RESEARCH ARTICLE

Molecular study of C^w/C^x antigens and frequency of Rh phenotypes in southeast Brazilian blood donors

Sidneia S. Costa | Thamy C. Souza Silva | Akemi Kuroda Chiba | Bruno R. Cruz | Dante Mário Langhi Junior | José O. Bordin

Discipline of Hematology and Hemotherapy, Universidade Federal de São Paulo – UNIFESP, São Paulo, Brazil

Correspondence

José O. Bordin, Disciplina de Hematologia e Hemoterapia, Universidade Federal de São Paulo, São Paulo, Brazil.

Email: jobordin@unifesp.br

Funding information

Universidade Federal de São Paulo – UNIFESP

Background: The C^w (RH:8), C^x (RH:9), and MAR (RH:51) antigens are encoded by alleles at the Cc locus of the Rh system, where C^w and C^x are considered low-frequency antigens and antithetical to the high-frequency antigen MAR. The frequency of C^w (RH:8) is approximately 2% in Caucasians, 1% in Black people, 4% in Finns, and 9% in Latvians. The aim of this study was to determine the frequency of RhD+ phenotypes in a population of southeast Brazilian blood donors and to perform a molecular study to distinguish the *RHCE**Ce.08.01 and *RHCE**Ce.09 alleles, responsible for the C^w and C^x expressions, respectively.

Methods: We investigated 11,536 RhD+ Brazilian blood donors. All samples were phenotyped for D, C, c, E, e, and C^w. In the C^w+ samples, a molecular analysis was performed to detect the nucleotide substitutions A122G and G106A, which determine the C^w and C^x antigens, respectively.

Results: C^w antigen was found in 110 (0.95%) samples in the following phenotypes: DC^we/dC^we (72/0.62%), DC^we/DC^we (30/0.26%), and DC^we/DC^wE (8/0.07%). Among 110 C^w+ samples, 108 showed the A122G nucleotide substitution associated with *RHCE**Ce.08.01 allele and 2 samples the G106A substitution associated with the *RHCE**Ce.09.01 allele.

Conclusion: This study showed the prevalence of the RhD+ phenotype in the Brazilian population, and that through the molecular study, it was possible to differentiate the *RHCE**Ce.08.01 and *RHCE**Ce.09.01 alleles. The phenotype frequency was similar from Black people (1%) and different from Caucasians, Finns, and Latvians.

KEYWORDS

blood group, C^w/C^x antigens, genotyping, Rh phenotypes, *RHCE* alleles

1 | INTRODUCTION

The Rh system is considered the most complex blood group system owing to the presence of numerous polymorphisms and clinically significant alleles. The five major Rh antigens—D, C, c, E, and e—are responsible for eight haplotypes that determine the individual phenotypic status. The prevalence of a blood group antigen is useful to describe the occurrences of a permanent

inherited characteristic at the phenotypic level in any given population.^{1–4}

The C^w (RH:8), C^x (RH:9), and MAR (RH:51) antigens are encoded by alleles at the Cc locus of the Rh system, where C^w and C^x are considered low-frequency antigens and antithetical to the high-frequency antigen MAR.^{1,5}

The amino acid changes in the first extracellular loop of the RhCe protein determine the expression of the C^w antigen with

Gln41Arg substitution (A122G) and C^x with Ala36Thr substitution (G106A), encoded by exon1 of the *RHCE* gene. Therefore, the MAR antigen is thought to involve amino acid residues 36 to 41 of the RhCE polypeptide and to be a conformation-dependent antigen.^{1,5}

The MAR antibody, commonly found in Finns, has an antithetical relationship to both anti-C^w and anti-C^x. Through serological methods, these three antigens behave as they were determined by an allelic series of genes.⁵ The distinction between C^w and C^x antigens is only possible by molecular methods.

The frequency of C^w is approximately 2% in Caucasians, 1% in Black people, 4% in Finns, and 9% in Latvians. Most C^w+ are C+ and has been associated with D(C)C^we, D(C)C^wE, (C)C^we, (C)C^wE, DC^w-, and C^wce haplotypes. The C^w antigen was rarely described in haplotypes lacking D and/or C antigen.¹

C^x+ individuals are C+, except in the rare haplotype C^xce^se V-VS+, found in individuals from Somalia. C^x has been associated with D(C)C^xe, (C)C^xe, and C^xce^s haplotypes.¹

The aim of this study was to determine the frequency of Rh phenotypes in a population of southeast Brazilian blood donors and to perform a molecular study of individuals with C^w phenotype, to distinguish the *RHCE**Ce.08.01 and *RHCE**Ce.09 alleles, responsible for the C^w and C^x expressions, respectively.

2 | MATERIALS AND METHODS

2.1 | Serological studies

We investigated 11,536 RhD+ blood donors, from the city of São Paulo, in the southeast region of Brazil. The Rh phenotyping was performed by hemagglutination in gel cards using monoclonal antibodies against D, C, c, E, e, and C^w antigens (Grifols, Diagnostic S.A., Spain).

2.2 | Molecular studies

Genomic DNA was extracted from blood samples from all C^w+ individuals according to the kit protocol (QIAamp DNA Blood Mini Kit, Qiagen GmbH, Hilden, Germany). The blood samples were analyzed by polymerase chain reaction (PCR-ASP) with sequence-specific primers to detect the nucleotide substitution (A122G) in exon 1 of the *RHCE* gene.⁵

The PCR-ASP A122G-negative sample was also analyzed by MLPA[®] (Multiplex ligation-dependent probe amplification) method (MRC Holland, Amsterdam, the Netherlands) to determine the *RHCE**Ce.08.01 allele. To detect the nucleotide substitution (G106A), in exon 1 of the *RHCE* gene (*RHCE**Ce.09 allele), we performed the gene sequencing by the Sanger method.⁶ The sequences were analyzed using a sequencing kit (BigDye Terminator v1.1, Applied Biosystem, Weiterstadt, Germany) and a genetic analyzer (ABI PRISM 3100, Applied Biosystem, Foster City, CA, USA).

TABLE 1 Rh phenotype frequencies among 11 536 Brazilian blood donors

Rh phenotypes	ISBT Nomenclature	Frequency	%
Dce/dce (R ₁ r)	RH:1,2,-3,4,5,-8	4.338	37.6
Dce/dce (R ₀ r)	RH:1,-2,-3,4,5,-8	1.924	16.68
DcE/dce (R ₂ r)	RH:1,-2,3,4,5,-8	1.679	14.55
DCe/DCe (R ₁ R ₁)	RH:1,2,-3,-4,5,-8	1.675	14.52
DCe/DCe (R ₁ R ₂)	RH:1,2,3,4,5,-8	1.431	12.40
DcE/DCe (R ₂ R ₂)	RH:1,-2,3,4,-5,-8	302	2.62
DCe/DCe (R ₁ R ₂)	RH:1,2,3,-4,5,-8	63	0.55
DcE/DCe (R ₂ R ₂)	RH:1,2,3,4,-5,-8	14	0.12
DC ^w e/dC ^w e (R ₁ ^w r)	RH:1,2,-3,4,5,8	72	0.62
DC ^w e/DC ^w e (R ₁ ^w R ₁)	RH:1,2,-3,-4,5,8	30	0.26
DC ^w e/DC ^w E (R ₁ ^w R ₂)	RH:1,2,3,4,5,8	8	0.07

3 | RESULTS

3.1 | Frequency of Rh phenotypes

Table 1 shows the phenotype distribution. The presence of C^w antigen was observed in 110 of 11,536 (0.95%) samples, with the following Rh phenotypes: DC^we/dC^we, DC^we/DC^we, and DC^we/DC^wE.

3.2 | Molecular investigation of C^w and C^x

Among 110 C^w+ samples, 108 (98.18%) showed the A122G nucleotide substitution associated with *RHCE**Ce.08.01 allele. In 2 of 110 (1.81%) samples, we were not able to detect A122G nucleotide substitution by PCR-ASP, neither by MLPA[®] method. These 2 samples were submitted to gene sequencing analysis detecting the G106A substitution associated with the *RHCE**Ce.09.01 allele (Table 2).

4 | DISCUSSION

The Rh phenotyping in RhD+ blood donors is very important for transfusion medicine. The distribution of Rh phenotypes within a population helps to determine the prevalence of these antigens and the occurrence of corresponding antibodies. However, when these phenotypes are compared among distinct populations, differences that concern the racial group can be observed.

Brazil has a mixed population due to the colonization of Europeans, Africans, and Amerindians. The progression of this colonization was highly diversified in different regions of the country and consequently reflected a variation in the genetic composition of this population. The city of São Paulo is in the southeast region of the country, with predominant ancestral characteristics of European origin (62.9%), with the contribution of Africans (25.5%) and Amerindians (11.6%).⁷

When we compared the prevalence of Rh phenotypes (DCE) found in this study with other populations, we observed that the Dce (R₁) phenotype (Dce/dce—R₁r and DCe/DCe—R₁R₁) was 37.6% and 14.52%, respectively, similar to the Caucasian population

TABLE 2 Rh phenotypes and C^w/C^x alleles

Rh phenotypes	RHCE*Ce.08.0 (C ^w)	RHCE*Ce.09.01 (C ^x)
DC ^w e/dC ^w e (R ₁ ^w r)	70	2
DC ^w e/DC ^w e (R ₁ ^w R ₁)	30	—
DC ^w e/DC ^w E (R ₁ ^w R ₂)	8	—

(42%).^{1,2} In addition, the DcE (R₂) phenotype (DcE/dce—R₂r and DcE/DcE—R₂R₂), 14.55% and 2.62%, respectively, was also similar to the Caucasian population (14%).^{1,2} Regarding the R₀ (Dce/dce) phenotype, the prevalence in Caucasians (4%) and Black people (44%) is different from our findings (16.7%).^{1,2} Some rare phenotypes found in our study, such as DCE/DcE (R₁R₂), DCE/DCE (R₁R₂), and DcE/DCE (R₂R₂) with 12.40%, 0.55%, and 0.12% frequency, respectively, showed similarity to the Caucasian population (12.5%, 0.2%, and 0.04%).²

The frequency found for C^w+ phenotype in our study was 0.95%, which were genotyped as RHCE*Ce.08.01 allele (0.94%) and RHCE*Ce.09 allele (0.017%). The phenotype frequency was similar for Black people (1%) and different for Caucasians (2%), Finns (4%), and Latvians (9%).¹ In other Western European countries, this frequency was 2.5% and 1.2% in India.^{8,9} There are no data regarding the frequency of RHCE*Ce.08.01 and RHCE*Ce.09 allele genotypes among individuals presenting the C^w+ phenotype.

This study also demonstrated that C^w phenotyping is unable to distinguish the critical epitopes for the expression of the C^w and C^x antigens. However, the molecular analysis is necessary to identify the alleles responsible for the expression of C^w and C^x phenotypes.

The anti-C^w is considered a clinically significant antibody and can cause mild to moderate hemolytic disease of the newborn and mild to severe immediate or delayed hemolytic transfusion reactions.^{10,11} However, regarding blood transfusion, there is no difficulty in finding a compatible donor due to the low frequency of these antigens.

In conclusion, we demonstrated in this study the prevalence of RhD+ phenotypes and the genetic diversity associated with these phenotypes in a blood donor population in the southeast of Brazil.

ACKNOWLEDGMENTS

We would like to thank the Santa Casa de Misericórdia de São Paulo for conceding the blood samples from the donors included in this study.

ORCID

Sidneia S. Costa <http://orcid.org/0000-0001-6684-890X>

REFERENCES

1. Reid ME, Lomas-Francis C, Olsson ML. *The Blood Group Antigens. FactsBook*. 3th edn. San Diego, CA: Academic Press, Elsevier; 2015:213-216.
2. AABB. *Technical Manual*, 17th edn. In: Roback JD, ed. Bethesda, MD: American Association of Blood Bank; 2000:437-462.
3. Sarkar RS, Philip J, Mallhi RS, Yadav P. Proportion of Rh phenotypes in voluntary blood donors. *Med J Armed Forces India*. 2013;69:330-334.
4. Makroo R, Gupta R, Bhatia A, Rosamma NL. Rh phenotype, allele and haplotype frequencies among 51,857 blood donors in North India. *Blood Transf*. 2014;12:36-39.
5. Mouro I, Colin Y, Sistonen P, Le Pennec PY, Cartron JP, Le Van Kim C. Molecular basis of Rh C^w (Rh8) and RhC^x (Rh9) blood group specificities. *Blood*. 1995;3:1196-1201.
6. Legler TJ, Maas JH, Köhler M, et al. RHD sequencing: a new tool for decision making on transfusion therapy and provision of Rh prophylaxis. *Transfus Med*. 2001;11:383-388.
7. Manta FSN, Pereira R, Vianna R, et al. Revisiting the genetic ancestry of Brazilians using autosomal AIM-Indels. *PLoS ONE*. 2013;8:1-10.
8. Lejon Crottet S, Henny C, Tinguely C, et al. Serological screening and molecular characterization of C^w antigen (Rh8) in Swiss blood donors. *Vox Sang*. 2014;107(Suppl 1):172.
9. Orzińska A, Guz K, Michalewska B, Brojer E. Molecular screening of the C^w antigen for typing donors compatible with patients with anti-MAR-like antibodies. *Blood Transfus*. 2016;14:573-576.
10. Jain A, Elhence P, Tripathi A, Pandey H, Agarwal P. Anti-C^w: in a young female patient. A case report with review of literature and frequency of low incidence C^w (Rh8) antigen in north India. *Indian J Hematol Blood Transfus*. 2014;30(Suppl 1):440-444.
11. Malik S, Moiz B. Clinical significance of maternal anti-C^w antibodies: a review of three cases and literature. *J Pak Med Assoc*. 2012;62:620-621.

How to cite this article: Costa SS, Souza Silva TC, Chiba AK, Cruz BR, Langhi Junior DM, Bordin JO. Molecular study of C^w/C^x antigens and frequency of Rh phenotypes in southeast Brazilian blood donors. *J Clin Lab Anal*. 2018;32:e22570. <https://doi.org/10.1002/jcla.22570>