



Original article

Lack of association between Kidd blood group system and chronic kidney disease

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ABSTRACT

Background: The Kidd blood group system has three antigens, Jk^a, Jk^b and Jk³, found on red blood cells and on endothelial cells of the inner lining of blood vessels in the renal medulla. These are known as urea transporter B (UT-B). Researchers have found that individuals carrying the Jk(a – b–) or Jk-null (UT-B null) phenotypes have a lower urine-concentrating capability and risk of severe renal impairment. This study evaluated the distribution of the Kidd phenotypes in patients with chronic kidney disease and a possible association of Kidd antigens with the development of renal disease.

Methods: Jk^a and Jk^b antigens were phenotyped using the gel column agglutination test (ID-cards Bio-RAD) in 197 patients with chronic kidney disease and 444 blood donors, as the control group. The phenotype and antigen frequencies between patients and controls were evaluated using the Chi-square method with Yates correction and logistic regression after adjustments for gender and age.

Results: No differences were observed between the Kidd phenotypes frequency distribution between patients with chronic kidney disease and blood donors [Jk(a – b+) = 22.3% and 27.2%; Jk(a + b–) = 30.5% and 24.3%; Jk(a + b+) = 47.25% and 48.4%, respectively].

Conclusion: The distribution of Kidd phenotypes found in the studied population is expected for Caucasians; Jk^a and Jk^b antigens and phenotypes were not found to be related to susceptibility for chronic kidney disease.

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Introduction

The red blood cell (RBC) membrane contains many anchored surface proteins and proteins that cross the lipid bilayer carrying different blood group antigens. Currently, 36 systems¹ of RBC groups have been described according to the International

Society of Blood Transfusion (ISBT) (<http://www.isbtweb.org>). Among them, the Kidd blood group system (JK; ISBT 009) has been recognized as clinically important since its identification in 1951.²

Antigens of the Kidd blood group system are expressed on type 3 glycoproteins, also known as the urea transporter B

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(UT-B). This protein contains 389 amino acids and passes ten times through the lipid bilayer with both the N terminus and C terminus being intracellular. Three antigens have been found (Jk^a, Jk^b and Jk3) on the neighboring fourth extracellular loop and three phenotypes, Jk(a + b-), Jk(a - b+), and Jk(a + b+), are common among different populations. The Jk(a - b-) phenotype is rare in most populations. It was first found in a Filipina of Spanish and Chinese ancestry with the antibodies usually being detected after transfusions or pregnancies. After immunization, anti-Jk3 can be found in patients with the Jk(a - b-) recessive phenotype, causing acute and delayed hemolytic transfusion reactions.^{3,4}

The Jk glycoprotein is coded by the *Solute carrier family 14, member A1 (SLC14A1)* gene, a member of the urea-transporter gene family, located on chromosome 18q12-q21 and organized into 11 exons. The two major codominant alleles of the gene, JK*A and JK*B, have similar frequencies in Caucasian populations (0.51 and 0.49, respectively) and define the Jk^a and Jk^b antigens, respectively. The Jk^a/Jk^b polymorphism is defined by 838 A>G in exon 9 (Asp280Asn substitution), the other two single nucleotide polymorphisms (SNPs) cause no changes to the amino acid sequence.⁵

The Kidd antigens or UT-B are expressed on RBCs and the endothelium of the vasa recta and epithelial surfaces of the renal inner medulla, as well as in non-renal tissues and endothelial cells.⁶⁻⁸ The Kidd protein is a major transporter of urea across the RBC membrane and this rapid process helps maintain the osmotic stability of the cell. Null phenotype individuals lack this transporter. Within the Kidney, the urea transporter enables the renal medulla to maintain a high concentration of both urea and urine, as well as conserve water. Individuals with the Jk(a - b-) phenotype have lower urine-concentrating ability.⁹⁻¹¹ In UT-B-null mice, long-term UTB deficiency was associated to severe renal dysfunction and structural damage.¹² UT-B isoforms are also important in several cellular functions, including urea nitrogen salvage in the colon, nitric oxide pathway modulation in the hippocampus, and normalization of the cardiac conduction system.¹³

Chronic kidney disease (CKD) is a major public health problem, defined as abnormalities of kidney structure and/or function, present for at least three months with implications on health.¹⁴ The adverse outcomes of the disease include loss of kidney function, cardiovascular disease and premature death. Besides environmental factors, genetic abnormalities are also involved¹⁵ including variations in the MYH9 (encoding non-muscle myosin IIA heavy chain),¹⁶ APOL1 (apolipoprotein L1),¹⁷⁻¹⁹ NPHS1 (nephrin),²⁰ and SHROOM3 (shroom family member 3)²¹ genes.

The relationship between the Kidd antigens and chronic kidney disease remains unknown. Therefore, the aim of this study was to investigate the distribution of Kidd phenotypes in patients with the chronic kidney disease and a possible association of Kidd antigens with the development of renal disease.

Methods

The ethical and methodological aspects of this study were approved by the Research Ethics Committee on Human

Beings from the Maringa State University (COPEP-UEM # 1.141.385/2014, CAAE 43117115.0.0000.0104, according to the Resolution of the Brazilian Council on Health-CNS 466/12.

Subjects

This retrospective case-control study enrolled 197 unrelated patients with chronic kidney disease (CKD group) and 444 unrelated blood donors as a control group, living in the same geographical area as the patients. The individuals were attended and immunophenotyped between 2013 and 2015 at the Regional Blood Bank of Pato Branco, southwest region of Parana (located in the southern region of Brazil at 26°13'46"-09"S and 52°40'16"-09"W).

Serologic tests

Red blood cell phenotyping for Kidd blood group systems was performed on a gel card (ID-Perfil II - k-Kp^a-Kp^b-Jk^a-Jk^b-ctl) using monoclonal antibodies according to the manufacturer's instructions (Diamed ID-Cards, DiaMed® AG, Switzerland). RBCs were suspended in Bromelin solution (BioRad ID-Diluent 1) at a final concentration of 1:21 or 5%.

Statistical analysis

The antigen and phenotype frequencies were estimated and the data was tested for their fit to the Hardy-Weinberg equilibrium²² by calculating the expected frequencies of the genotypes and comparing them with the observed values. The Student's t-test was used to compare differences between groups. Statistical comparisons between these groups were performed and the estimated risk of developing CKD in individuals who have genetic polymorphisms was calculated by determining the Odds Ratio (OR) with a 95% of confidence interval (CI) adjusted for gender and age. Association tests were carried out to identify the codominant, dominant, recessive, overdominant and log-additive genetic inheritance models. *p*-Values ≤ 0.05 by the Chi-square test with Yates correction and logistic regression were considered statistically significant. All statistical analyses were performed using the software OpenEpi program Version 2.3.1 (<http://www.openepi.com>) and SNPStats²³ (<http://bioinfo.iconcologia.net/index.php>).

Results

The Kidd phenotype frequency distribution in the studied populations was in Hardy-Weinberg equilibrium (*p*-value = 0.48: CKD and *p*-value = 0.51: controls).

The characteristics of patient and control subjects are described in Table 1. The CKD patients were between 45 and 90 years old (62.8 ± 13.9) and from both genders (male: 55.8%; female: 44.2%). Regarding ethnicity, all patients declared themselves Caucasians. The control group was formed by 444 individuals between 18 and 64 years old (30.6 ± 11.1; *p*-value < 0.0001) from both genders (1:1) with 98.4% declaring themselves Caucasians. Because CKD was observed only in individuals > 45 years old, the control group was subdivided

Table 1 – Characteristics of the CKD patient and control individuals.

	Patients (n = 197)		Controls (n = 444)	
	Total (>45 years old) n = 197 n (%)	Total n = 444 n (%)	<45 years old n = 349 n (%)	>45 years old n = 94 n (%)
Gender^a				
Male	110 (55.8)	219 (49.3)	163 (46.7)	56 (59.6)
Female	87 (44.2)	225 (50.7)	186 (53.3)	38 (40.4)
Age (years)				
Mean	62.8 ± 13.9	30.6 ± 11.1 ^b	31.1 ± 7.2 ^b	52.5 ± 5.4 ^b
Ethnic group				
Caucasian	104 (100)	437 (98.4)	342 (98.0)	94 (100)
Black	0	6 (1.3)	6 (1.7)	0
Mulatto	0	1 (0.3)	1 (0.28)	0

^a p-Value >0.05 (between-group comparison by χ^2 test).
^b p-Value <0.0001 (between patients and controls. Student's t-test).

Table 2 – Distribution of Jk antigen and phenotype frequencies in CKD patients and controls (total and >45 years old).

	CKD Patients n = 197 n (%)	Control Total (n = 444) n (%)	Control >45 years old (n = 94) n (%)
Phenotypes			
Jk(a – b+)	44 (22.3)	121 (27.2) ^a	29 (30.8) ^e
Jk(a + b–)	60 (30.5)	108 (24.3) ^b	23 (24.5) ^f
Jk(a + b+)	93 (47.2)	215 (48.4) ^c	42 (44.7) ^g
Jk antigens			
Jk ^a	213 (54.0)	431 (49.0) ^d	90 (48.0) ^h
Jk ^b	181 (46.0)	457 (51.0)	98 (52.0)

^a p-Value = 0.32; ^b p-Value = 0.55; ^c p-Value = 1; ^d p-Value = 0.16 (CKD patients vs. controls – total of samples).
^e p-Value = 0.55; ^f p-Value = 0.18; ^g p-Value = 1; ^h p-Value = 0.16 (CKD patients vs. controls >45 years old).

(n = 94) for analyses, considering the minimum age reported by patients. In this group, the mean age was 52.54 ± 5.36 years (p-value < 0.0001), and 58.9% were males and 41.1% females with all of them being self-declared Caucasians.

The distributions of antigen and phenotype frequencies for the Kidd blood group systems in CKD patients and controls (total group and group >45 years old) are shown in Table 2. The phenotype frequencies for the Kidd blood group system in CKD patients were 22.3% for Jk(a – b+), 30.5% for Jk(a + b–) and 47.2% for Jk(a + b+). For controls (total and >45 years old, respectively) the phenotype frequencies were 27.2% and 30.8% for Jk(a – b+), 24.3% and 24.5% for Jk(a + b–), and 48.4% and 44.7% for Jk(a + b+). The null phenotype was not observed in any of the groups.

Differences in distributions of the Jk^a and Jk^b antigen and phenotype frequencies were not observed when case and control groups were compared according to inheritance models or after logistic regression stratified by age.

Discussion

In this association study, patients with CKD on hemodialysis were analyzed in order to investigate the distribution of JK phenotypes in patients with CKD and a possible influence

of Jk^a and Jk^b antigens and phenotypes in the development of renal disease. However, differences between Jk^a or Jk^b antigen and phenotype frequencies were not observed between patients and controls.

The patients' ages ranged from 45 to 95 years old (55.8 ± 13.9) and the predominant gender and ethnic group were male (62%) and Caucasian (100%). When the age range of CKD patients was compared with that of the control group, significant differences were found. This was because the control group was formed of blood donors from the Pato Branco Blood Bank with ages ranging between 18 and 64 years (BRASIL, ordinance 2712 of November 12, 2013). Therefore, the great majority of control individuals (78.8%) were between the ages of 18 and 44, and none of the CKD patients was under 45 years of age. On the other hand, 44.7% of the patients were between the ages of 65 and 90, but none of the control group were this old. In order to circumvent these differences in age distribution, a control subgroup was defined with only individuals over 45 years of age. However, differences between Jk^a or Jk^b antigen and phenotype frequencies were not observed between the two control groups (total of individuals and >45 years old).

Non-matching between patients and controls with respect to age may be a weakness of this study. This bias may occur because in complex diseases many genetic and environment factors contribute to the outcome; individuals from the

control group with an age of under 45 are possible candidates for developing renal dysfunction in the future. On the other hand, in case-control studies, ideally there should be at least one control per case,²⁴ and the control group of individuals older than 45 years had fewer patients than the CKD Group. However, the Jk^a or Jk^b antigen and phenotype frequencies in both control groups of this study were similar to those frequencies found in another Brazilian population,²⁵ thereby validating the results in this study.

The urea transporter in the kidney enables its medulla to maintain a high concentration of both urea and urine, as well as conserve water. Individuals with the Jk(a-b-) phenotype had lower urine-concentrating ability. In animal models, long-term UTB deficiency was associated with severe renal dysfunction and structural damage. On the other hand, genetic variations of the *SLC14A1* gene were associated with bladder cancer and, some genotypes were associated with higher morbidity,²⁶⁻²⁹ and rejection after renal transplant.³⁰ However, we did not find any null alleles in CKD patients or controls that could be identified as a risk factor for the disease.

In this study, more than 50% of the CKD patients were homozygous carrying either Jk(a+b-) or Jk(a-b+) phenotypes, in agreement with the results reported in the literature for Caucasians.²⁵ Furthermore, no differences were observed between patients and controls regarding the distribution of Jk^a or Jk^b antigen and phenotype frequencies. Thus, Kidd phenotypes were not associated to CKD.

Conclusion

The distribution of Kidd phenotypes found in the studied population was as expected for Caucasians and therefore, Jk^a and Jk^b antigens and phenotypes were not found to be associated to the development of CKD.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

- Möller M, Joud M, Storry JR, Olsson ML. ErythroGene: a database for in-depth analysis of the extensive variation in 36 blood group systems in the 1000 Genomes Project. *Blood Adv*. 2016;1(3):240-9.
- Allen F, Diamond L, Niedziela B. A new blood group antigen. *Nature*. 1951;167:482.
- Daniels G. Other blood groups. In: Roback JD, Combs MR, Grossman BJ, Hillyer CD, editors. *Technical manual*. 16th ed. Bethesda, MD: American Association of Blood Banks; 2008. p. 411-36.
- Onodera T, Sasaki K, Tsuneyama H, Isa K, Ogasawara K, Satake M, et al. JK null alleles identified from Japanese individuals with Jk(a-b-) phenotype. *Vox Sang*. 2014;106(4):382-4.
- Hamilton JR. Kidd blood group system: a review. *ImmunoHematology*. 2015;31(1):29-35.
- Sands JM, Gargus JJ, Fröhlich O, Gunn RB, Kokko JP. Urinary concentrating ability in patients with Jk(a-b-) blood type who lack carrier-mediated urea transport. *J Am Soc Nephrol*. 1992;2(12):1689-96.
- Inoue H, Jackson SD, Vikulina T, Klein JD, Tomita K, Bagnasco SM. Identification and characterization of a Kidd antigen/UT-B urea transporter expressed in human colon. *Am J Physiol Cell Physiol*. 2004;287(1):C30-5.
- Stewart G. The emerging physiological roles of the SLC14A family of urea transporters. *Br J Pharmacol*. 2011;164(7):1780-92.
- Sands JM. Molecular mechanisms of urea transport. *J Membr Biol*. 2003;191(3):149-63.
- Timmer RT, Klein JD, Bagnasco SM, Doran JJ, Verlander JW, Gunn RB, et al. Localization of the urea transporter UT-B protein in human and rat erythrocytes and tissues. *Am J Physiol Cell Physiol*. 2001;281(4):C1318-25.
- Lucien N, Sidoux-Walter F, Roudier N, Ripoché P, Huet M, Trinh-Trang-Tan MM, et al. Antigenic and functional properties of the human red blood cell urea transporter hUT-B1. *J Biol Chem*. 2002;277(37):34101-8.
- Zhou L, Meng Y, Lei T, Zhao D, Su J, Zhao X, et al. UT-B-deficient mice develop renal dysfunction and structural damage. *BMC Nephrol*. 2012;13:6.
- Shayakul C, Cléménçon B, Hediger MA. The urea transporter family (SCL14): physiological, pathological and structural aspects. *Mol Aspects Med*. 2013;34(2-3):313-22.
- Chapter 1: Definition and classification of CKD. *Kidney Int Suppl* (2011). 2013;3(1):19-62.
- Pattaro C, Teumer A, Gorski M, Chu AY, Li M, Mijatovic V, et al. Genetic associations at 53 loci highlight cell types and biological pathways relevant for kidney function. *Nat Commun*. 2016;7:10023.
- Nelson GW, Freedman BI, Bowden DW, Langefeld CD, An P, Hicks PJ, et al. Dense mapping of MYH9 localizes the strongest kidney disease associations to the region of introns 13 to 15. *Hum Mol Genet*. 2010;19(9):1805-15.
- Freedman BI, Murea M. Target organ damage in African American hypertension: role of APOL1. *Curr Hypertens Rep*. 2012;14(1):21-8.
- Parsa A, Kao WH, Xie D, Astor BC, Li M, Hsu CY, et al. APOL1 risk variants, race, and progression of chronic kidney disease. *N Engl J Med*. 2013;369(23):2183-96.
- Chen TK, Estrella MM, Parekh RS. The evolving science of apolipoprotein-L1 and kidney disease. *Curr Opin Nephrol Hypertens*. 2016;25(3):217-25.
- Bonomo JA, Ng MC, Palmer ND, Keaton JM, Larsen CP, Hicks PJ, et al. Coding variants in nephrin (NPHS1) and susceptibility to nephropathy in African Americans. *Clin J Am Soc Nephrol*. 2014;9(8):1434-40.
- Khalili H, Sull A, Sarin S, Boivin FJ, Halabi R, Svajcer B, et al. Developmental origins for kidney disease due to Shroom3 deficiency. *J Am Soc Nephrol*. 2016;27(10):2965-73.
- Guo SW, Thompson EA. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*. 1992;48(2):361-72.
- Sole X, Guino E, Valls J, Iniesta R, Moreno V. SNPStats: a web tool for the analysis of association studies. *Bioinformatics*. 2006;22(15):1928-9.

24. Dorak TM. Basic population genetics. Available from: <http://www.dorak.info/genetics/popgen.html> [cited 21.02.17].
25. Guelsin GA, Sell AM, Castilho L, Masaki VL, de Melo FC, Hashimoto MN, et al. Genetic polymorphisms of Rh, Kell, Duffy and Kidd systems in a population from the State of Paraná, southern Brazil. *Rev Bras Hematol Hemoter.* 2010;33(1):21-5.
26. Garcia-Closas M, Ye Y, Rothman N, Figueroa JD, Malats N, Dinney CP, et al. A genoma-wide association study of bladder cancer identifies a new susceptibility locus within SLC14A1, a urea transporter gene on chromosome 18q12.3. *Hum Mol Genet.* 2011;20(21):4282-9.
27. Rafnar T, Vermeulen SH, Sulem P, Thorleifsson G, Aben KK, Witjes JA, et al. European genome-wide association study identifies SLC14A1 as a new urinary bladder cancer susceptibility gene. *Hum Mol Genet.* 2011;20(21):4268-81.
28. Koutros S, Baris D, Fischer A, Tang W, Garcia-Closas M, Karagas MR, et al. Differential urinary specific gravity as a molecular phenotype of the bladder cancer genetic association in the urea transporter gene, SLC14A1. *Int J Cancer.* 2013;133(12):3008-13.
29. Ran J, Wang H, Hu T. Clinical aspects of urea transporters. *Subcell Biochem.* 2014;73:179-91.
30. Rourk A, Squires J. Implications of the Kidd blood group system in renal transplantation. *Immunohematology.* 2012;28(3):90-4.