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...
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 del/del genotype was associated with the outcome and with the study factor at p < 0.05. 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). 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 < 0.05. 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.

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 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 (%) | | |
| 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, in Table 3, no association of NKG2C genotypes with HIV/HCV coinfection was observed (data available for 370 individuals). Considering HIV co-infected individuals genotyped (n = 15), 80% were NKG2C WT homozygous and 20% were heterozygous. No NKG2C deletion homozygous was found in the HIV co-infected group, probably due to the small number of HIV 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.

**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].

Informed consent 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 < 0.05. 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.
In our wild-type homozygous Long-Term Non-Progressor individuals investigated in the context of other viral infections, such as HCMV, the ability. Nonetheless, the same genetic variant has also been hypothesized that the presence of this deletion in homozygosis NKG2C del/del genotype in the PLWH group, and authors previously demonstrated how polymorphisms may have different ethnic differences between our cohort and the few other populations evaluated concerning NKG2C deletion and HIV infection could be responsible for 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 2. NKG2C genotype and allele frequencies among HIV-infected individuals and control group stratified by ethnicity

| Ethnicity      | NKG2C Genotype       | WT/WT n (%) | WT/del n (%) | del/del n (%) | p-value | WT (%) | del (%) | p-value |
|----------------|----------------------|-------------|--------------|---------------|---------|--------|---------|---------|
| African-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) |         |
| European-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      | 245 (64)    | 127 (33)     | 13 (3)        | 0.093   | 617 (80) | 153 (20) | 0.763   |

WT wild-type, del deletion

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

| Ethnicity      | NKG2C Genotypes—HIV/HCVa | WT/WT n (%) | WT/del n (%) | del/del n (%) | p-valuea |
|----------------|--------------------------|-------------|--------------|---------------|----------|
| 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                    | 56 (58)     | 39 (40)      | 2 (2)         | 0.15     |
|                 | Co-infected individuals  | 180 (66)    | 82 (30)      | 11 (4)        |          |

WT wild-type, del deletion

aData available for 370 individuals.
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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