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Ethnic diversity in Chilean blood groups: A comprehensive analysis of genotypes, phenotypes, alleles and the immunogenic potential of antigens in northern, southern and central regions

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Abstract

Background and Objectives: The available information on blood groups in the Chilean population is derived from studies on aboriginal cohorts and routine serological test results. The purpose of this study is to conduct a comprehensive analysis of genotypes, phenotypes and blood group alleles in donors from northern, central and southern Chile using molecular methods.

Materials and Methods: Overall, 850 samples from donors in northern, central and southern Chile were genotyped. Allelic, genotypic and antigenic frequencies were calculated and compared among regions. Of these, 602 samples were analysed by haemagglutination, and discrepancies found between phenotypes and genotypes were investigated. The immunogenic potential of antigens was calculated by the Giblett equation, using the antigenic frequencies of donors from Santiago and the alloantibody frequencies of patients from the same region.

Results: Alleles of low prevalence, variant alleles and those responsible for the absence of high-prevalence antigens were found. Significant differences were observed between the antigenic frequencies of the three regions. Discrepancies between serologic and molecular results were mostly attributed to the molecular background affecting antigen expression. In the calculation of the immunogenic potential of antigens, the highest value was attributed to the Di^a antigen.

Conclusion: These findings represent the first molecular characterization of blood group antigens in Chileans. Our results highlight the necessity of using molecular tools to explore the genotypes underlying variant phenotypes, low-frequency antigens and antigens lacking specific antisera that cannot be detected by haemagglutination. Additionally, they emphasize the importance of understanding the distribution of blood groups among different populations.

Keywords
antigenic frequencies, blood group antigen, genotype blood groups

Highlights
- Antigens, alleles and genotypes of clinical importance not previously described were found.
- The presence of these phenotypes impacts transfusion safety, leads to problems in pre-transfusion studies and increases the difficulty in finding compatible red blood cell units.
- There are significant statistical differences among the three areas studied, which could have an impact on alloimmunization.
- In the calculation of the immunogenic potential of antigens, the highest value was attributed to the Di^a antigen.

INTRODUCTION

There are more than 300 polymorphic antigens on the red blood cell (RBC) membrane that can be incompatible between a blood donor and a transfusion recipient or between a mother and her child during pregnancy, potentially causing alloimmunization and eventually leading to haemolytic transfusion reaction (HTR) or haemolytic disease of the fetus and newborn (HDFN), respectively [1]. Blood transfusion plays a crucial role in the treatment of various diseases; approximately 15% of inpatients undergo blood transfusion, and about 1% of transfused products result in severe HTR in alloimmunized patients due to blood group incompatibility [2]. Between 2017 and 2021, the US Food and Drug Administration (FDA) reports that 21% of transfusion-related deaths were caused by HTR resulting from blood group incompatibility [3]. Despite existing strategies to mitigate this adverse transfusion effect, prevention of RBC alloimmunization remains an unsolved challenge.

Alloimmunization is a complex adverse event related to transfusion, resulting from diverse factors such as donor and patient antigen mismatches, the recipient's immunological status, underlying inflammation, the immunomodulatory effects of transfused erythrocytes on the recipient's immune system and genetic factors.

In Chile, patients undergoing transfusion therapy are at high risk of alloimmunization since most receive only ABO and RhD-matched blood [4]. The ethnic background of both patients and donors can impact transfusion outcomes. Therefore, understanding of the blood groups present in the population is critical for implementing extended phenotype/genotype matching, aiming to reduce alloimmunization rates and improve patient outcomes [4].

No molecular studies of blood groups have been performed in the Chilean population to detect clinically significant genotypes and to predict phenotypes for which there are no antisera. The existing information comes from prospective studies carried out mainly in small groups of aborigines, which have anthropological objectives or from retrospective information gathered from the results of routine serological studies conducted in different blood banks, these studies are limited by the variable availability of reagents between blood banks of different health centres, and serological studies are restricted to identifying antigenic specificities for which antisera exist. Additionally, they are conditioned by the sensitivity of the technique, which may not detect weak variants [5–9]. Knowledge of the blood groups present in a population is essential for selecting the most appropriate reagents and phenotyping techniques to ensure patient safety. As serological reagents have limitations, it is relevant to conduct studies using molecular methods.

Chileans have varying proportions of European, Native American and, to a lesser extent, West African and East Asian ancestry [10]. Given the ethnic background of the Chilean population and the recent increase in immigration to Chile, it is possible to find previously undescribed genotypes, alleles and phenotypes due to the lack of molecular methods, and it is also plausible that there are significant differences in blood group frequencies in different regions of the country, variations that could increase the risk of alloimmunization [11].

As a comprehensive molecular analysis of blood group genotypes, alleles and phenotypes in the Chilean population has not yet been performed, this study represents the first in-depth examination using genotyping techniques. Consequently, the objectives of this study were to provide data on the genotypic, allelic and antigenic frequencies of 11 blood systems in a cohort of Chilean blood donors from the Northern, Central and Southern regions, to compare the frequencies between regions and with those documented in Europeans and Afro-descendants, to evaluate the concordance between the serological phenotype and the phenotype predicted from the genotype.

Additionally, we estimated the immunogenic potential of clinically relevant antigens.

MATERIALS AND METHODS

Blood samples

Three different groups of Chilean donors were enrolled in this study. A total of 850 blood samples were randomly collected from three regions: 212 from Arica (Northern), 515 from Santiago (Central) and 123 from Punta Arenas (Southern). Two etylenediaminetetra-acetic acid (EDTA) samples were collected from each donor, samples from cities in northern and southern Chile were shipped by air for processing in the Immunohematology and Blood Group Molecular Laboratories of the Santa María Clinic Blood Bank, located in Santiago, in the Central zone, Metropolitan region.

The inclusion criteria were (1) to be Chilean, considering Chilean nationality and fixed residence in Chile, (2) to meet the requirements to donate blood according to the general technical regulation NGS146 that regulates the care of donors in Chile and (3) to have signed the informed consent form agreeing to participate in the study.

Ethical approval was obtained from the ethics committee at the Tarapacá University, Arica, I region (North zone); Santa María Clinic, Metropolitan region (Central zone); Magallanes University, Punta Arenas, XII region (South zone) and the Chile University, Santiago to Chile.

Molecular methods

DNA was extracted from the EDTA-anticoagulated whole blood samples using the QIAamp DNA mini kit (Qiagen, USA). DNA concentration was measured on the Qubit fluorometer and was acceptable in the range between 10 and 100 ng/μL. Extracted DNA was stored at −80°C until testing.

Two commercial platforms were employed to genotype the samples: HEA BeadChip (Bioarray Solutions, Immucor, Warren, NJ, USA) and ID CORE XT (Grifols, Spain) [12, 13]. A total of 602 samples (212 from Arica, 328 from Santiago and 123 from Punta Arenas city) were genotyped by the HEA Beadchip, which analyses 38 RBC antigens of the following 11 blood group systems: RH, KEL, JK, FY, MNS, DI, DO, CO, LU, LW and SC using 24 single-nucleotide polymorphism (SNPs). The 187 remaining samples from Santiago were genotyped by IDCORE XT, utilizing Luminex xMAP technology that includes 29 SNPs responsible for the expression of 37 RBC antigens of the following 10 blood group systems (RH, KEL, JK, FY, MNS, DI, DO, CO, YT and LU).

RHD and *RHCE* gene variants from samples exhibiting discrepancies between molecular and serological methods were analysed by the RHD and RHCE BeadChips (Bioarray Solutions, Immucor) [14] in a reference laboratory.

Results for all blood group systems were expressed in accordance with the International Society of Blood Transfusion (ISBT) nomenclature of blood groups, alleles and phenotypes [15]. Rare blood group donors are defined as those that are negative for high-prevalence antigens (<1:1000).

Serological methods

The 602 samples genotyped with the HEA BeadChip platform [12] were previously serologically phenotyped by tube or using the automated NeoGalileo equipment (Immucor, Norcross, GA, USA) with monoclonal and polyclonal antisera according to the manufacturer's instructions for the following blood group antigens: Rh (C, c, E, e), KEL (k), JK (Jk^a, Jk^b), FY (Fy^a, Fy^b), DI (Di^a), MNS (M, N, S, s) and LU (Lu^a, Lu^b). The 187 samples that were genotyped using ID CORE XT [13] were not phenotyped through serological methods because we did not have all the necessary antisera available when the samples were processed.

Predicted phenotypes based on genotypes were compared with results from serological phenotyping, and any discrepancies were analysed. A discrepancy was defined as a situation where genotype results were positive but serology results were negative, or vice versa.

Statistical analysis

Antigenic and allelic frequencies for each region were estimated using direct counting and presented as percentages. Fisher's test was employed to assess whether statistically significant differences existed among antigenic frequencies of Chilean blood donors in the three regions studied. Furthermore, the RBC antigenic frequencies of each region were compared with those reported for individuals of Caucasian and Afro-descendant backgrounds. p-values <0.05 were considered indicative of significance.

Calculation of the immunogenic potential of antigens

For this calculation, the 'Giblett equation' was applied [16]. This equation included in the analysis only those RBC antigens for which both antigen and clinically significant antibody frequency data were available. RBC antigen frequencies predicted from the genotype data collected in Santiago were used. Alloantibody frequencies were obtained from 502 patients with one or more irregular antibodies identified at the same centre. Data were obtained from the statistics module of the Hematos software used in the Blood Bank during the same period as the donors' genotyping. The alloimmunized patients included in the study were from the same region as the donors.

The 'Giblett equation' is a widely used method for estimating the immunogenic potential of blood group antigens. This calculation involves dividing the total number of antibodies of a specific type by

the probability that an antigen-negative individual will receive transfusions of antigen-positive red blood cells. The resulting value is then normalized relative to the immunogenic potential of K antigen by dividing it by the corresponding value for K [16].

RESULTS

In the 850 donors studied from the 3 zones of the country, we found 55 different genotypes and 35 alleles from 11 blood group systems. Within these, uncommon alleles coding for low-frequency antigens (LFA), absence of high-frequency antigens (HFA) and partial and weakly expressed antigens were found.

In the Rh system, 14 genotypes were identified, 6 of which included the *RHCE*01.20.01* allele or the *RHCE*01.20.03* allele, all of which were in heterozygosis. Table 1 displays the number of donors and genotypes in which each allele was found, along with the corresponding nucleotide, amino acid changes and associated phenotypes.

Eight genotypes were found in the FY system. Among these, five genotypes were observed in 54 (6.3%) donors with the *FY*02W.01* allele or the *FY*02N.01* allele, of which 13 (1.5%) were carriers of the *FY*02W.01* allele, and 41 (4.8%) of the *FY*02N.01* allele, two (0.2%) of the latter had a homozygous genotype (*FY*02N.01/FY*02N.01*) and were therefore classified as carriers of the Fy null phenotype. Table 2 displays the donors and their respective genotypes containing either the *FY*02N.01* or *FY*02W.01* alleles.

In the other systems studied, donors with infrequent genotypes and alleles were also identified. Table 3 shows the homozygous genotypes responsible for the absence of HFA in KEL, LU, DI, YT blood group systems, found in Chilean donors. Additionally, other rare alleles associated with LFA were detected. Table 4 displays the alleles, genotypes and LFA occurrences found in the donors studied.

None of the alleles, genotypes and phenotypes described in Tables 1–4 had been previously described in Chileans. Details of the genotypes and alleles identified in all donors studied and the frequencies at which they were found in the three areas studied are shown in Tables S1 and S2.

Antigens predicted from genotypes: Frequencies and statistical analysis

Table 5 displays the frequencies of blood system antigens: RhCE, KEL, JK, FY, MNS, LU, DI, CO, DO, LW and SC obtained from molecular studies of blood donors from the northern (Arica), central (Santiago) and southern (Punta Arenas) regions of Chile. To the left of Table 5 is

TABLE 1 Nucleotide and amino acid change, phenotypes and genotypes associated with *RHCE*01.20.01* and *RHCE*01.20.03* alleles from the Chilean donors.

Allele name ISBT	Nucleotide change	Predicted amino acid change	Phenotypes	Donors (*n*)	Genotypes with *RHCE*01.20* alleles
*RHCE*01.20.01*	733 C>G	Leu245Val	c + partial, e + partial, V + VS+, hr^B pos., weak or neg.	15	*RHCE*01/RHCE*01.20.01* *RHCE* 02/RHCE*01.20.01* *RHCE*03/RHCE*01.20.01* *RHCE*04/RHCE*01.20.01*
*RHCE*01.20.03*	48 G>C 733 C>G 1006 G>T	Trp16Cys Leu245Val Gly336Cys	c + partial, e + partial, V−VS+, hr^B−	4	*RHCE*01/RHCE*01.20.03* *RHCE*03/RHCE*01.20.03*

TABLE 2 Nucleotide and amino acid change, phenotypes and genotypes associated with *FY*02W.01* and *FY*02N.01* alleles from the Chilean donors.

Allele name ISBT	Nucleotide change	Predicted amino acid change	Phenotypes	Donors (*n*)	Genotypes
*FY*02W.01*	265 C>T	Arg89Cys	Fy^b weak	8	*FY*01/FY*02W.01*
				5	*FY*02/FY*02W.01*
*FY*02N.01*	-67 T>C	0	Fy^a	29	*FY*01/FY*02N.01*
			Fy^b	10	*FY*02/FY*02N.01*
			Silent	2	*FY*02N.01/FY*02N.01*

TABLE 3 Rare phenotypes of the KEL, LU, DI and YT blood systems present in Chilean donors.

System	Phenotypes	Genotypes	Nucleotide change	Predicted amino acid change	Donors (*n*)
KEL	K+k−	*KEL*01.01/KEL*01.01*	578 C>T	Thr193Met	1
DI	Di(a+b−)	*DI*01/DI*01*	2561 C>T	Pro854Leu	2
LU	Lu(a+b−)	*LU*01/LU*01*	230 G>A	Arg77His	1
YT	Yt(a−b+)	*YT*02/YT*02*	1057 C>A	His353Asn	2

TABLE 4 Alleles encoding LFA, antigens and genotypes detected in Chilean donors.

System	Allele encoding LFA	LFA	Genotypes	Donors (*n*)
RhCE	*RHCE*01.20.01*	V+VS+	*RHCE*03/01.20.01*	15
			*RHCE*01/01.20.01*	
			*RHCE*02/01.20.01*	
			*RHCE*04/01.20.01*	
	*RHCE*01.20.03*	VS+	*RHCE*01.39/01.20.03*	4
			*RHCE*03/01.20.03*	
KEL	*KEL*01.03*	Kp^a	*KEL*01.03 /KEL*01.04*	18
	*KEL*02.06*	Js^a	*KEL*02.06/KEL*02.05*	2
MNS	*GYP*501*	Mi^a	*GYPB*03/GYP*501*	1
DI	*DI*01*	Di^a	*DI*01/DI*02*	2
			*DI*01/DI*01*	28
LU	*LU*01*	Lu^a	*LU*01/LU*01*	1
			*LU*01/LU*02*	25
CO	*CO*02*	Co^b	*CO*01/CO*02*	40
SC	*SC*02*	Sc2	*SC*01/SC*02*	5

Abbreviation: LFA, low-frequency antigen.

a map of Chile, highlighting in red the areas from which the samples were obtained.

In the 187 samples studied from Santiago donors by the ID CORE platform, RBC antigen frequencies were also calculated for: Mi^a (0.5%), Yt^a (98.9%) and Yt^b (10.7%).

As expected, due to the heterogeneous nature of the miscegenation that formed the Chilean population across the country, significant statistical differences were observed in the frequencies of eight antigens across five blood group systems (RhCE, FY, JK, MNS and DO) among Chilean blood donors residing in the three regions studied. Table 6 presents the *p*-values resulting from the statistical comparisons, with statistically significant differences highlighted in bold. Antigenic frequencies with values of 0 or 100% were excluded from the analysis.

In all the statistical analyses performed, significant statistical differences were found between all the frequencies obtained in the three areas studied, and those reported in Caucasians and Afro-descendants. The above except for the LU and DO blood system antigens between Punta Arena–Caucasians and Punta Arenas–Afro-descendants and in the DO system antigens between Arica–Afro-descendants' frequencies.

Discrepancies between genotyping and phenotyping

Six hundred and six samples from the three areas genotyped by the HEA Bead Chip platform were subsequently phenotyped with antisera. Discrepancies between genotype and phenotype were observed in 10 samples from Arica and Santiago for four antigens (Fy^b, C, e and M). All identified discrepancies were attributed to negative results in the serological method and positive results in the molecular method. The concordance rate between serological and molecular methods is presented in Table 7. In three of the six donors with discrepancies in C, the cause could not be identified with the studies conducted. In a subsequent sample from donor 4, the serological study was repeated with 2 anti-C clones, yet the C antigen remained undetected. Consequently, sequencing is required to resolve this discrepancy.

The *RH*01.01 allele present in donor 5 encodes a weak e antigen. However, the serological test returned a positive result, likely because the RH*01.01* allele can lead to weaker expression of the e antigen, depending on the serological methodology employed. For donors 5 and 6, no additional samples were obtained to repeat the serological study, and the discrepancies with the *RHCE* genotype (RHCE Beadchip) were left unexplained. In both cases, sequencing is necessary to resolve the discrepancies.

The immunogenic potential of antigens

Figure 1 illustrates the frequencies of antigens and antibodies specific to the antigens K, Di^a, E, C, e, c, Fy^a, Kp^a, Jk^a, Lu^a, Jk^b, S, Fy^b and s, which were used for calculating immunogenicity. The corresponding values, multiplied by 1 factor of 100 are depicted in Figure 2, with the highest value attributed to the Di^a antigen.

DISCUSSION

This study represents the first implementation of molecular methods to characterize the genotypes, alleles and antigens of blood groups in donors from the Northern, Central and Southern zones of Chile. Within this investigation, previously unreported antigens lacking

TABLE 5 Antigenic frequencies of the blood systems: RH, KEL, JK, FY, MNS, LU, DI, CO, DO, LW and SC studied in donors from northern, central and southern Chile.

The RBC antigen frequencies (%)																		
Arica	**C**	**E**	**c**	**e**	**V**	**VS**	**K**	**k**	**Kp^a**	**Kp^b**	**Js^a**	**Js^b**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**M**	**N**
***n* = 212**	73.1	49.1	74.1	87.7	0.9	1.9	4.2	100	1.4	100	0.5	100	84.9	57.5	67	84.9	88.2	55.7
	S	**s**	**U**	**Lu^a**	**Lu^b**	**Di^a**	**Di^b**	**Co^a**	**Co^b**	**Do^a**	**Do^b**	**Hy**	**Jo^a**	**LW^a**	**LW^b**	**Sc1**	**Sc2**	
	42	92.5	100	1.4	100	4.2	100	100	3.8	51.9	92	100	100	100	0	100	0.5	
Santiago	**C**	**E**	**c**	**e**	**V**	**VS**	**K**	**k**	**Kp^a**	**Kp^b**	**Js^a**	**Js^b**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**M**	**N**
***n* = 515**	60.2	38	74.9	95.5	1.7	2.1	6.0	99.8	1.2	100	0.2	100	73.6	68	70.7	82.7	86	61.5
	S	**s**	**U**	**Lu^a**	**Lu^b**	**Di^a**	**Di^b**	**Co^a**	**Co^b**	**Do^a**	**Do^b**	**Hy**	**Jo^a**	**LW^a**	**LW^b**	**Sc1**	**Sc2**	
	51.8	92.6	100	3.5	99.8	3.9	99.6	100	6.2	59.4	88.5	100	100	100	0	100	0.2	
Punta Arenas	**C**	**E**	**c**	**e**	**V**	**VS**	**K**	**k**	**Kp^a**	**Kp^b**	**Js^a**	**Js^b**	**Fy^a**	**Fy^b**	**Jk^a**	**Jk^b**	**M**	**N**
***n* = 123**	82.1	43.1	81.3	94.3	3.3	3.3	3.3	100	1.6	100	0	100	80.5	74	63.4	90.2	85.4	57.7
	S	**s**	**U**	**Lu^a**	**Lu^b**	**Di^a**	**Di^b**	**Co^a**	**Co^b**	**Do^a**	**Do^b**	**Hy**	**Jo^a**	**LW^a**	**LW^b**	**Sc1**	**Sc2**	
	32.5	93.5	100	4.1	100	0.8	100	100	1.6	60.2	86.2	100	100	100	0	100	2.4	

Abbreviation: RBC, red blood cell. In the gray rows are the names of blood group antigens included in the study.

TABLE 6 *p*-Value obtained from statistical analyses between antigenic frequencies of Arica–Santiago, Santiago–Punta Arenas and Arica–Punta Arenas.

p-Value								
Antigens	**C**	**E**	**e**	**Fy^a**	**Fy^b**	**Jk^a**	**S**	**Do^b**
Arica–Santiago	0.472	**0.006**	**0.005**	**0.005**	**0.006**	0.379	**0.018**	**<0.001**
Santiago–Punta Arenas	**0.007**	0.34	0.64	0.287	0.277	**<0.001**	**0.0001**	**0.027**
Arica–Punta Arenas	**0.031**	**<0.001**	0.058	0.362	**0.003**	**<0.001**	0.103	0.095

Note: Statistically significant differences are highlighted in bold.

TABLE 7 Concordance rate between serological and molecular antigen detection.

Antigen	Donors (*n*)	Concordance rate (%)	Genotypes
Fy^b	3	99.5	*FY*01/FY*02W.01*
C Donors 1, 2 and 3 are also VS+ and e antigen discrepant	6	99.0 (c) 99.5 (e)	Donors 1 and 2:*RHCE*03/RHCE*01.20.03* Donor 3: *RHCE*01/RHCE*01.20.03* Donor 4: *RHCE*01/RHCE*01* Donor 5: *RHCE*01/RHCE*01.01* Donor 6: *RHCE*03/RHCE*01*
M	1	99.8	It was not possible to obtain a new sample to repeat.

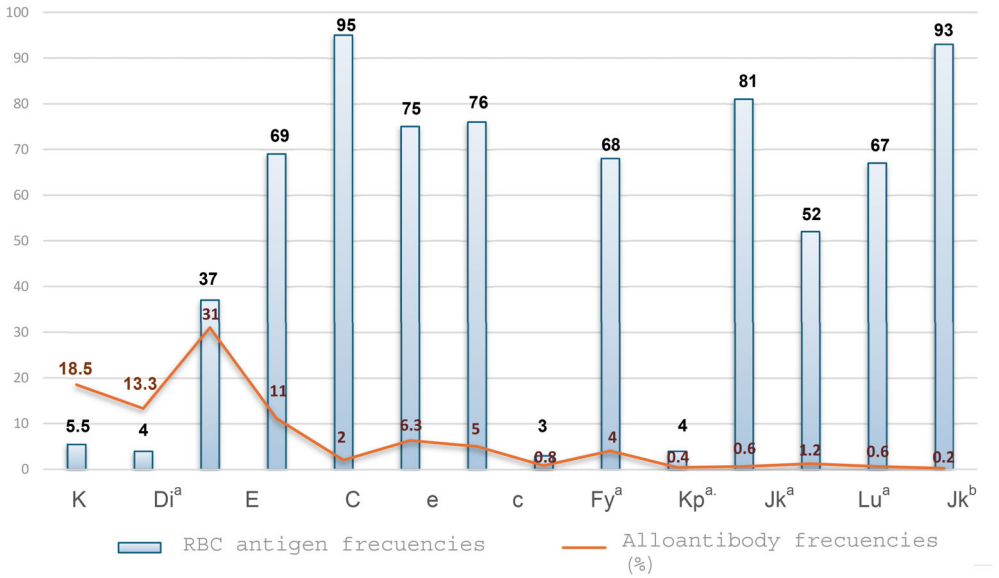

FIGURE 1 Erythrocyte antigen frequencies in Santiago donors and alloantibody frequencies in Santiago patients.

corresponding antisera, as well as LFA, weak variants, null phenotypes and rare blood group phenotypes, were recognized. Notably, genotypes harbouring alleles that affect antigen expression or inactivate antigen expression in the RH and FY systems were identified. Specifically, alleles *RHCE*01.20.01* and *RHCE*01.20.03* were identified, producing partial phenotypes of the c and e antigens on the RhCE protein. Additionally, these alleles are responsible for the expression of the LFA V and VS. *RHCE*01.20.01* also associated with the expression of hr^B antigen, unlike *RHCE*01.20.03*, which lacks hrB expression [17]. The *FY*01W.01* allele, identified in 13 donors, resulted in weak antigen expression, which may not always be detected by serological methods. Conversely, the *FY*02N.01* allele, present in 41 donors, is a silent allele. This allele features a T>C change within the promoter region of the gene, located 33 bp upstream of the erythroid transcription start point and 67 bp upstream of the major translation start codon (position −67). This mutation occurs within a GATA consensus sequence, disrupting the binding of the erythroid-specific GATA-1 transcription factor and consequently preventing gene expression in erythroid cells while maintaining expression in other cell types [18]. Furthermore, two donors exhibited the Fy null phenotype

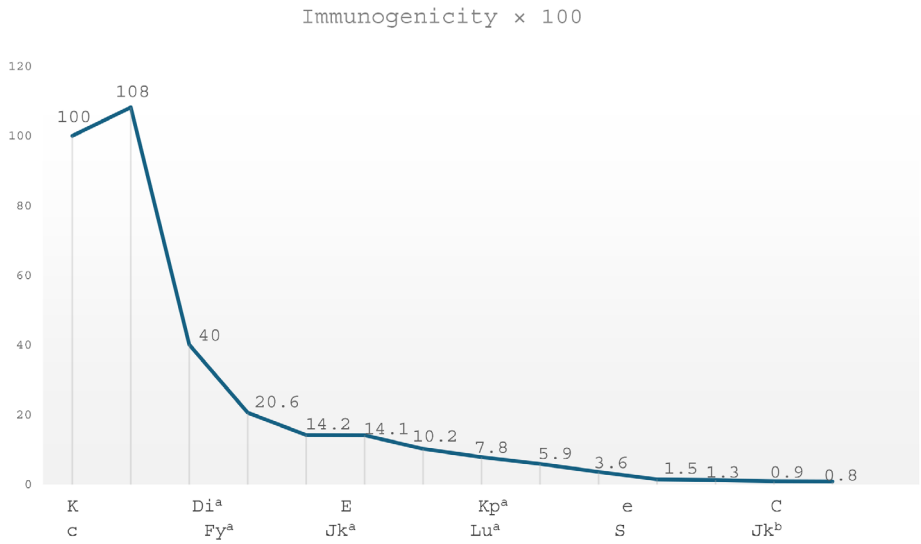

FIGURE 2 The calculation of the immunogenic potential of antigens performed with the antigenic frequencies of donors from Santiago and the frequency of antibodies of the same specificity from patients treated at the same institution.

due to a homozygous genotype for the *FY*02N.01* allele. While this phenotype is common among Africans, it is rare in other populations.

In the blood systems KEL, LU, DI, YT homozygous genotypes responsible for the absence of HFA, also known as rare, were identified. The identification and recruitment of blood donors with rare phenotypes, is crucial for providing safe transfusion therapy to patients with these phenotypes. While there are highly effective rare blood donor registry programmes worldwide, not all regions have the same level of availability. In Chile, only the Santa Maria Clinic has such a registry, which is partly formed by the donors identified in this study. Additionally, there is an Ibero-American initiative to establish a registry of rare blood donors capable of supplying Latin American countries and providing rare blood to patients in the region who need it [19].

The molecular study facilitated the detection of LFA, for which corresponding antisera are unavailable. All identified LFAs have been implicated in acute or delayed HTR or HDFN and had not been previously described in Chileans. The presence of the V, VS and Js^a antigens can be attributed to the West African ancestry of Chileans which accounts for close to 2.5% [10]. The donor who presented the Mi^a antigen indicated that he is unaware of having any Asian ancestry, noting that his parents, grandparents and great-grandparents are Chilean, with the exception of his great-grandmother, who was Italian. However, it is important to consider that Chileans have approximately 1.7% East Asian ancestry, likely a result of the Asian individuals who arrived during the colonization of Chile [10]. Similarly, the presence of the Di^a antigen is attributed to the Native American ancestry of the Chilean people, which comprises 38.7% [10], the higher frequency of this antigen in the northern zone aligns with previous findings reported by Etcheverry et al. [5] who observed that only the Atacameños (Amerindian-natives of the northern zone) presented the Di^a antigen, with a frequency of 12.5%.

LFA Sc2 was detected in all three regions studied, with the frequency found in Punta Arenas (2.4%) being higher than those reported in other countries, such as Canada, England, Germany, Czech Republic and Japan, where frequencies range from 0.5% in Japan to 1.7% in Canada [1]. While there are few reports of HTR and HDFN caused by anti-Sc2, it is noteworthy that one of the three reports of HDFN originated from Chile [20]. Due to the limited availability of molecular methods to detect these antigens in Chile, the role of LFA in HTR and HDFN may be underestimated. Additionally, identifying specific antibodies against LFA poses a challenge, as these antigens are rarely present in commercial RBCs. Failure to detect them may result in HTR in a previously alloimmunized patient.

Statistically significant differences were found between the frequencies of certain antigens in the RhCE, FY, MNS, DI, JK and DO blood systems across the three regions. These differences can be attributed to the heterogeneity of the Chilean population, characterized by a mixture in different proportions throughout the country of various native Amerindian peoples, European and African ancestry [5, 10].

Disparities in antigen frequencies among the three regions of the country increase the risk of alloimmunization. This risk is particularly significant given Chile's geography layout—a long and narrow country—where the most advanced healthcare facilities are concentrated in Santiago, located in the Central zone. Consequently, patients requiring organ transplants, haematopoietic progenitor transplants, cardiovascular surgery and other transfusion-dependent procedures from the Northern and Southern zones, are often transferred to Santiago. This find may suggest that patients from the North and South of the country face an increased risk of alloimmunization when transfused with RBC from donors in the Central zone, although haemovigilance data are required to confirm this.

Discrepancies between serological and molecular studies in 10 donors revealed the presence of weakly expressed antigens that are not detectable by serology. Three of these donors presented the allele *FY*01W.01*, which encodes a weak Fy^b antigen. This highlights that for certain antigens with weak expression, the serological method lacks the necessary sensitivity. Additionally, three discrepant samples in the C

antigen revealed the *RHCE*01.20.03* variant allele of the *RHCE* gene that had not been previously documented in Chileans, underscoring the importance of molecular methods to identify such variants.

Understanding immunogenicity is crucial for prioritizing which antigens should be routinely studied and determining the level of compatibility that RBCs should have in transfusion. In assessing the immunogenic potential of antigens, the Di^a antigen yielded the highest value, which is a significant finding, especially given that this antigen and its corresponding antibody are not routinely identified in Chile. This result strongly supports the inclusion of Di^a antigen testing in the typing of Chilean donors and underscores the need for mandatory use of red blood cells capable of detecting anti-Di^a antibodies.

In conclusion, this study conducted using molecular methods represents the first comprehensive and invaluable dataset on the phenotype, genotype and alleles present in Chileans, which cannot be studied by serological methods alone. This information can play crucial roles, the provision of compatible blood for alloimmunized patients, guiding the prioritization of antigens for further study and potentially establishing a rare donor programme in Chile. The insights gained from this research significantly contribute to enhancing transfusion practices and blood banking strategies in the Chilean context.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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