ARTICLE

Assessment of NKG2C copy number variation in HIV-1 infection susceptibility, and considerations about the potential role of lacking receptors and virus infection

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Human Immunodeficiency Virus (HIV) infection dynamics is strongly influenced by the host genetic background. NKG2C is an activating receptor expressed mainly on Natural Killer (NK) cells, and a polymorphism of copy number variation in the gene coding for this molecule has been pointed as a potential factor involved in HIV infection susceptibility. We evaluated the impact of the *NKG2C* deletion on HIV-1 susceptibility, with or without HBV/HCV co-infection, in a total of 780 individuals, including 385 HIV-infected patients and 395 healthy blood donors. *NKG2C* deletion genotyping was performed by standard PCR. To our knowledge, this is the first study to access the impact of complete *NKG2C* deletion among HIV-infected Brazilian individuals. The frequency of *NKG2C* deletion (range: 19–22%) was similar in cases and controls. No association of NKG2C deletion with HIV-1 susceptibility or influence on clinical features, HBV or HCV co-infection was observed in the evaluated population. Our findings suggest that *NKG2C* deletion, and the consequent absence of this receptor expression, does not directly impact HIV susceptibility, HBV/HCV-co-infection in the studied population, suggesting that other signaling pathways might be triggered and perform similar functions in cell activity in the absence of this specific receptor, preventing the development of disadvantageous phenotypes. Larger cohorts and studies involving protein expression are necessary to confirm our findings.

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INTRODUCTION

The Human Immunodeficiency Virus (HIV) is classified into two major subtypes, HIV-1 and HIV-2. While the first one shows the highest infectivity and is responsible for the global epidemic, the latter is mainly endemic in west Africa [1, 2]. HIV-related mortality has been decreasing worldwide but still represents a major public health issue, especially in low and middle-income countries, where factors such as impaired access to treatment, lack of public health policies, stigma, and discrimination are responsible for the reduced effectiveness of clinical protocols [3, 4]. Additionally, while numbers of AIDS-related deaths and new HIV cases have been decreasing worldwide, an opposite trend can be seen in eastern Europe and central Asia, where these parameters increased more than 30% in the last 10 years. Furthermore, the HIV incidence did not change in Latin America, although HIV mortality has declined 21% from 2010 to 2020 [4].

Among risk factors influencing HIV infection, the host genetic background is known to strongly impact overall HIV susceptibility and disease progression [5]. In this context, pharmacogenetic studies observed remarkable differences in HIV drug treatment response associated with genes from several distinct biological pathways [6]. Furthermore, distinct genetic variants have been pointed out as potential factors influencing HIV susceptibility and disease [7], but additional studies are required to understand how these alterations in the host immune response are related to HIV pathogenesis. Besides the *CCR5Δ32* allele from the chemokine receptor-5 (CCR5) gene, which in homozygosis protects humans from HIV infection and is the best-known gene variant that affects HIV infection [8, 9], other loss-of-function variants such as the killer cell lectin-like receptor-2 (KLRC2, also known as NKG2C) gene deletion have been associated with HIV infection risk, suggesting that the absence of NKG2C expression impairs viral immune response [10, 11].

The *KLRC* (*NKG2*) gene family is located within the NK (Natural Killer) cell complex in human chromosome 12 and encodes seven proteins. NKG2A and NKG2B act as inhibitory receptors, whereas NKG2C, NKG2D, NKG2E, and NKG2H are activating NK receptors [12, 13]. NKG2F function is unknown although it binds to DAP12 potentially providing activating signals. After signaling this complex is retained intracellularly [14], while CD94 is known to form dimers with multiple members of the NKG2 family such as NKG2A, -2B, -2C, -2E, and -2H [13]. The activating receptor *NKG2C*/CD94 acts as a receptor to the human leukocyte antigen-E (HLA-E). The receptor is expressed primarily on NK, γδ-T cells, and some

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subsets of CD8 + T cells [15]. The *NKG2C* deletion has been correlated with the absence of expression in homozygous individuals, while an intermediate phenotype is observed in heterozygous [10]. Since NKG2C and NKG2A co-modulate NK cell function by recognizing HLA-E, *NKG2C* deletion may impair cytotoxic and immunomodulatory response through inefficient immune cell activation [16, 17]. The role of the NKG2C+ subset of NK and T cells has been studied in multiple viral infections, such as Hepatitis B Virus (HBV), Human Cytomegalovirus (HCMV), and HIV [18–22]. Despite the extent of data gathered over the years, the impact of *NKG2C* deletion on HIV infection is still unclear, since different groups have not been able to replicate results on different populations. Our study evaluated the impact of *NKG2C* deletion in a cohort of 780 Brazilian individuals, divided into 385 HIV-infected individuals and 395 controls from two geographic regions of Brazil, in order to achieve a better comprehension of the influence of NKG2C on HIV susceptibility.

MATERIALS AND METHODS

Patients and data collection

Blood samples were obtained from 395 healthy blood donors and 385 HIV+ individuals. The control group was composed of HIV, HBV, and HCV seronegative individuals from two different Brazilian cities, Porto Alegre (the capital of the southernmost state of Brazil) and Rio de Janeiro (capital of one of the main Brazilian states located in the southeast region). All HIV+ patients were under HAART (highly active antiretroviral therapy) treatment as previously described [23] and were enrolled in the South Brazilian HIV Cohort (SOBRHIV) in Porto Alegre. These cities were selected since both have similarly admixed populations. Clinical data of the patients (i.e., co-infection by Hepatitis B and C) were obtained by reviewing the medical records. This study was approved by the Ethics Committees from all medical centers involved and all patients and controls provided written informed consent. DNA samples were obtained from peripheral blood using the salting-out method [24] and the *NKG2C* gene deletion was genotyped with conventional PCR as previously optimized by Moraru et al. [25]. To ensure the quality of our results, all amplification experiments included internal controls with known genotypes, and 10% of the DNA samples were randomly tested with 100% concordance with initial data.

Statistical analysis

Categorical variables were evaluated through the Chi-square test. Asymmetric distribution of continuous variables was evaluated through the Mann–Whitney *U* test and represented by the median and the 25th–75th percentile. Undetectable viral load was considered as the number of <50 viral copies/mL. Adherence to Hardy-Weinberg equilibrium was evaluated as previously described by Rodriguez et al. [26]. The strength of association between the genetic marker and the outcome was evaluated by adjusted binary logistic regression. Potential confounding factors were evaluated and entered in the logistic regression models only if they were associated both with the outcome and with the study factor at $p < 0.20$. All analyses were performed by SPSS v.18.0 for Windows (SPSS Inc., Chicago, Illinois, USA). For all instances, a *p*-value < 0.05 was considered statistically significant.

RESULTS

The demographic and clinical characteristics of the study group are shown in Table 1. A significant difference in male/female frequency between groups, with a major representativity of men in the control group (63.3% vs. 36.7; $p = 0.021$) was observed (data available for 756 individuals). All patients included in this study were under HAART treatment and 96.1% of them showed undetectable viral load (<50 copies/mL), and the CD4 + T cell count (cells/mm^3) median was 504.5 (362.5–687.0). Also, a statistical difference in ethnicity proportion was observed between groups ($p = 0.013$). Since *NKG2C* wild-type (WT) allele frequency was higher among European-derived controls than African-derived controls (84% vs. 74%; $p < 0.001$), the impact of *NKG2C* deletion was assessed in the overall group and according to ethnicity.

Table 1. Demographic and clinical features of HIV-infected individuals and controls

	HIV-infected individuals ($n = 385$)	Control group ($n = 395$)
Age [median (25–75%)]	42 (37–49)	43 (37–49)
Gender *n* (%)[a]		
Male	212 (55.1)	235 (63.3)
Female	173 (44.9)	136 (36.7)
Ethnicity *n* (%)		
Euro-derived	219 (56.9)	259 (65.6)
African-derived	166 (43.1)	136 (34.4)
T-CD4+ [cells/mm³, median (25–75%)]	504.5 (362.5–687.0)	NA
HIV viral load [median (25–75%)]	292 (110–2169)	NA
Undetectable HIV viral load *n* (%)	370 (96.1)	NA
Time on HAART (months), median (25–75%)	28 (16–46)	NA
Total HAART time, median (25–75%)	66 (32–104)	NA
HBV co-infection, *n* (%)	15 (4.1)	NA
HCV co-infection, *n* (%)	97 (26.2)	NA
Smoking, *n* (%)	109 (28.3)	NA
Alcohol consumption, *n* (%)	79 (20.5)	NA

HAART highly active antiretroviral therapy, *NA* data not available, *SD* standard deviation

[a]Data available for 756 individuals

The genotype and allele frequencies of the evaluated individuals were in Hardy-Weinberg equilibrium. No differences in allele and genotype frequencies between HIV-infected and controls were observed (Table 2), thus suggesting that *NKG2C* deletion has no direct impact on HIV-infection risk in our population. Also, as shown in Table 3, no association of *NKG2C* genotypes with HIV/HCV co-infection was observed (data available for 370 individuals). Considering HBV co-infected individuals genotyped ($n = 15$), 80% were *NKG2C* WT homozygous and 20% were heterozygous. No *NKG2C* deletion homozygous was found in the HBV co-infected group, probably due to the small number of HBV co-infected patients. No statistical differences were observed when compared to the HIV/HBV co-infected individuals (data available for 364 individuals).

Since this is the first study to evaluate *NKG2C* deletion in a Brazilian HIV cohort, we compared our results to those previously published concerning other human populations [10, 11, 27–35]. *NKG2C* genotype and allelic frequencies of previous studies are given in Table 4. In the present study, we found that the frequency of *NKG2C* del/del genotype was around 4%, ranging from 0 to 10% in previous studies. Besides, the allele frequency of *NKG2C* deletion reported by other studies ranges from 3 to 30%, compared to 20% found in our study.

DISCUSSION

In the present study, no association of *NKG2C* deletion with HIV susceptibility nor HB/HCV co-infection was observed. Importantly, our results differ from similar published studies that found *NKG2C* deletion to be a risk factor for HIV infection [10, 11]. Thomas et al. observed a statistically significant higher frequency of *NKG2C*

Table 2. *NKG2C* genotype and allele frequencies among HIV-infected individuals and control group stratified by ethnicity

Ethnicity	NKG2C Genotype				NKG2CAllele		
	WT/WT *n* (%)	WT/del *n* (%)	del/del *n* (%)	*p*-value	WT (%)	del (%)	*p*-value
European-derived							
HIV-infected individuals	140 (64)	72 (33)	7 (3)	0.214	352 (80)	86 (20)	0.105
Control group	185 (71)	67 (26)	7 (3)		437 (84)	81 (16)	
African-derived							
HIV-infected individuals	105 (64)	55 (33)	6 (3)	0.093	265 (80)	67 (20)	0.068
Control group	77 (57)	46 (34)	13 (9)		200 (74)	72 (26)	
All individuals							
HIV-infected individuals	245 (64)	127 (33)	13 (3)	0.093	617 (80)	153 (20)	0.763
Control group	262 (66)	113 (29)	20 (5)		637 (80)	153 (20)	

WT wild-type, *del* deletion

Table 3. Evaluation of NKG2C deletion on HIV/HCV co-infection risk

Ethnicity	*NKG2C* Genotypes—HIV/HCV[a]			
	WT/WT *n* (%)	WT/del *n* (%)	del/del *n* (%)	*p*-value[a]
European-derived				
Co-infected individuals	24 (60)	16 (40)	–	0.37
No co-infected	110 (65)	53 (31)	7 (4)	
African-derived				
Co-infected individuals	32 (56)	23 (40)	2 (4)	0.31
No co-infected	70 (68)	29 (28)	4 (4)	
Total				
Co-infected individuals	56 (58)	39 (40)	2 (2)	0.15
No co-infected	180 (66)	82 (30)	11 (4)	

WT wild-type, *del* deletion
[a]Data available for 370 individuals

wild-type homozygous in Long-Term Non-Progressor individuals (LTNP) compared to other progression categories [10]. In our study, progression to AIDS could not be assessed due to the indication of HAART initiation in all HIV-positive patients, independently of the CD4 + T cell counts, thus, this parameter was not checked. Information regarding treatment status and duration was also not available. Another group recently assessed the impact of the same genetic variant among people living with HIV (PLWH) and subjects who remained uninfected even after multiple HIV exposures. Results indicated a higher frequency of the *NKG2C* del/del genotype in the PLWH group, and authors hypothesized that the presence of this deletion in homozygosis could be associated to increased susceptibility to HIV by impairing NK cell response to virus infection [11]. Although we do not have information specifically regarding environmental exposure to HIV, mainly due to the nature of our cohort (healthy blood donors), we could speculate that subjects enrolled in our control group have not been exposed, or have a low exposure, to HIV. This feature should be taken into consideration when discussing the results of our study.

Few studies have evaluated *NKG2C* deletion in HIV susceptibility. Nonetheless, the same genetic variant has also been investigated in the context of other viral infections, such as HCMV, HSV-1, H1N1, and RSV [27–35]. Although data is conflicting (Table 4), it has been suggested that the lack of NKG2C expression caused by the gene deletion impairs the control of HCMV viremia and disease [31, 34], and significantly impacts the development of severe SARS-CoV-2 infection [35].

'Natural gene knockouts' are frequently observed among different populations, and redundancy of function between genes is suggested to compensate for eventual loss-of-function variants; ultimately leading to no disadvantageous phenotypes [36]. Interestingly, NK cell maturation triggered by HCMV infection was demonstrated to occur even in the absence of NKG2C [37], and similar studies reported that individuals lacking NKG2C expression display normal immune response towards HCMV infection [38, 39]. Thus, alternative routes might exist, leading to similar functions and cell activity when this specific receptor is lacking. Given that, it is feasible to speculate that population ethnicity could also be playing an important role in how this genetic variant impacts HIV infection. Interestingly, our group has previously demonstrated how polymorphisms may have different clinical outcomes depending on the genetic/ethnic background of the evaluated individuals [40]. Although it is generally accepted that the Brazilian population is highly admixed, encompassing Amerindian, African, and European components, the European component is preponderant in different Brazilian regions [41–43]. In fact, according to a study based in a panel of 40 validated ancestry-informative insertion-deletion DNA polymorphisms, the genetic composition of the Brazilian population is rather uniform in its miscegenation in different regions of the country [43]. The characteristic miscegenation of the Brazilian population and its potential consequences were discussed extensively by our group in a recent review (see ref. [44]). Nevertheless, genetic/ethnic background differences between our cohort and the few other populations evaluated concerning *NKG2C* deletion and HIV infection could be responsible by the discordant results. Therefore, further assessments of the impact of *NKG2C* deletion in viral infections among different populations are highly recommended.

Besides studies assessing the *NKG2C* genotype, total numbers of NKG2C+ cells in the context of viral infections have also been evaluated [18, 19, 21, 22, 42–47]. Of note, it was demonstrated that this subset is significantly increased in HIV-infected patients when compared to healthy controls [22], and a higher number of NKG2C + γδ T cells was observed in HIV-infected patients [46]. However, opposite results reported no differences in CD8+ NKG2C + T cells counts comparing HIV+ patients and healthy controls [19]. Additionally, studies enrolling HIV+ patients with concomitant infections suggested that increase in NKG2C + NK and CD8 + subpopulations might be a response to an underlying co-infection of HCMV, and not necessarily to HIV itself [18, 21, 47]. Similar data were reported by groups evaluating NKG2C+ cells in chronic hepatitis, strongly suggesting that underlying HCMV infection is the factor responsible for the expansion of this subset

Table 4. Summary of the studies evaluating the association of NKG2C copy number variation in distinct infectious diseases

Reference	Viral infection	Country	Individuals *N* (Controls + Patients)	NKG2C Del/del Control *N* (%)	Genotype frequency Patients *N* (%)	NKG2C deletion Control *N* (%)	Alelle frequency Patients *N* (%)	Association
[25]	HSV-1	Spain	137 + 163	6 (4)	7 (4)	60 (22)	69 (21)	NSS
[29]	H1N1 and RSV	Mexico	300 + 131	2 (1)	0 H1N1-infected 1 RS-V1 infected (2)	62 (10)	12 H1N1-infected (9) 12 RS-V1 infected (10)	NSS
[30]	HPV	Spain	295 + 572	15 (5)	31 (5)	119 (20)	219 (19)	NSS
[28]	HCMV	Spain	19 + 26	1 (5)	0	10 (26)	9 (17)	NSS
[31]	HCMV	Gambia	0 + 181	–	19 (11)	–	106 (29)	Alterations in NK subpopulations and antibody titers
[33]	HCMV	Spain	313 + 360	19 (6)	22 (6)	132 (21)	168 (23)	NSS
[32]	HCMV	Mexico	72 + 104	0	1 (1)	4 (3)	11 (5)	NSS
[34]	HCMV	Austria	49 + 49	0	2 (4)	10 (10)	32 (33)	Increased risk
[35]	COVID-19	Austria	260 + 361	5 (2)	14 (4)	96 (18)	198 (27)	Increased severity of symptoms
[10]	HIV	Germany	280 + 433	13 (5)	33 (8)	97 (17)	196 (23)	Increased risk
[11]	HIV	Canada	157 + 434	0	11 (3)	60 (19)	169 (20)	Increased risk
Current study	HIV	Brazil	395 + 385	20 (5)	13 (3)	153 (20)	153 (20)	NSS

Frequencies of patients groups are independent of subclinical classifications. Percentages are rounded without decimals

Del deletion, *NSS* not statistically significant, *HSV* Herpes simplex Type 1, *RSV* Respiratory sincytial virus, *HPV* Human papillomavirus, *HCMV* Human cytomegalovirus, *HIV* Human immunodeficiency virus

in HBV/HCV-infected patients [48–50]. Given the lack of information regarding HCMV status in our cohort, this issue could not be taken into consideration. We also highlight that most of the previous studies regarding HCMV-co-infection did not evaluate the *NKG2C* deletion; moreover, we highly encourage further studies to access the role of *NKG2C* copy number variation on HIV/HCMV-co-infection.

In conclusion, no association between *NKG2C* deletion and HIV susceptibility nor HBV/HCV co-infection was observed. To our knowledge, this is the first study to evaluate the contribution of *NKG2C* deletion in a Brazilian population, and also the third worldwide in an HIV context. Of note, we are aware that phenotypic expression of NKG2C also deserves attention, and the lack of data regarding protein expression is a limitation of our study. Given controversial data gathered throughout the years, it is still unclear whether or how NKG2C influences HIV susceptibility and disease progression. Therefore, studies evaluating larger populations, as well as integrating genetics and functional aspects are necessary to understand the relation between this receptor and HIV infection and progression.

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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