584)*	0.550 (449)*
	GG	0.250 (232)	0.250 (16)	0.735 (486)*	0.376 (189)*	0.362 (177)*	0.306 (125)*
	CG	0.510 (473)	0.484 (31)	0.247 (163)*	0.425 (214)*	0.470 (230)	0.488 (199)
	CC	0.240 (223)	0.266 (17)	0.018 (12)*	0.199 (100)	0.168 (82)*	0.206 (84)

SNP: single nucleotide polymorphism; n: individuals.
Comparisons were made using the allele and/or genotype frequencies in Mexico City population as the reference group, *$p \leq 0.05$.
[a] Mexican ancestry from Los Angeles California USA population.

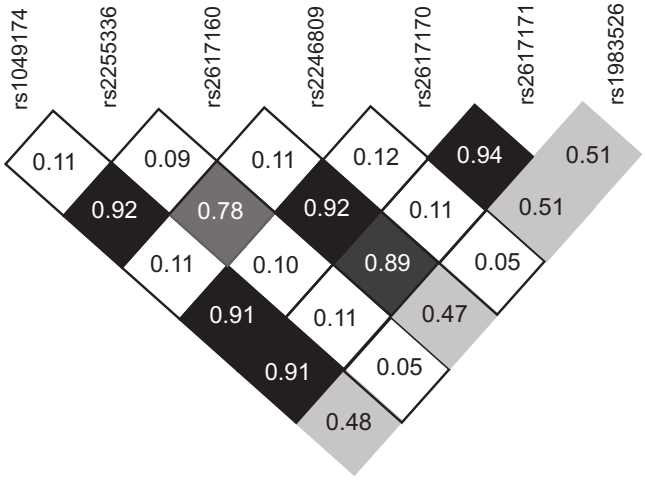

Fig. 1. Identification of haplotype block-1 variant (hb-1v). Genotyping data were linkage disequilibrium analyzed using Haploview 4.0 software. The haplotype generation was considered when $r^2 > 0.80$. In the linkage disequilibrium plot, the value inside of each row represents r^2 value and the color of each row depends on the r^2 value. The black elements indicate that SNPs in the intersection showed $r^2 > 0.80$, dark gray elements $r^2 < 0.80$ and > 0.60, gray elements $r^2 < 0.60$ and > 0.40, light gray elements $r^2 < 0.40$ and > 0.20 and white elements $r^2 < 0.20$.

Table 4
Haplotype block-1 variant (hb-1v) frequencies.

Haplotype[a]	n = 928 Frequency (n)
CTCG	0.610 (1132)
GATC	0.356 (661)
CTCG/CTCG	0.364 (338)
CTCG/GATC	0.460 (427)
GATC/GATC	0.114 (106)

n: individuals.
[a] The order of the SNPs in the haplotypes is according to the positions in the chromosome (rs1049174 C/G, rs2617160 A/T, rs2617170 C/T and rs2617171 C/G).

4. Discussion

The distribution of the allele, genotype, and haplotype frequencies of rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526 in Mexico City was unknown. These variants have been associated with the cytotoxic activities of NK cells and CD8+ T lymphocytes [19,20,25]. Therefore, we characterized the previously identified SNP frequencies. Nevertheless, it is well established that hb-1v, based on LD analysis. The new haplotype comprised rs1049174, rs2617160, rs2617170, and rs2617171.

The SNP frequencies of our population were compared with those of the Mexican population living in Los Angeles, California, USA [24].

Although both populations have the same ancestry, the frequency of SNPs was different between the two groups. While the alleles C (rs1049174), T (rs2617160), C (rs2617170), and G (rs2617171) were less frequent in our population, the frequency of other SNPs was similar. This difference could be attributed to the fact that the Mexican population of Los Angeles originated from different places in the country, whereas the samples studied in the present study were obtained from the same site. The admixture of the Mexican population varies according to the geographical area. Thus, it may be observed that the European ancestry predominates in the north and west of Mexico (66.7–95%), whereas the center and southeast are dominated by the Amerindian ancestry (37–50%) [26]. Mexico City population includes 69.2% of Amerindian genes, 30.2% of European genes, and 0.6% of African genes [27]. We could not compare the allele and genotype frequencies of our population with Mexican Amerindian population because no genotyping data are available for the SNPs studied in the database of Human Genome Diversity Project [28]. Regarding African, European, and South Asian populations, the allele frequencies were totally different from those studied in the present study [24]. We also compared the allele frequencies with the Japanese population because these SNPs were studied for the first time in this population [19]. As expected, the frequencies were different and statistically significant. The frequencies of the six SNPs in our population were higher than those in the Japanese population, whereas the frequency of G (rs1983526) allele was low. Genotype analysis results revealed CG (rs1049174), GG (rs2255336), AT (rs2617160), GG (rs2246809), CT (rs2617170), CG (rs2617171), and CG (rs1983526) as the most frequent genotypes in our population. However, the comparison of our results with the Mexican ancestry from Los Angeles California, African, European, South Asian, and Japanese populations revealed the differences in the genotype frequency distribution in each population [19,24]. This frequency distribution diversity within and between populations are governed by random (genetic drift) and deterministic (natural selection) forces [29,30].

Genotyping of all the SNPs in the human genome may be an expensive and unnecessary process, given the existence of LD among thousands of SNPs. LD refers to the nonindependence of alleles at different sites; thus, the alleles implied construct a haplotype [23]. The SNPs studied in the present report showed LD and allowed for the generation of an haplotype [19]. However, NKG2 haplotype generation has never been analyzed in our population; thus, LD analysis of the studied SNPs was performed to determine their nonrandom association. LD analysis results revealed some interesting observations. We found that rs1049174, rs2617160, rs2617170, and rs2617171 are in LD because they showed r^2 values ≥ 0.80 and consequently constituted a haplotype. This haplotype was named as hb-1v because rs1983526 was not present in this haplotype unlike the observation reported for hb-1 in Japanese population [19]. In accordance with our results, rs1983526 showed no LD with the SNPs mentioned above in the Mexican ancestry from Los Angeles, California population [24]. Therefore, the hb-1v is restricted to *KLRC4-KLRK1* region (rs1049174, rs2617160, rs2617170, and rs2617171). The SNPs rs2255336 and rs2246809 presented similar frequencies in our population, and a weak LD ($r^2 = 0.78$) was observed. However, as the r^2 value between these SNPs did not exceed the given threshold (0.80) we estimated the D′ (0.89) value. Therefore, the value of r^2 between these SNPs may not necessarily indicate the lack of LD for these markers but more likely reflects the low power to detect LD [31,32]. It has been reported that LD differences between populations are the result of historical events [33]. In fact, a single mutation event may give rise to a mutated allele in a specific population, and this allele may have spread and changed in frequency over time owing to some evolutionary events such as admixture, genetic drift, mutation, migration, recombination rate, and natural selection. These events have resulted in specific patterns of LD for each population [34–36].

The comparison between hb-1v and hb-1 haplotypes revealed some interesting observations. For hb-1v, two predominant haplotypes were identified based on the allele content. The most common haplotype was CTCG, followed by GATC haplotype (rs1049174, rs2617160, rs2617170, and rs2617171 in both haplotypes). The allele of the fourth SNP (rs2617171) was different between major hb-1v haplotype allele (CTCG) and LNK1 for hb-1 (CTCC). Interestingly however, the frequencies of these haplotype alleles in Mexicans and Japanese were very similar (0.610 and 0.615, respectively) [19]. A fragment of KLRC4 gene from intron 2 to intron 3 was thus sequenced, and included rs2617170 (exon 3) and rs2617171 (intron 2). Based on our results, we corroborate that G allele (rs2617171) generated CTCG haplotype and that C allele (rs2617171) generated GATC haplotype in Mexicans. This difference between major hb-1v haplotype allele and LNK1 variant of hb-1, associated with low overall NK cell and CD8 T lymphocytes cytotoxic activity, suggests a minor role for rs2617171 as tag SNP [19].

Although different SNPs are present within NKC, we decided to study the allele, genotype, and haplotype frequency distribution of only seven SNPs (rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526) owing to their association with the cytotoxic functions of NK and T CD8+ cells [19,20,25]. The existence of LD among SNPs may allow for the selection of a tag SNP to represent the remaining SNPs [23,37]. In this context, rs1049174 has been characterized as a tag SNP to hb-1 haplotype [25,38]. The SNP rs1049174 is located in the 3′-untranslated region (3′-UTR) of *KLRK1* gene, which is a targeting site for the microRNA (miR-1245) negative regulator of NKG2D receptor expression [25]. NKG2D is an activating and co-stimulatory receptor expressed on NK and CD8 T lymphocytes [7]. The interaction between this activating receptor and its ligands (MICA, MICB, and ULBPs) mediates killing through the perforin cytotoxic pathway [8]. Although the hb-1 haplotype includes rs1983526, which is located within the promoter region of the *KLRC1* (NKG2A) gene, remains unclear whether this SNP participates in the regulation of NKG2A expression. Even, the hb-1 haplotype has been related to the cytotoxic function of NK and CD8 T lymphocytes due to regulation of NKG2D receptor expression [19,20,25]. The fact that hb-1v haplotype is restricted to *KLRC4-KLRK1* region further supports an hypothesis in which the relationship between these haplotypes and lymphocyte cytotoxicity involves NKG2D and not NKG2A regulation. Rs1049174 genotype has been related to some pathologic conditions, including recurrent miscarriage, cervical and colorectal cancer, as well as to improvement in response of some drugs [19,25,38–42]. Moreover, several other studies related rs2617170 genotype to the clinical course of Behcet's disease and chronic hepatitis B infection [43–45]. Our results further confirm the usefulness of rs1049174 or rs2617170 as alternative tag SNPs that could be used for future genetic association studies of human diseases. In addition, rs2255336 GG genotype has been related to the development of cervical cancer, systemic lupus erythematosus and to inefficient response to anti-tumor necrosis factor therapy in rheumatoid arthritis [42,46,47]. The high frequency of this genotype in Mexico City Mexicans offers future opportunities for disease association studies in this population.

In conclusion, our study is the first approach to describe the allele, genotype, and haplotype frequencies of rs1049174, rs2255336, rs2617160, rs2246809, rs2617170, rs2617171, and rs1983526 in Mexico City. The present study demonstrates that rs1049174, rs2617160, rs2617170, and rs2617171 constitute hb-1v and provides the basis for future association studies.

Declaration of Competing Interest

The authors declare no conflict of interest in this work.

Acknowledgements

The study was supported by Coordinación Nacional de Investigación en Salud, IMSS, México, grants FIS/IMSS/PROT/G14/1322, FIS/IMSS/PROT/MD17/1689 and CONACYT SALUD-2014-1-233422. Wendy Guadalupe Vázquez González is a doctoral student from Programa de Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de

México (UNAM) and was supported by Consejo Nacional de Ciencia y Tecnología (CONACYT) 440851 and IMSS 051-2012.

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