#### **IMMUNOHEMATOLOGY**

# Original article

# **Genomic characterization of clinically** significant blood group variants in **Aboriginal Australians**

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**Background** - Hematological disorders are often treated with blood transfusions. Many blood group antigens and variants are population-specific, and for patients with rare blood types, extensive donor screening is required to find suitable matches for transfusion. There is a scarcity of knowledge regarding blood group variants in Aboriginal Australian populations, despite a higher need for transfusion due to the higher prevalence of renal diseases and anemia.

Materials and methods - In this study, we applied next-generation sequencing and analysis to 245 samples obtained from Aboriginal Australians from South-East Queensland, to predict antigen phenotypes for 36 blood group systems.

Results - We report potential weak antigens in blood group systems RH, FY and JR that have potential clinical implications in transfusion and pregnancy settings. These include partial DIII type 4, weak D type 33, and Del RHD (IVS2-2delA). The rare Rh phenotypes D+ C+ E+ c- e+ and D+ C+ E+ c+ e- were also detected.

Discussion - The comprehensive analyses of blood group genetic variant profiles identified in this study will provide insight and an opportunity to improve Aboriginal health by aiding in the identification of appropriate blood products for population-specific transfusion needs.

**Keywords:** blood transfusion, population genetics, rare blood types, next-generation sequencing.

### INTRODUCTION

Blood group antigens are the primary indicator to assess the compatibility between blood donor and recipient in transfusion medicine. Pre-transfusion testing of patients with a transfusion requirement (obstetric, surgery, hematological disorders) is of vital importance to facilitate blood group antigen matching in order to provide safe and compatible blood and blood products for transfusion. Blood group antigen typing methods are also employed in the diagnosis, treatment and management of other disorders of antigen incompatibility, including hemolytic disease of the fetus and newborn (HDFN), and solid organ transplantation<sup>1,2</sup>. Extended blood group genotyping and matching between

donors and patients reduces the risk of alloimmunization and, for already alloimmunised patients, it is critical to meet ongoing transfusion needs3. Interestingly with regard to donor screening, Gleadall et al. identified 2-6 times more compatible donors for patients with multiple red cell (RBC) alloantibodies, including hard-to-transfuse patients, by employing a dense donor-typing approach<sup>4</sup>. Aboriginal Australians experience a higher incidence of renal disease and anemia than the general Australian population<sup>5</sup> which results in an increased dependency and frequency of blood transfusions. Most blood donors in Australia have European ancestry6; therefore understanding the diverse nature of blood group antigens and their potential for adverse transfusion reactions in Aboriginal people is vital for the provision of safe and equitable transfusion approaches and hemovigilance. Cultural considerations relating to blood, historical disadvantage and previous inappropriate research approaches have led to genuine concerns from the Aboriginal Australian population about participation in medical research. As a result, there are relatively few studies investigating diversity in blood groups within the Aboriginal Australian population.

Between the 1940's and the early 1970's, approximately 19 blood group antigens from a small number of Aboriginal Australian populations were studied using serology8-14. Relatively few blood group systems had been described since this period, whereas by 2023 the International Society of Blood Transfusion (ISBT) recognises 360 blood group antigens and over 1,800 allele within 45 blood group systems. NGS technologies have the potential to detect all polymorphisms in each individual's genome, including null alleles, novel variants, complex gene rearrangements, and copy number variations<sup>15,16</sup>. NGS-based blood group profiling studies in Western desert<sup>8</sup> and Tiwi Island populations17 have also shown that the prevalence of various blood group alleles varies between Aboriginal Australians and other population groups worldwide. Previous research has emphasized the significance of blood group characterization in diverse ethnic and cultural groups as antigen prevalence differs amongst global populations<sup>18,19</sup>. Up to date blood group frequency data is important to guide blood donor recruitment to support patients requiring blood transfusion and

minimise hemolytic transfusion reactions (HTR's) in diverse populations<sup>18-20</sup>.

Harnessing NGS technology to minimise risk and improve health outcomes for Aboriginal Australian patients requiring frequent transfusions is essential, provided culturally acceptable, respectful processes are addressed. The study was designed and conducted in collaboration with Aboriginal organizations and their representatives, and Aboriginal governance was integrated throughout the project. In this report, we present the NGS findings for the most clinically significant groups, the ABO (ISBT 001), RH (ISBT004) and KEL (ISBT 006) systems and discuss predictions for weakened or rare antigens in systems including FY (ISBT 008), JR (ISBT 032). Overall findings are compared with previous population studies.

#### **MATERIALS AND METHODS**

## Study design and recruitment of participants in the South East Queensland Aboriginal (SEQA) population cohort

An Aboriginal Advisory Committee (AAC) established to maintain Aboriginal governance and culturally safe research practices throughout this study. The AAC consisted of Aboriginal researchers, stakeholders, consumers, and community members. Respectful consultation and community engagement was conducted prior to any sample collection. Any feedback or suggestions for improvement that emerged from this consultation process were incorporated into the final study design and protocol. Potentially eligible participants were invited to one-on-one consultations with Aboriginal research staff at the participating Aboriginal Community Controlled Health Organisation, Carbal Aboriginal Medical Services, Queensland. As part of the consenting process, it was ensured that participants understood this study and had the opportunity to ask any questions or raise their concerns prior to providing informed consent. If they agreed, all participants provided written informed consent. In collaboration with Australian Red Cross Lifeblood, sample receipt, transport, serological testing, nucleic acid extraction and blood group targeted sequencing was conducted under the governance of the AAC. Safe storage and respectful handling of samples was also ensured, under the leadership of the AAC and according to appropriate guiding principles<sup>21</sup>.

#### **Data collection**

### SEQA population serological phenotyping and Targeted Exome Sequencing (TES)

ABO, and Rh (D, C, E, c,e) phenotyping was undertaken for all samples using monoclonal typing reagents (Immulab, Parkville, Australia) by tube direct hemagglutination technique. 27/127 red cell samples that failed to react with Epiclone 2 anti-D (IgM DVI-) by tube direct hemagglutination were tested using an anti-D monoclonal blend (IgG/IgM, Immucor [Norcross, GA, USA]) by tube indirect antiglobulin test at 37°C (IAT). Additional phenotyping for the K antigen was undertaken for 127 samples using by direct hemagglutination technique at 37°C using monoclonal anti-K (IgM clone MS-56, Immulab). Samples that typed positive for the K antigen (No.=7) were tested for the k antigen using a polyclonal typing reagent by IAT at 37°C (Immulab)<sup>22,23</sup>. Genomic DNA was extracted for all samples and submitted for targeted blood group exome (TES) sequencing on the Illumina MiSeq (Illumina Inc., San Diego, CA, USA) as previously described by Roulis et al., 2020<sup>22</sup>.

#### The 1000 genomes project phase 3

The 1KGP3 project includes 2,504 whole genome sequencing (WGS) samples from 26 population groups classified into five super populations. WGS binary alignment map (BAM) files (a compressed binary format with reference genome alignment information) were accessed through the 1KGP3 project FTP server<sup>24</sup>.

# Blood group profiling and population genomic analyses

BAM and VCF files for each individual SEQA sample, and individuals in the 1KGP3 cohort were submitted for blood group genotype and phenotype prediction using the first iteration of an automated interpretative algorithm, RBCeq version V1.0.0.1<sup>25</sup>. RBCeq V1.0.0.1 considers 36 blood group systems and two transcription factors encoded by 44 genes (*Online Supplementary* **Table SI**; **Figure 1**)<sup>25</sup>. We also compared the blood group variant profiles of the SEQA population with those reported in other Aboriginal Australian populations<sup>9-14,17</sup> including Western Desert Aboriginal Australians (WD)<sup>8</sup> Tiwi Islanders (TI)<sup>17</sup> and Northern Territory (NT)<sup>26</sup> where predicted genotyping profiles or NGS data was available.

#### RESULTS

# Overview of comparative data for ABO (ISBT 001), RH (004), LU (005), KEL (006) and SC (013)

Phenotypes predicted by RBCeq were concordant with serology for ABO, Rh and KEL, except in two cases where the predicted ABO phenotype did not match serology. Manual analysis of the VCF for these two samples suggests that the RBCeq prediction is correct.

For ABO (ISBT 001), the group O phenotype prevalence is >50% in all Aboriginal populations except in WD, where it is 14%. The prevalence of groups B (7.76%) and AB (1.22%) was higher in SEQA as compared to other Aboriginal populations, while these groups were absent in the WD population. Three individuals had predicted B1 (ABO\*B.01/ABO\*O.01.02) or B3 (ABO\*B3.02/ABO\*O.01.35) alleles. The A antigen was predicted to have a >2 fold higher prevalence in the WD (86%) in comparison with SEQA and other Aboriginal populations. Additionally, 17 individuals were predicted to have the A2 phenotype in SEQA and one in the TI population which is not reported in WD and NT (Table I). For the Rh system, the predicted RhD negative phenotype was 9.38% which was >3 fold higher compared to other Aboriginal populations (0-3%).

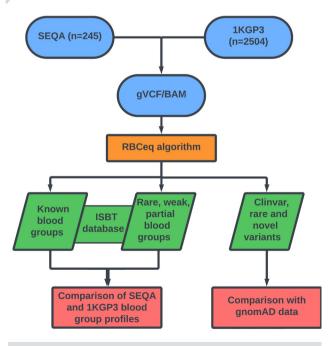


Figure 1 - An overview of the workflow used to comprehensively characterise population-specific blood group variants and predicted phenotypes

In contrast, previous studies have reported a much lower prevalence of D- in the NT Aboriginal population at 1.2%<sup>27</sup>, whilst the frequency in the WD population was reported at 3%<sup>8</sup> and D- was completely absent in the TI population<sup>17</sup> (**Table I**).

With respect to the LU system, the predicted Lu(a+b+) phenotype was only present in the SEQA population and absent in other Aboriginal populations (**Table I**). In comparison with 1KGP3, the predicted phenotype Au(a-b+) (LU\*02.19) in the SEQA population is 6.53%, which was lower than that observed in African and European populations (**Table II**). Alloantibodies formed against the K antigen are the second major cause of HDFN in Australia after anti-D. Alloantibodies against KEL antigens have been reported in Aboriginal Australian ICU patients<sup>26</sup>, as well as in thalassemic patients of non-Indigenous Australian heritage<sup>28</sup>.

The KEL\*01/02 genotype predicting the K+k+ phenotype was 8.57% among the SEQA population, and was absent in all other Aboriginal populations (**Table I**). When comparing this genotype in population groups in 1KGP3, the prevalence is 7.55% in European, whereas it is absent in the East Asian population. The K+k+ phenotype has been reported at a prevalence of 2% amongst the African population<sup>29</sup> (**Table II**).

We also detected two individuals in the SEQA cohort, one in the European population and two in the South Asian population carrying heterozygous  $SC^*o2$  (Sc2+) alleles. The *ERMAP* c.169G>A variant encoding for the  $SC^*o2$  variant was not detected in either the WD or TI population datasets<sup>8,17</sup> (**Table II**).

### Investigation of Rh predictive phenotype

Our investigation revealed that 90.61% (No.=222) of the SEQA population were homozygous for the RHD gene,

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Blood group antigens	SEQA prediction % (No.=245)	Western desert %8 (No.=72)	Tiwi islanders % <sup>17</sup> (No.=457)	Northern Territory % <sup>26</sup> (No.=2,130)	Literature % (1940-1970 serology) <sup>9-14</sup>	Comment <sup>9-14</sup>
0	52.24 (No.=128)	14 (No.=10)	81.18 (No.=371)	51.9 (No.=1,191)	NA	-
A	38.77 (No.=95)	86 (No.=62)	18.59 (No.=85)	14.1 (No.= 876)	NA	-
В	7.76 (No.=19)	0	0.21 (No.=1)	2.4 (No.=52)	NA	-
АВ	1.22 (No.=3)	0	0	0.5 (No.=11)	2	-
D+	90.61 (No.=222)	97 (No.=70)	100 (No.=457)	98 (No.=2,104)	-	-
D-	9.38 (No.=23)	3 (No.=2)	0	1.2 (No.=26)	-	-
Lu(a+b+)	4.08 (No.=10)	0	0	-	0	7.5% in European <sup>50</sup>
K+k+	8.57 (No.=21)	0	0	-	NA	-

Table I - Comparison of predicted blood group antigens in SEQA population with other Aboriginal Australian populations

Table II - Distribution of predicted genotypes for LU, KEL and SC, and its comparison with 1KGP3

Blood group	Predicted phenotype	RBCeq prediction (%)						
system and ISBT number		SEQA % (No.=245)	AFR % (No.=661)	AMR % (No.=347)	EAS % (No.=504)	EUR % (No.=503)	SAS % (No.=489)	
LU 005	LU2, LU18-, LU19 or Au(a-b+)	6.53 (No.=16)	19.21 (No.=127)	6.91 (No.=24)	0.99 (No.=5)	10.34 (No.=52)	4.91 (No.=24)	
KEL 006	KEL1, KEL2 or K+k+	8.57 (No.=21)	0.45 (No.=3)	4.32 (No.=15)	-	7.55 (No.=38)	1.23 (No.=6)	
SC 013	Sc1+, Sc2+	0.82 (No.=2)	-	-	-	0.2 (No.=1)	0.41 (No.=2)	

AFR: African; AMR: American; EAS: East Asian; EUR: European; SAS: South Asian.

**RBCeq prediction** Literature<sup>29,31,33</sup> **Blood** group **Predicted** system and phenotype SEQA % AMR % SAS % European % AFR % EAS % EUR % Asian % AFR % **ISBT** number (No.=245) (No.=661) (No.=347) (No.=504) (No.=503) (No.=489) RHD D 90.61 96.52 93.95 99.80 83.90 94.48 85 99 92 004 RH:1 (No.=222) (No.=628) (No.=326) (No.=503) (No.=422) (No.=462) RHD D-9.38 3.48 6.05 0.2 16.10 5.52 15 1 8 004 RH:-1 (No.=23)(No.=23)(No.=21)(No.=1)(No.=81) (No.=27)

**Table III** - Distribution of the RhD positive (D or RH:1) and negative (D-) phenotype

AFR: African; AMR: American; EAS: East Asian; EUR: European; SAS: South Asian.

with two individuals predicted as hemizygous for *RHD* gene encoding the alleles weak D Type3: *RHD*\*0*IW*.03 and Del RHD (IVS2-2delA): *RHD*\*53, which were phenotyped as altered D antigen and D- by serology. The SEQA cohort exhibited the second-highest proportion of D- resulting from complete deletion of the *RHD* gene (9.38%, No.=23), after the EUR cohort (16.10%, No.=81), while the lowest prevalence of this type was observed in the EAS population (0.2%, No.=1) (**Table III**).

The *RHCE* gene encodes for C/c and E/e antigens, which are inherited together, separately from RhD antigens, however are considered together in 8 distinct Rh haplotypes<sup>30</sup>. Rare Rh phenotypes predicted in the SEQA cohort were compared with previously published reports<sup>31</sup>. The D+ C+ c+ E+ e- (0.41%) and D+ C+ E+ c- e+ (2.86%) phenotypes which were predicted in the SEQA population, both containing the  $R_z$  haplotype, are also rare in other populations (**Table IV**) and were absent in the 1KGP3 dataset. The D+ C- E- c+ e+ phenotype, containing the  $R_o$  haplotype, is common in the African population (45.8%) and has a lower frequency in the

**Table IV** - RH blood group predictive phenotype prevalence in SEQA and other population datasets from literature<sup>31</sup>

Observed and predicted	RBCeq prediction	Literature <sup>31</sup>		
phenotype	SEQA %	European %	Asian %	African %
D+ C+ E+ c+ e-	0.41 (No.=1)	0.1	0.4	Rare/-
D+ C+ E+ c- e+	2.86 (No.=7)	0.2	1.4	Rare/-
D+ C- E- c+ e+	0.41 (No.=1)	2.1	0.3	45.8
D+ C- E+ c+ e-	4.48 (No.=11)	2.3	4.4	0.2
D+ C+ E- c+ e+	30.61 (No.=75)	34.9	8.5	21

SEQA cohort (0.41%) and Asian populations (0.3%) (**Table IV**). The SEQA cohort exhibited a higher prevalence of predicted D+ C- E+ c+ e- (4.48%) phenotype than any other population, while the D+ C+ E- c+ e+ phenotype prevalence was highest in European (34.9%) and SEQA cohorts (30.61%) respectively (**Table IV**) $^{32,33}$ .

# Predicted distribution of weak and partial antigens in the SEQA population

The predicted RhD partial DIII type 4 (*RHD\*o3.04*) was observed in two individuals (No.=2) and predicted Weak D Type3 (*RHD\*o1W.3*) was observed in one SEQA and European individual. The *RHD\*53* allele, associated with clinically significant Del RHD (IVS2-2delA) phenotype was predicted in one individual of the SEQA population and has been previously reported in Australia<sup>34</sup> but was not observed in any population of the 1KGP3 dataset. Weak D Type33(*RHD\*o1W.33*) has been associated with anti-D formation<sup>35</sup>, and here we report for the first time the presence of weak D Type33 at a frequency of 0.82% (No.=2), not only in SEQA but in any Australian aboriginal population (**Table V**).

In comparing the SEQA and IKGP3 datasets, we detected the FY\*02W.01 allele that encodes the Fy(a-b+<sup>W</sup>) FY<sup>X</sup> phenotype in 1.63% (No.=4) of the SEQA population, 1.44% (No.=5) of the American population (Colombia and Puerto Rico), 0.2% (No.=1) of the South Asian population, while it was missing from African population (**Table V**). The FY<sup>X</sup> gene frequency has previously been reported as 0.015% in a small proportion of Europeans<sup>36</sup> and was absent in the WD population dataset<sup>8</sup>.

While HTRs due to Anti-Jr(a) are rare, cases of severe and sometimes fatal HDFN have been reported<sup>37,38</sup>. The *ABCG2\*OIW.OI* allele, which encodes the Jr(a+w) predicted phenotype, was higher in the East Asian population (7%, No.=40) compared to other populations, including SEQA, where the prevalence was in the range of 1-2%.

# Comparison of SEQA blood groups with those of other Australian Aboriginal populations

Historically, the Fy(a+) phenotype in the FY system was reported to be 100% prevalence in the Aboriginal population, with the presence of Fy(b+) unreported<sup>9,39</sup>. In comparing SEQA data to WD and TI populations, we predicted a 20% prevalence of Fy(a–b+) in the SEQA population, compared to 1.39% in the WD and 0.88% in TI populations respectively (**Table VI**).

The Knops blood group (KN) alloantibodies can be difficult to identify, as they often mimic or mask the presence of other antibodies and therefore need to be correctly identified before dismissing their significance. 8.16% (No.=20) of samples in this dataset were homozygous for the variant c.4223C>T encoding the KN\*01.-05 allele, thereby resulting in a prediction of the Yk(a-) phenotype. The Yk(a-) phenotype is common among most global populations and was detected at a frequency of 37.5% in the WD population and 10.07% in the TI population.

# RBCeq blood group profiling of SEQA population reveals rare and novel variants

We additionally characterised variants that have not been formally identified as blood group alleles by the ISBT but were predicted to potentially impact structural formation (Figure 2). We detected four variants with disease-associated polymorphism with functional evidence (DFP) and functional polymorphism (FP) related to blood group variation and reduced protein expression: rs117351327 (KLF1), rs5036 (DI - ISBT 010), rs150221689 (LAN - 033), and rs12721643 (JR - 032). rs5036 is a Band 3 Memphis electrophoretic variant in SLC4A1 in the Diego system40. rs150221689 has been associated with reduced LAN expression41 and rs12721643 was associated with reduced JR protein expression as reported by HGMD. We also detected one variant associated with the FY blood group system (ACKR1:rs118062001) reported in ClinVar (ClinvarID: 1174942), however the impact of this variant is unknown. RBCeq identified nine computationally

**Table V** - Distributions of predicted weak and partial antigens

Blood group	Predicted phenotype	RBCeq prediction						
system and ISBT number		SEQA % (No.=245)	AFR % (No.=661)	AMR % (No.=347)	EAS % (No.=504)	EUR % (No.=503)	SAS % (No.=489)	
RHD 004	Partial DIII type 4	0.82 (No.=2)	0.60 (No.=4)	-	-	-	-	
RHD 004	Del RHD (IVS2-2delA)	0.41 (No.=1)	-	-	-	-	-	
RHD 004	Weak D Type 33	0.82 (No.=2)	0.45 (No.=3)	-	-	-	-	
RHD 004	Weak D Type3	0.41 (No.=1)	-	-	-	0.19 (No.=1)	-	
FY 008	Fy(a-b+ <sup>W</sup> ), Fy <sup>x</sup>	1.63 (No.=4)	-	1.44 (No.=5)	-	1.19 (No.=6)	0.2 (No.=1)	
JR 032	Jr(a+W)	1.22 (No.=3)	-	1.73 (No.=6)	7.94 (No.=40)	1.19 (No.=6)	0.61 (No.=3)	

 ${\it AFR: African; AMR: American; EAS: East Asian; EUR: European; SAS: South Asian.}$ 

Table VI - Comparison of predicted blood group antigens in SEQA population with other Aboriginal Australian populations

Blood group antigens	SEQA prediction % (No.=245)	Western desert % <sup>8</sup> (No.=72)	Tiwi Islanders % <sup>17</sup> (No.=457)	Northern territory % <sup>26,27</sup> (No.=2,130)	Literature (1940-1970 serology) <sup>9-14</sup>	Comment <sup>9-14</sup>
Fy(a-b+)	20 (No.=49)	1.39 (No.=1)	0.88 (No.=3)	-	0	100% Fy(a+) in Bentinck, Mornington and Forsyth Islanders <sup>39</sup>
Yk(a-)	8.16 (No.=20)	37.5 (No.=27)	10.07 (No.=46)	-	NA	Clinically significant

predicted novel and potentially antigenic non-synonymous variants present in exonic regions for the KN (022), GLOB (028), LAN (033), I (027), IN (023), LU (005) and H (018) blood group systems (**Figure 2**; *Online Supplementary* **Table SII**).

### **DISCUSSION**

Aboriginal Australians are 15-30 times more likely to develop end-stage renal disease requiring kidney transplantation<sup>42,43</sup>, with associated increased transfusion requirements. Blood group profiling at the population

level provides insight into the frequency of blood group alleles and antigens represented in the population and can help in sourcing the most appropriate antigenmatched blood for transfusion. In this study, we utilised RBCeq<sup>25</sup> to provide a comprehensive predicted genotype and phenotype for 36 blood groups, as well as identifying rare and novel blood group variants in the SEQA cohort. The findings of the study demonstrate that rare blood group genotypes of the SEQA population are not only distinct from European populations, which make up the majority of blood donors, but also illustrate the diversity

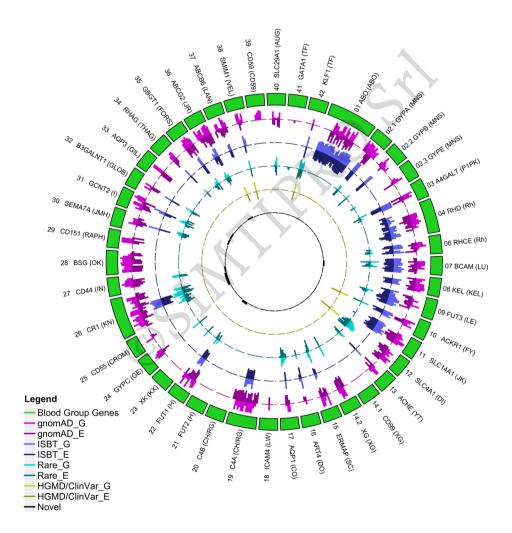


Figure 2 - Circos plot showing the distribution of genetic variants in RBC antigen-encoding genes and their frequencies. The outer ring (green) represents the RBC encoding genes. Box length represents the number of variants observed. G denotes gnomAD genome frequency, while E denotes gnomAD exome frequency. The outer purple (light/dark) circle indicates the distribution of variant frequencies across different blood group genes from the gnomAD data. The blue (light/dark) circle indicates the number of variants with ISBT allele designations relative to all gnomAD variants. The aqua (light/dark) circle indicates the distribution of gnomAD rare non-ISBT variants. The dark yellow circle indicates the number of non-ISBT variants annotated by the ClinVar/HGMD database. The black circle shows the distribution of the number of novel variants.

of blood group antigen prevalence among different Aboriginal Australian populations.

The ABO system is the prime determinant for transfusion matching, and in comparing the ABO group variant profile of the SEQA population with that of other Aboriginal Australian populations we found the highest prevalence of B (7.76%) phenotypes in SEQA, as compared to WD/TI/NT populations<sup>8,17,26</sup>. Universal plasma donors are AB phenotype, and this is the rarest of the four ABO phenotypes with prevalence of 3-5% in all populations<sup>33</sup>. The AB phenotype was reported at 1.22% in the SEQA population, higher than reported in NT but lower than previous reports in Indigenous Australians from Queensland (2%)<sup>9</sup>.

The RhD blood group is the second most clinically significant blood group after ABO, and extremely immunogenic. Partial D proteins lack certain RhD epitopes and individuals identified as partial D should ideally receive D- blood products. However, these individuals can be mistyped as being D+, which places them at risk of alloimmunization44. Additionally, anti-D alloantibodies from a mother with a partial D phenotype can place an RhD-positive foetus at risk of HDFN. The RhD typing strategies used in Germany and other European countries protect carriers of Partial D categories IV and VI from anti-D immunization but not carriers of other partial D antigens45. In our analysis, we detected variants in two individuals from the SEQA population encoding for the Partial D Category III type 4 phenotype, Weak D Type 33, and also one individual with a clinically significant Del RHD(IVS2-2delA) and one with Weak D Type3 phenotype. Earlier studies have identified these phenotypes in the Australian population but they have not been found in the 1KGP3 dataset except in African and European populations<sup>34</sup>. Additionally, typing for C/c and E/e antigens in the RhCE should be considered for neonates at risk of HDFN<sup>29</sup>, and for individuals with regular transfusion needs, as antibodies towards these antigens, particularly anti-c, are often encountered29. We reported the population-scale prevalence of the rare phenotypes D+C+E+c-e+ and D+C+E+c+e-in the SEQA cohort. The importance of accurately determining RH system phenotypes has been particularly illustrated in a study examining the prevalence of Rhc alloimmunization in a retrospective study of Aboriginal and Torres Strait

Islander peoples admitted to intensive care in the Northern Territory<sup>26</sup>.

Insights into the prevalence of these rarer Rh phenotypes in the SEQA cohort reveals transfusion management for the Aboriginal population requires a different approach to prevent alloimmunization. The provision of rare blood requires further attention and culturally safe engagement with Aboriginal communities. Australia's population is increasingly more diverse because of migration and the blood group landscape is reflective of this change. Recent reports have highlighted a decrease in D-blood donors but an increased demand for D- blood, within a population that is becoming increasing D+27,46. With respect to the SEQA cohort, we observed higher prevalence D- (9.38%, No.=23), in contrast to recent reports from the Northern Territory where 1.2% of Australian Aboriginal peoples were found to be D-. This disparity reiterates that blood group profiles vary between geographically diverse Australian Aboriginal peoples and more comprehensive blood group characterization studies are imperative to understand blood group prevalence, facilitating blood supply planning and effective hospital blood inventory management.

In examining our dataset for rare variants, we observed the variant for the Scianna blood group antigen Sc2+ as heterozygous in two individuals in the SEQA cohort, whilst the 1KGP3 dataset had one in the European population, and two in the South Asian population. The Scianna blood group is comprised of two high frequency (Sc1+ and Sc3+) and two low frequency (Sc2+ and Sc4+) antigens<sup>32</sup>. As only 0.82% of the SEQA cohort carry the Sc2+ antigen, there is a risk of alloimmunization to individuals without this low frequency antigen. Anti-Sc2 has been shown to cause HDFN on rare occasions, and the first case of a severe HTR to anti-Sc2 has also recently been reported<sup>32</sup>. Additionally, we report the presence of the Duffy FYX allele in the SEQA population, as well as European and South Asian populations. The FY<sup>x</sup> allele encodes a weakly-expressed Fybantigen, which is not always detected by monoclonal anti-Fyb47, and mistyping of this antigen may lead to delayed HTRs<sup>47</sup>. The SEQA population had higher predicted prevalence of clinically significant K+k+, Fy(a-b+), and Lu(a+b+) phenotypes as compared to other populations, while the variant encoding KEL:31(KYO+) antigen was detected in the Western Desert population but was absent in the SEQA population. A major limitation of the study is that additional red cell serology to confirm the presence of low incidence antigens predicted by RBCeq was not undertaken, and investigations of hybrid Rh and MNS changes were not performed.

The HGMD professional database, a manually curated database for locating disease-causing mutations, was used to explore the functional polymorphism of variants. The findings demonstrated that the HGMD-reported variations rs5036 (DI), rs150221689 (LAN), and rs12721643 (JR) are linked to reduced gene and protein expression. Further investigation is needed to explore the antigenic effect of these functional variants. Additionally, we identified nine novel non-synonymous variants using *in silico* predictive techniques.

Understanding the unique blood group profiles of Aboriginal populations is an essential step forward for equitable transfusion research, to inform transfusion professionals with the knowledge to provide safe transfusion support for First Nations peoples. This genomic research highlights the importance of study co-design with Aboriginal peoples, and that further steps forward are required to translate the findings to ensure transfusion care is delivered in a culturally safe and supported environment. The proper management and care of Aboriginal patients requiring blood transfusions is a delicate yet essential procedure, that aims to improve their health, wellbeing and quality of life7. Advancement in genomic technologies is increasingly transforming disease diagnosis, treatment, and prevention. The benefit of this progress, however, is inequitably provided for Aboriginal populations, who continue to experience poor health outcomes<sup>48</sup>. By establishing Aboriginal governance, co-design and culturally appropriate methodologies, this study highlights the importance of ethically appropriate and scientifically relevant research. The findings from this study provide clinically significant and valuable information that can potentially benefit transfusiondependent Aboriginal Australians and elevate further research work in the field to assist in developing holistic transfusion approaches mindful of the thoughts, feelings and beliefs of Aboriginal peoples with respect to blood<sup>49</sup>.

### **ETHICS APPROVAL**

The study was co-designed and approved by the AAC who established culturally safe and ethical practices. The study

was approved by the Australian Red Cross Lifeblood Ethics Committee (Reference number: 2018#17), as well as the Queensland University of Technology Human Research Ethics Committee (2021-3941-4347).

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### **AUTHORS' CONTRIBUTIONS**

Concept and design: SHN, RF, DI, ER, CH, SJ, CD. Data Analysis and validation SJ, CD, ER, CH. Visualization: SL, SJ. DNA sample collection and processing: TC, RG, ER, CD. First draft of the manuscript: SJ, CD, ER, CH, RF, SHN. Manuscript revision: SJ, ER, CD, CH, RF, SHN, MT, AB, MP, DI, BN. All authors have read and approved the final manuscript.

The Authors declare no conflicts of interest.

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