

SHORT COMMUNICATION

GATA GENOTYPE AND FY*B(-67T>C) POLYMORPHISM AS A CHEAP AND RELIABLE TOOL TO EVALUATE ETHNICITY IN BRAZILIAN PATIENTS SUBMITTED TO AUTOLOGOUS TRANSPLANTATION

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INTRODUCTION

Although race and ethnicity are socially constructed concepts, collecting high quality data on these characteristics is crucial for clinical research.¹ Notably, in genetic association studies, a particular potential confounding factor is different ethnic background among patients, that can be related both to the genes and the outcome of interest – a bias referred to as population stratification.²

In Brazil, to describe race we commonly use the National Institute of Geography and Statistics (IBGE) classification, so-called “self-declared color”, referring to individuals declaring their own racial or ethnic identity. The classification is divided in five categories (white, black, brown, yellow and indigenous), but it is acknowledged that defining ancestry by phenotypic characteristics, especially in countries with highly mixed population like Brazil, can be misleading and imprecise.³

The association between FY*B(-67T>C) polymorphism (rs2814778) in the GATA-box erythroid promoter region of Duffy antigen receptor chemokine

gene and African ancestry is well known, defining the Duffy-null genotype.⁴

At the present study we aimed to evaluate the relationship between the GATA FY*B(-67T>C) polymorphism and self-declared race in Brazilian patients undergoing autologous transplantation and verify the feasibility of using this test as a more reliable tool to evaluate ethnicity in this population.

MATERIAL AND METHODS

We conducted a study to evaluate the association of candidate-gene polymorphisms and toxicities after autologous stem-cell transplantation (ASCT) in adult patients with lymphoma or myeloma. All patients submitted to ASCT in two Brazilian transplant centers (Hospital das Clínicas, Faculdade de Medicina University of São Paulo and Hospital Sirio-Libanês) from 2015 to 2021 with available DNA to study genetic polymorphisms were included. This study was approved by Ethics Committee of both institutions (CAPPesq and CEP) and carried out according to the criteria established by the Declaration of Helsinki with its modifications.⁵ Considering our population,

it was imperative to properly eliminate the potential bias of population stratification and we decided to use GATA polymorphism besides self-declared race with this aim.

This cohort includes patients with available data on self-declared race and GATA polymorphism. Demographics and clinical data were collected from the transplant unit databases by investigators blind to the results of polymorphisms analysis. GATA genotyping was performed using commercial antisera (Lorne Laboratories, Danehill, UK) and DNA was extracted from blood or marrow samples using commercial kits (PureLink® Genomic Invitrogen, Carlsbad, CA, USA) following manufacturer's instructions. FY*B(-67T>C) allele genotyping was performed as described elsewhere.⁶

STATISTICAL ANALYSIS

Self-declared race was compared to GATA genotype (T/T, T/C and C/C) and the presence of GATA FY*B(-67T>C) polymorphism using Chi2 and Fisher tests. The departure from Hardy-Weinberg equilibrium (HWE) was tested by Chi2. Patients self-declared as black, brown, yellow or indigenous according to IBGE classification were grouped as "non-white patients". Survival curves were constructed using the Kaplan-Meier method, and the log-rank test was used to assess differences between curves. For neutrophil and platelet engraftment, cumulative incidence function was estimated by the Aalen-Johansen method and compared using the Gray test. Statistically significant levels were set at $p \leq .05$. Analyses were held on STATA software, version 18.0.

RESULTS

A total of 217 patients were included in this analysis. Median age at transplantation was 56 years (21-79) and 130 patients (59.9%) were male. Most patients were self-declared white ($n=175$, 80.6%) and the most common genotype was GATA67T/T (wild type/wild type) ($n=142$, 65.4%). There was no deviation from HWE in this population ($p=0.129$). Patients' demographic and clinical characteristics are described in Table 1.

Among the self-declared white patients, 73.1% were genotyped as GATA67T/T and 4.0% as GATA-67C/C, while among the non-white patients 21.4% were genotyped as GATA-67C/C ($p<.0001$). The allele FY*B(-67T>C) frequency was 0.22 in the whole population; it was identified in 26.9% of the self-declared white patients and in 66.6% of the non-white ($p<.0001$). Table 1 presents data regarding GATA

genotype and GATA FY*B(-67T>C) allele frequency according to self-declared race.

Cumulative incidence of neutrophil and platelet engraftment in 30 days were 98.6% and 88.6%, respectively. Twelve-month overall survival was 86.6% (95% CI 79.9 - 91.2%), with no difference according to self-declared race (86.8% vs 85.5% for white and non-white patients, respectively, $p=0.81$) or the presence of the allele FY*B(-67T>C) (85.2% vs 89.9% for non-mutated and mutated patients, respectively, $p=0.27$).

DISCUSSION

This study shows that GATA FY*B(-67T>C) polymorphism is more prevalent in the non-white population among Brazilians submitted to ASCT. While two thirds of self-declared non-white patients presented the polymorphism, it was identified in a minority of white patients. GATA-67C/C genotype, meaning the presence of GATA FY*B(-67T>C) polymorphism in homozygosis, was identified in 21.4% of self-declared non-white patients, while it was very rare in the self-declared white group.

The presence of GATA FY*B(-67T>C) is a marker of African ancestry. This polymorphism prevents the expression of FY*B on erythrocytes' surface, leading to Fy (a-b-) phenotype, which induces protection against malaria infection.⁷ Our group previously described that GATA-67C/C genotype, a simple and low-cost test, is more accurate than self-declared race in diagnosing benign ethnic neutropenia, a condition also associated to African ancestry.⁴ The global GATA FY*B(-67T>C) allele frequency described at dbSNP (rs2814778) varies from 0 to 0.98 depending on the population, with very low frequencies in populations of North-Europe and high frequencies in Middle-East and Africa, as expected.⁸ In Brazil, GATA FY*B(-67T>C) allele frequency is 0.18.⁹

Most studies of genetic association in the field of hematopoietic stem cell transplantation don't properly address differences on ethnicity among patients, probably because most of them are white of European ancestry and in this scenario population stratification is unlikely.¹⁰⁻¹² Also, collecting data on race or ethnicity is illegal in some countries.¹³ However, in mixed populations like Brazilians, self-reporting race is inaccurate. According to recent data, 42.8% of Brazilians are self-declared white, 10.6% black and 45.3% brown with huge differences according to the region. Besides the risk of presenting misleading results, collecting proper data on race or ethnicity is important to guide health policies also regarding

HSCT. The Center for International Blood and Marrow Transplant group demonstrated that Hispanics and non-Hispanic blacks had lower stem cell transplantation utilization rate compared with non-Hispanic whites.¹⁴

No differences in clinical outcomes following ASCT were noted based on self-declared race or the presence of the GATA FY*B(-67T>C) polymorphism in this study, similar to the findings of a recent study that

investigated the influence of race on outcomes after ASCT in multiple myeloma patients.¹⁵

In conclusion, our study shows that GATA FY*B(-67T>C) polymorphism is associated to self-declared race in Brazilian patients submitted to ASCT. Given the importance of correctly evaluating ancestry in genetic association studies in transplantation, GATA FY*B(-67T>C) polymorphism can be used as a more accurate tool in populations with mixed ethnic/racial background.

Table 1. Patients' demographic and clinical characteristics, GATA genotype and presence of FY*B(-67T>C) according to self-declared race*

	White n=175		Non-white n=42		p- value
	n	%	n	%	
Median age in years (range)	55 (20-79)		57 (27-71)		
Gender					
Male	101	57.7	29	69.0	
Female	74	42.3	13	31.0	
Diagnosis					
Gamopathy	92	52.6	29	69.0	
Lymphoma	83	47.4	13	31.0	
GATA Genotype					
GATA67T/T	128	73.1	14	33.4	<.0001
GATA67C/T	40	22.9	19	45.2	
GATA67C/C	7	4.0	9	21.4	
FY*B(-67T>C) allele					
Present	47	26.9	28	66.6	<.0001
Absent	128	73.1	14	33.4	

*Self-declared race according to Brazilian Institute of Geography and Statistics (IBGE) classification: white (n=175), black (n=20), brown (n=20), yellow (n=1), indigenous (n=1). For these analyses patients self-declared black, brown, yellow or indigenous were grouped as "non-white".

REFERENCES

1. Mathur R, Rentsch CT, Venkataraman K, et al. How do we collect good-quality data on race and ethnicity and address the trust gap? *Lancet*. 2022;400(10368):2028-30.
2. Attia J, Ioannidis JP, Thakkinstian A, et al. How to use an article about genetic association: B: Are the results of the study valid? *JAMA*. 2009;301(2):191-7.
3. Osorio RG. O Sistema Classificatório de "Cor ou Raça" do IBGE. Texto para discussão. 2003;(996):1-53.
4. Dinardo CL, Kerbauy MN, Santos TC, et al. Duffy null genotype or Fy(a-b-) phenotype are more accurate than self-declared race for diagnosing benign ethnic neutropenia in Brazilian population. *Int J Lab Hematol*. 2017;39(6):e144-6.
5. World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA*. 2013;310(20):2191-4.
6. Denomme GA, Rios M, Reid ME. Molecular Protocols in Transfusion Medicine [Internet]. San Diego: Academic Press; 2000 [cited 2023 Oct 31]. Available from: <https://www.sciencedirect.com/book/9780122093708/molecular-protocols-in-transfusion-medicine#book-description>

7. Parasol N, Reid M, Rios M, et al. A novel mutation in the coding sequence of the FY*B allele of the Duffy chemokine receptor gene is associated with an altered erythrocyte phenotype. *Blood*. 1998;92(7):2237-43.
8. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM, Sirotkin K. dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res*. 2001;29(1):308-11.
9. Naslavsky MS, Scliar MO, Yamamoto GL, et al. Whole-genome sequencing of 1,171 elderly admixed individuals from São Paulo, Brazil. *Nat Commun*. 2022;13(1):1004.
10. Rocha V, Porcher R, Fernandes JF, et al. Association of drug metabolism gene polymorphisms with toxicities, graft-versus-host disease and survival after HLA-identical sibling hematopoietic stem cell transplantation for patients with leukemia. *Leukemia*. 2009;23(3):545-56.
11. Bochud PY, Chien JW, Marr KA, et al. Toll-like receptor 4 polymorphisms and aspergillosis in stem-cell transplantation. *N Engl J Med*. 2008;359(17):1766-77.
12. Trigo FM, Luizon MR, Dutra HS, et al. Interaction between IL-6 and TNF- α genotypes associated with bacteremia in multiple myeloma patients submitted to autologous stem cell transplantation (ASCT). *Leuk Res Rep*. 2014;3(2):76-8.
13. European Union. Guidance note on the collection and use of equality data based on racial or ethnic origin. [Internet]. LU: Publications Office; 2021 [cited 2023 Oct 23]. Available from: <https://data.europa.eu/doi/10.2838/06180>
14. Schriber JR, Hari PN, Ahn KW, et al. Hispanics have the lowest stem cell transplant utilization rate for autologous hematopoietic cell transplantation for multiple myeloma in the United States: A CIBMTR report. *Cancer*. 2017;123(16):3141-9.
15. Davis JA, Thurlapati A, Weeda E, et al. Impact of race and ethnicity on outcomes after autologous stem cell transplantation for patients with newly diagnosed multiple myeloma. *Ann Hematol*. 2024;103(1):251-8.