

by the -148C>T mutation, nor by any other putative mutation linked to this change. The -148C>T mutation in the erythroid-specific promoter of *PKLR* should, therefore, be considered a benign polymorphism. The basis for the lowered PK activity in the 3 individuals presented here thus remains to be established.

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Keywords: *PKLR*, promoter mutation, transcriptional regulation,
pyruvate kinase deficiency.

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Citation: de Vooght KMK, van Wijk R, van Wesel AC, van
Solinge WW. Characterization of the -148C>T promoter polymor-
phism in *PKLR*. *Haematologica* 2008; 93:1407-1408.
doi: 10.3324/haematol.12328

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Molecular studies reveal a concordant *KEL* genotyping between patients with hemoglobinopathies and blood donors in São Paulo City, Brazil

Kell is the most important blood group system after ABO and Rh because all frequently occurring Kell-specific antibodies must be considered clinically significant. The highly immunogenic Kell antigens are usually involved in red cell (RBC) alloimmunization that can cause either hemolytic transfusion reactions or perinatal hemolytic disease. Patients with hemoglobinopathies such as sickle cell disease (SCD) require frequent blood transfusions. Therefore, they often become alloimmunized and produce antibodies to low-prevalence Kell antigens, especially *KEL1*.¹⁻⁴ The Kell low-prevalence antigens are expressed due to single-nucleotide polymorphisms in the *KEL* exons and some of them show ethnic or racial specificity. *KEL1* is present in 9% of Caucasians and 2% of those of African descent, *KEL6* is expressed in 19.5% of Africans and in less than 0.01% of white Caucasians, while *KEL3* is found in 2.3% in Caucasians and is rare among Africans.¹ The incidence of alloimmunization, although multifactorial in etiology, is particularly high in SCD patients when compared with other multitransfused patients, primarily due to the disparity of phenotyping pattern distribution which is partly caused by ethnic or racial differences between the blood donor population and the SCD patient population.^{2,4} Extended antigen-matched RBC transfusion has been recommended for reducing the incidence of alloimmunization in patients with SCD. This practice adds greatly to the cost of providing blood components to these patients and can cause difficulties in finding RBC units for all patients.^{2,3} In order to evaluate differences in *KEL* genotyping distribution between blood donors and patients with SCD, and the benefit of providing extended antigen-matched RBC transfusion in multitransfused patients, we investigated the frequencies of different *KEL* genotypes in patients with hemoglobinopathies and blood donors in São Paulo City, the largest city in Brazil. The Ethics Committee Board approved the study.

Aliquots of EDTA-anticoagulant peripheral venous blood were collected from 108 Brazilian patients of African descent with hemoglobinopathies and 205 randomized blood donors. Genomic DNA was isolated from whole blood using a commercial kit (QIAamp DNA Blood mini kit, Qiagen, Hilden, Germany). Employing polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique, we performed the *KEL**1,2, *KEL**3,4,21 and *KEL**6,7 genotyping as previously described.^{5,7} The differences between the *KEL* genotype frequencies in the two groups were analyzed by Fischer's exact test. *p* values of 0.05 were considered statistically significant. The results of the frequencies for *KEL**1,2, *KEL**3,4,21 and *KEL**6,7 polymorphisms are summarized in Table 1. The *KEL**1/2 genotype was found in 6.5% (7/108) of patients with hemoglobinopathies and in 6.3% (13/205) of blood donors. The *KEL**3/4 genotype was observed in only 1.0% (2/205) of blood donors. The *KEL**6/7 genotype was found in 6.5% (7/108) of patients with hemoglobinopathies and in 4.4% (9/205) of blood donors. We also found 1 (0.5%) donor with the *KEL**6/6 genotype. No *KEL**21 allele, *KEL**1/1, or *KEL**3/3 genotypes were identified among the 313 subjects. There was no statistically significant difference between the frequencies of *KEL**1/2, *KEL**3/4 and *KEL**6/7 genotypes seen in

Table 1. Frequencies of KEL*1,2, KEL*3,4,21 and KEL*6,7 genotypes of patients with hemoglobinopathies and blood donors in Brazil.

Group (n)	KEL*1/2 n (%)	KEL*2/2 n (%)	KEL*3/4 n (%)	KEL*4/4 n (%)	KEL*6/6 n (%)	KEL*6/7 n (%)	KEL*7/7 n (%)
Hemoglobinopathies [†] (108)	7 (6.5)	101 (93.5)	0	108 (100)	0	7 (6.5)	101 (93.5)
Blood donors (205)	13 (6.3)	192 (93.7)	2 (1.0)	203 (99.0)	1 (0.5)	9 (4.4)	195 (95.1)
p value	> 0.999		0.547		0.430		

[†]HbSS:n=79; HbSC:n=19; HbS-Thal:n=6; HbCC:n=4

patients with hemoglobinopathies compared to those observed in blood donors.

Patients with hemoglobinopathies commonly require RBC transfusion to manage clinical complications such as anemia, acute chest syndrome, stroke, and splenic sequestration. Overall, RBC alloimmunization in transfused patients with SCD has a reported incidence of up to 40%. Alloantibodies against Kell antigens, especially anti-KEL1, have been observed as one of the most frequent antibodies in multitransfused SCD patients. Anti-KEL1 alloimmunization has a reported incidence of 28.1% up to 76.9% among alloimmunized SCD patients in North America and Europe,^{2-4,8,9} however, there are few data regarding the RBC alloimmunization rate in South American SCD patients. Table 2 summarizes the results of some studies evaluating RBC alloimmunization in patients with hemoglobinopathies. We have reported an overall RBC alloimmunization rate of 12.9% (11/85) in SCD Brazilian patients, and anti-KEL1 specific alloimmunization rate of 18.2% (2/11) among such subjects.¹⁰ A larger Brazilian study described an overall RBC alloimmunization rate of 9.9% (82/828) and anti-KEL1 specific alloimmunization frequency of 14.6% (12/82) among alloimmunized SCD patients,¹¹ suggesting a lower KEL alloimmunization risk for patients in Brazil.

A frequently recommended strategy for reducing the incidence of RBC alloimmunization in patients with SCD is to provide RBC transfusion matched for ABO, Rh (D), and additional blood group antigens, ranging from limited matching, i.e., C, E, and K antigens, to extended matching for as many as 8 additional antigens, i.e., C, c, E, e, K, S, Fy^a, Fy^b, and Jk^b.^{2,3} Besides the benefit of the phenotype matching protocols that would prevent alloimmunization in 52% to 70.8% of all immunized patients, these strategies may also diminish the risk of delayed hemolytic transfusion reactions, hyperhemolysis syndrome, and autoantibody formation. On the other hand, these protocols demand high cost, need access to uncommon RBCs phenotypes, and do not totally avoid the risk of alloimmunization, or the occurrence of delayed hemolytic transfusion reactions or hyperhemolysis syndrome.^{2,3}

Molecular techniques have been used in transfusion medicine as an alternative tool to serological methods in order to investigate patients who have been recently transfused, or when antibody reagents, such as anti-Js^a and anti-Js^b, are scarce or not commercially available.¹ Using molecular studies, we demonstrated that the frequency of the KEL*1/2 (6.5%) and KEL*6/7 (6.5%) genotypes among Brazilian patients with hemoglobinopathies differed from those usually observed in subjects of African descent. Moreover, the frequency of the KEL*1/2 (6.3%) and KEL*6/7 (4.4%) genotypes in blood donors differed from those to be expected in a Caucasian population or in subjects of African descent, but quite similar to the results observed in our patients with hemoglobinopathies. These

Table 2. Results of studies reporting RBC alloimmunization rate and anti-KEL1 specific alloimmunization frequency among alloimmunized SCD patients.

Author	Country	n	RBC alloimmunization rate	Anti-KEL1 frequency in alloimmunized patients
Rosse <i>et al.</i> ⁸	USA	1,814	18.6% (338/1,814)	28.1% (95/338)
Vichinsky <i>et al.</i> ⁴	USA	107	29.9% (32/107)	56.2% (18/32)
Moreira <i>et al.</i> ¹⁰	Brazil	85	12.9% (11/85)	18.2% (2/11)
Aygun <i>et al.</i> ²	USA	140	37.1% (52/140)	76.9% (40*/52)
Castro <i>et al.</i> ³	USA	351	39% (137/351)	38% (52/137)
Murao and Viana ¹¹	Brazil	828	9.9% (82/828)	14.6% (12/82)
Schonewille <i>et al.</i> ⁹	Netherlands	1,778 [†]	NR	30% (541/1778)

n: number of transfused patients; *Included anti-KEL7 (anti-Js^d);
[†]Alloimmunized patients (not only SCD); NR: not reported.

findings suggest a high rate of racial admixture in Brazilian patients of African descent with hemoglobinopathies, as well as in Brazilian blood donors, and possibly explain the lower risk of Kell alloimmunization seen in Brazilian SCD patients.^{10,11}

In conclusion, the observed concordant KEL genotyping donor-recipient population suggests that Brazilian patients are at a lower risk of Kell alloimmunization than that reported in studies from North America and Europe where the donor population is predominantly formed by white Caucasians. In addition, our data indicate that the requirement for extended antigen-matched RBC transfusion in multitransfused patients may not be cost-effective in Brazil.

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Funding: this study was partially supported by CNPq (Process n°
474995/03-8) and FAPESP (Process n° 05/55237-9).

Key words: KEL genotyping, sickle cell disease, transfusion
reactions, alloimmunization

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Citation: Boturão-Neto E, Chiba AK, Vicari P, Figueiredo MS,
Bordin JO. Molecular studies reveal a concordant KEL genotyping
between patients with hemoglobinopathies and blood donors in São
Paulo City, Brazil. *Haematologica* 2008; 93:1408-1410. doi:
10.3324/haematol.12766.

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Association of asymmetric dimethylarginine with sickle cell disease-related pulmonary hypertension

Pulmonary hypertension (PHT) occurs in approximately 30% of adult sickle cell patients and is associated with a high risk of early death. Hemolysis driven reductions in nitric oxide (NO) bioavailability resulting from NO scavenging by cell free hemoglobin and increased arginase activity are of importance in the pathophysiology of SCD related PHT.¹

Elevated plasma concentrations of asymmetric dimethylarginine (ADMA) contribute to limiting NO bioavailability in SCD.² ADMA and symmetric dimethylarginine (SDMA) derive from the irreversible post-translational methylation of arginine residues by protein arginine methyltransferases (PRMT) and are released as free amino acids upon proteolysis. ADMA (but not SDMA) competitively inhibits NO synthase (NOS) enzymes, thereby limiting NO production. ADMA is degraded by dimethylarginine dimethylaminohydrolases (DDAH) whereas SDMA is mainly cleared renally.³ Elevated plasma ADMA concentrations occur in several forms of PHT and are associated to PHT outcome.^{4,5} We investigated whether ADMA concentrations are associated with PHT in SCD.

Serum and EDTA plasma samples were available from adult sickle cell patients consecutively screened for PHT with echocardiography as previously reported.⁶ Mild and moderate-severe PHT are defined as tricuspid regurgitant jet flow velocity (TRV) of 2.5-2.9 m/s and TRV≥3 m/s respectively, with pulmonary-artery pressures considered

normal in patients with trace or no tricuspid regurgitation (with TRV assigned 1.3 m/s).¹ Plasma concentrations of ADMA, SDMA, amino acids and serum soluble vascular cell adhesion molecule-1 (sVCAM-1) levels were determined as previously described.^{2,7} For analysis, HbSS and HbSβ⁰-thalassemia patients were grouped together, as were HbSβ⁺-thalassemia and HbSC patients. *p*-values <0.05 were considered statistically significant (SPSS 12.0.2, SPSS Inc, Chicago, IL, USA). The study was carried out in accordance with the principles of the Declaration of Helsinki.

Two out of 19 PHT patients had moderate-severe PHT. Hydroxyurea use did not differ between patients with and without PHT and no patients used anticoagulation, calcium antagonists, endothelin receptor blockers or sildenafil. Between group comparisons were only per-

Table 1. Demographics and laboratory parameters in sickle cell patients with and without pulmonary hypertension.

	HbSS (n=40)/ HbSβ ⁰ -thalassemia (n=6)		<i>p</i>	HbSC (n=16) / HbSβ ⁺ -thalassemia (n=5)	
	PHT	PHT		PHT	PHT
N	28	18		20	1
Age (years)	33 (21-44)	28 (22-52)	0.80	29 (23-39)	41
Male:female	6:22	5:13		10:10	0:1
TRV (m/s)	2.0 (1.3-2.3)	2.7 (2.6-2.8)		2.1 (1.3-2.2)	2.6
sPAP*(mmHg)	21 (12-28)	34 (32-43)		21 (12-25)	31
Hb (mmol/L)	5.7 (5.0-6.2)	4.9 (4.2-5.9)	0.05	7.0 (6.6-7.6)	6.5
HbF (%)	10.6 (6.1-18.3)	5.9 (2.2-14.1)	0.13	1.0 (1.0-2.4)	4
LDH (U/L)	369 (300-515)	575 (388-846)	0.02	231 (202-359)	261
GFR (mL/min)**	151 (120-195)	120 (66-172)	0.10	140 (125-160)	114
ADMA (μmol/L)	0.57 (0.52-0.65)	0.63 (0.58-0.79)	0.01	0.50 (0.45-0.58)	0.48
SDMA (μmol/L)	0.47 (0.42-0.55)	0.51 (0.47-0.83)	0.07	0.46 (0.44-0.56)	0.47
Arginine (μmol/L)	45 (32-56)	46 (41-62)	0.26	67 (57-79)	56
Ornithine (mmol/L)	56 (42-66)	56 (45-75)	0.41	56 (49-62)	44
Citrulline (mmol/L)	23 (16-32)	27 (20-32)	0.66	29 (24-33)	32
Proline (mmol/L)	208 (162-257)	209 (176-234)	0.84	197 (148-247)	257
Arginine/ornithine	0.84 (0.66-1.0)	0.93 (0.72-1.15)	0.45	1.14 (0.86-1.21)	1.1
Arginine/citrulline	1.87 (1.66-2.49)	1.98 (1.43-2.65)	0.84	2.1 (1.6-2.3)	1.5
Arginine/proline	0.23 (0.18-0.31)	0.25 (0.19-0.35)	0.41	0.30 (0.21-0.37)	0.19
sVCAM-1 (ng/mL)	1089 (801-1239)	1542 (1119-1880)	0.007	851 (628-1011)	971

Data are presented as medians with their corresponding inter quartile range. A *p*-value <0.05 is considered statistically significant. *Right ventricular systolic pressure was estimated based on the modified Bernoulli equation (1) and considered to be equal to the systolic pulmonary artery pressure (sPAP) in absence of right ventricular outflow obstruction. **Glomerular filtration (GFR) rate calculated with Cockcroft and Gault-formula (males: creatinine clearance= 1.23xweight x (140-age)/serum creatinine, females: creatinine clearance = 1.03xweight x (140-age)/serum creatinine).