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# First report of a KELnull phenotype in Peru and a lesson of invisible genetic disparity

Ina Pérez[a,*], Lilian Castilho[b], Alfredo Goytendia[a], Erika Rojas[a], Jesús Bernal[a], José Alva[a], Julio Coyotupac[a], Christian Cortez[a], Carlos Munive[c], Indira Tirado-Hurtado[d], Joseph A. Pinto[d]

[a] Servicio de Banco de Sangre y Medicina Transfusional, Clínica Delgado - AUNA, Avenida Angamos Oeste, cuadra 4, esquina con Calle General Borgoño, Miraflores, Lima 18, Peru
[b] Molecular Immunohematology Laboratory Hemocentro, Universidade Estadual de Campinas (UNICAMP), Rua Carlos Chagas 480, Caixa Postal 6198, Barão Geraldo, 13081 - 970 Campinas, São Paulo, Brazil
[c] Departamento de Medicina Oncológica, Oncosalud - AUNA, Avenida Guardia Civil 571, San Borja, Lima 41, Peru
[d] Unidad de Investigación Básica y Traslacional, Oncosalud - AUNA, Avenida Guardia Civil 571, San Borja, Lima 41, Peru

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ABSTRACT

Blood banks in developing countries have limited capability to typify common blood groups creating disparities in the access to blood units for patients with rare blood genotypes. We report the case of a Peruvian woman with metastatic breast cancer with KELnull phenotype ($K_0$), a rare blood group characterized by the lack of expression of all Kell antigens on the red blood cells (RBCs). The molecular studies identified that the patient's RBCs were homozygous for the nonsense c.1546C > T mutation predicted to encode p.Arg516Ter (*KEL*02 N.17* allele), which confirmed the $K_0$ phenotype. We conducted a local and international search of compatible blood units. Finally, the Japanese Red Cross donated the blood units for the patient. We present here the first report for a $K_0$ phenotype in Peru and the challenging genetic disparities that many patients have to face to access to blood units in our country.

## 1. Introduction

Access to rare blood groups is very difficult and challenging for patients and the health system. Several initiatives including the register of rare donors could facilitate this access; however, these initiatives are scarce in developing countries [1,2].

The Kell blood group system is one of the most important blood groups in transfusion and obstetric medicine, after the ABO and Rh systems. It was discovered in 1946 by Coombs et al., when he isolated an antibody in the blood of a woman named Mrs. Kelleher whose newborn child had a severe hemolytic disease [3,4].

The Kell system is formed by the Kell and XK proteins, linked by a single disulfide bond, which are responsible for expressing all the antigens of this system. The *XK* gene (Xp21.1) encodes the XK protein, which it is characterized by crossing 10 times the membrane of the red blood cells (RBCs) and carries only one antigen, kx. While the highly polymorphic *KEL* gene (7q33) encodes the Kell protein, a type II membrane glycoprotein that carries 39 antigens [5]. The prevalence of these antigens varies according to the ethnic origin of the population [6], being K/k (KEL1/KEL2), $Kp^a/Kp^b$ (KEL3/KEL4), and $Js^a/Js^b$ (KEL6/KEL7) the most important [7,8].

There are different Kell phenotypes product of single nucleotide mutations leading to amino acid substitution, such as Gerbich-negative, $Kp^a$, Kmod, McLeod syndrome, and the KELnull ($K_0$); being these last two very rare phenotypes [9–11]. The McLeod syndrome is a neurological disorder with an X-linked recessive inheritance, caused by the lack of the XK protein and characterized by the low expression of Kell antigens and abnormal star shaped RBCs (acanthocytosis) [12].

The $K_0$ phenotype was discovered in 1957 by Chown et al. [13], and it is characterized by the total absence of the Kell protein and Kell antigens on RBCs; however, the RBCs have a normal discoid shape but with less expression of the XK protein. $K_0$ is the result of single nucleotide mutations that alter the reading frame causing the premature termination of translation of the protein or an alternative splicing. Currently, 37 $K_0$ alleles are known, which must be present in an individual in homozygous (both alleles affected by the same mutation) or compound heterozygote (alleles with different mutations) state to cause the null phenotype [7].

Although $K_0$ individuals have these mutations in the *KEL* gene, they are completely healthy. Nevertheless, they can produce anti-KEL5 (anti-

* Corresponding author.
*E-mail address:* iperez@auna.pe (I. Pérez).

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Ku), an antibody that reacts against many epitopes in the Kell glycoprotein, if they are exposed to RBCs with Kell antigens by transfusion and/or pregnancy, which can cause severe hemolytic transfusion reactions and hemolytic disease of the newborn. Therefore, $K_0$ individuals they should only be transfused with $K_0$ blood products [7,14].

$K_0$ phenotype is very rare, with an approximate frequency of 1:15,000 to 1:25,000 individuals [15]. Despite this, it has been reported in different populations of the world such as in Europeans (0.007%), Japanese (0.008%), Chinese (0.00228%), Austrians (0.00017%), Africans, and Indians. Nevertheless, there are few studies and reports in Latin America [7].

Scarce information about the local prevalence of uncommon blood groups, the lack of local resources for an extended blood typing and the absence of registries of donors with rare blood groups create genetic disparities to access to a compatible blood transfusion. This is an issue not adequately addressed in the scientific literature.

We present here the first description of a case of $K_0$ phenotype in Peru in a woman with metastatic breast cancer and discuss the disparities and difficulties to access to units of rare blood groups.

## 2. Materials and methods

### 2.1. Phenotyping by serology

Phenotypes were determined by haemagglutination in gel cards (AG Profile I, II, III) with monoclonal antibodies specific for 25 antigens belonging to the 9 main blood group systems at the Blood Bank of the Clínica Delgado - AUNA (Lima - Peru). These tests were confirmed in duplicate (Fig. 1). Due to the lack of local resources to conduct a comprehensive molecular study in order to determine the genotype of the patient's blood group, we decided to send the patient's DNA to an international reference laboratory.

### 2.2. Genotyping and sequencing

Genomic DNA was isolated from peripheral blood and it was sent to the Molecular Immunohematology Laboratory Hemocentro at UNICAMP (São Paulo - Brazil) for molecular studies. *KEL* alleles were genotyped with the HEA v1.2 BeadChip (Bioarray, Immucor, Warren, NJ), obtaining the phenotype K–k+, Kp(a–b+), Js(a–b+). Due to the discrepancy between *KEL* phenotyping and genotyping, sequence analysis was conducted. The 19 *KEL* exons, the 3 *XK* exons and intron-exon boundaries were sequenced using the Sanger dideoxy method as previously described [16].

## 3. Case report

### 3.1. Case presentation

The patient was a 59 years old Peruvian woman diagnosed with breast cancer in 2005, and currently with multiple metastatic sites in lung, bone, liver, and skull. Due to this, she underwent treatment with several chemo and radiotherapy lines since her diagnosis. Her obstetric history revealed that she had been pregnant five times. Her first pregnancy ended in spontaneous abortion, the second one was a full-term healthy girl, the third one ended in fetal death at 34 weeks, the fourth one resulted in a full-term healthy girl, and the last pregnancy was a preterm boy who needed an exsanguinous transfusion. Although she has undergone three prior surgeries (hysterectomy, mastectomy, and cholecystectomy) and five pregnancies, she has no history of previous blood transfusions.

**Table 1**
Comparison of column agglutination methods.

| | Grifols | BIO-RAD |
|---|---|---|
| Cell I | 4+ | 4+ |
| Cell II | 4+ | 4+ |
| Cell III | 4+ | 3+ |
| Diego | 4+ | no available |

The patient was admitted to the hospital because of a febrile syndrome, sepsis with respiratory and urinary focus, and severe anemia (hemoglobin: 6.9 g/dl); so her treating oncologist recommended a blood transfusion. The patient was previously typed as blood group O and RH positive, thus pre-transfusional tests with erythrocytes from donors with the same ABO and RH phenotypes was conducted. However, the patient's serum was incompatible (4+ reactions) with more than 48 blood units, so a more detailed phenotyping was carried out (Table 1, Figs. 2 and 3). No compatible donor was found among his closest relatives.

### 3.2. The blood typing

Serological test showed that the patient has the following phenotype: blood group O, RH positive (D+, C+, c-, E-, e+), K(-), k(-), $Kp^a$(-), $Kp^b$(-), $Jk^a$(-), $Jk^b$(+), M(+), N(-), S(-), s(+), $Fy^a$(+), $Fy^b$(+), $P^1$(+), $Le^a$(-), $Le^b$(+), $Lu^a$(-), and $Lu^b$(-) (Fig. 1). The patient's RBCs typed negative for the main Kell blood group antigens; moreover, immunohematological analyzes identified anti-Ku antibodies, typically found in immunized $K_0$ individuals.

*KEL* genotyping showed homozygosity for a previously found change in exon 14, c.1546C > T mutation, on *KEL*02* allele background (*KEL*02 N.17*) leading to a premature stop codon (p.Arg516Ter) associated with a $K_0$ phenotype. No other types of mutations were identified in the *KEL* gene and there was no any mutation in the XK gene. Altogether these results confirmed that the patient has the $K_0$ phenotype.

### 3.3. The transfusion

We look for compatible blood units in local blood banks. Unfortunately, this type of blood could not be located in Peru since there are no databases, no Blood Bank for Rare Blood Units, and no Regional Reference Laboratories for Immunohematology to facilitate

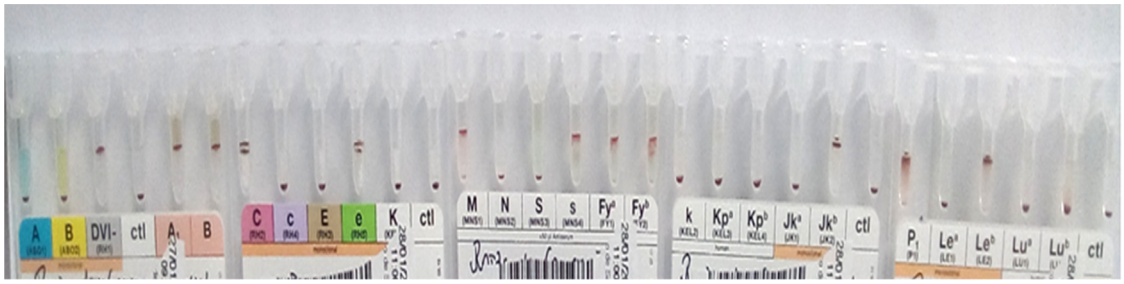


**Fig. 1.** Blood group. The gel cards of extended phenotype in gel microcolumns show the blood group O, RH positive (D+, C+, c-, E-, e+), K(-), k(-), $Kp^a$(-), $Kp^b$(-), $Jk^a$(-), $Jk^b$(+), M(+), N(-), S(-), s(+), $Fy^a$(+), $Fy^b$(+), $P^1$(+), $Le^a$(-), $Le^b$(+), $Lu^a$(-), and $Lu^b$(-).

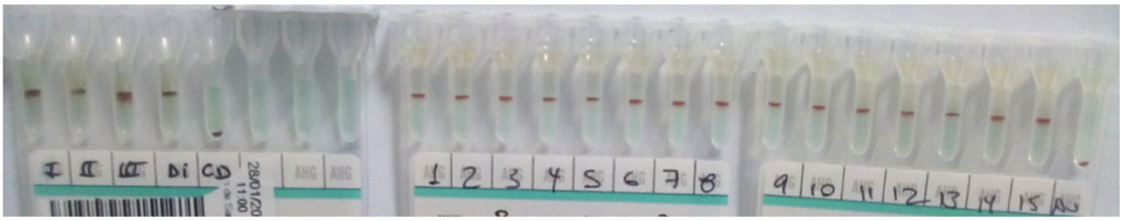

**Fig. 2.** Antibody Screening. Direct Coombs and Panel ID antibodies tests through microcolums.

the search. So we proceeded to conduct a search in the neighboring countries of South America, as well as in other continents.

Finally, we received a positive response from the Rare Blood Group Laboratory of the Japanese Red Cross in Osaka, who has a registry of $K_0$ individuals. They donated 3 units of blood $K_0$, and once they arrived in Peru, we proceeded to execute the protocol of repetition of regulatory quality control checks and also repeat the extended phenotype. The patient finally received the units of blood $K_0$, without any transfusion reaction.

## 4. Discussion

Studies about inequalities in the access to healthcare resources are frequently and historically addressed to ethnicity, race or socio-economic status; however, there is scarce discussion about the genetic disparities that many patients have to face. One genetic disparity frequently faced in the health system is the access to rare blood units.

The $K_0$ phenotype is a very rare blood group, defined by the total absence of the Kell protein and Kell antigens on RBCs; so it is a real challenge to find a donor with this phenotype even in populations with a similar genetic background. Currently, it have been reported 37 $K_0$ alleles that are carriers of single nucleotide mutations that cause premature termination of translation of the protein or an alternative splicing resulting in the null phenotype. However, despite the fact that $K_0$ individuals present these mutations, which at the molecular level inactivate the activity of the Kell enzyme, they are healthy. This suggests that either the function of this protein is not essential for the organism or there are compensatory mechanisms of other proteins from the same family [7,9].

At present, there is no published database on molecular information or the frequency of $K_0$ cases in Peru. In this study, we reported the first case of $K_0$ phenotype in Peru in a woman with the c.1546C > T mutation in exon 14 of the *KEL* gene (*KEL*02 N.17* allele) in a homozygous state, which generates a premature stop codon (p.Arg516Ter). This mutation has been previously been found as a cause of $K_0$ phenotype in two heterozygous individuals, one reported by a German research group [17] and the other in a Taiwanese blood donor [16]. No other type of mutations was found in the patient, not even in the *XK* gene, so the Mcleod phenotype was discarded.

Through immunohematological analysis, we detected anti-Ku antibodies in the patient, probably produced by exposure to Kell antigens during her pregnancies. It is suspected that this may have been the cause of her miscarriage, her fetal loss, and that her son was born with severe anemia (who required exanguineo transfusion according to familiar references). Likewise, we could find hidden anti-E antibodies in the same reaction, so one of the three $K_0$ units that were donated could not be transfused to the patient since it was E + . If we had not performed screening tests and panel characterization of irregular antibodies with elution techniques, the patient probably would have had an intense hemolysis reaction, so we decided not to use this unit. This is not an isolated event since most of the $K_0$ individuals have been identified after producing antibodies [17]; therefore, this shows us how important is the identification of rare phenotypes, the creation of a national database of rare blood groups, and a stock of frozen blood products.

From this case, we realized that the regional system of Latin America was not prepared to quickly refer cases of patients with suspected rare groups. The management of such patients generally poses a major challenge, so it is necessary that we design short and long-term strategies to provide them quick and efficient care. Unfortunately, this is oversimplified to a problem of scarcity of rare blood units and it is not adequately addressed as a case of genetic disparity that many patients around the globe have to face in order to obtain a compatible blood unit.

Peru has no national reference laboratories of immunohematology (much less molecular immunohematology); in addition, there are few accredited laboratories. An accredited system would be a great strength because it validates the creation of new methods, facilitates the creation of new working algorithms, as well as the creation of a databases.

Latin American centers that are accredited to maintain a "diamond" standard must have the ability to open up among peers in the region. There should be a standard criterion for registering and calling an individual with a rare blood group, whose data should be connected to the working parties of the International Society of Blood Transfusion (ISBT). Finding a rare donor is mainly technical, but it also requires the administrative and financial support of the center that decides to make the report and the call to resolve it. In addition, educational awareness programs must be implemented since many resources are invested to make determinations of blood group phenotypes.

## 5. Conclusions

In this study we described the first case of $K_0$ phenotype in Peru caused by a nonsense c.1546C > T mutation in exon 14 of the *KEL* gene (*KEL*02 N.17* allele), who probably got immunized during her pregnancies. It is necessary to implement the phenotyping and

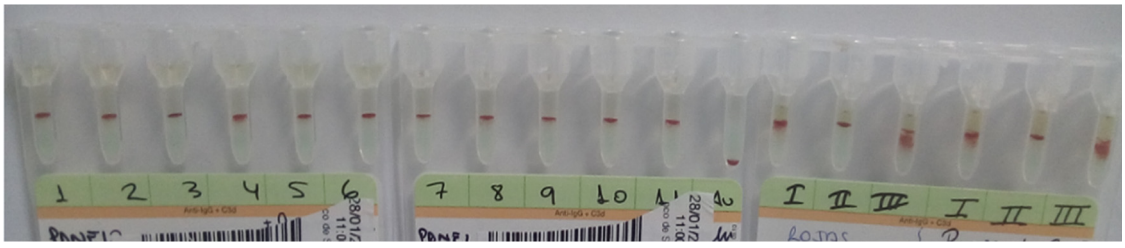

**Fig. 3.** Antibody Screening, Direct Coombs and Panel ID antibodies tests through ID-Card LISS/Coombs. The antibody panel containing anti-IgG and anti-C3d within the gel matrix reveals strong alloimmunization. Here we see an intensity of 3+ to 4+ of reaction.

genotyping of rare antigens in the routine, mainly in special cases such as cancer and pregnancy, to avoid hemolytic reactions. Policymakers should adequately address the shortage of rare blood units as a case of genetic inequalities to conduct more efforts to solve this problem.

## Author contributions

IP, AG, IT-H, and JP contributed to the conception and design of this work. IP, AG, and CM gathered all the clinical information. ER, JB, JA, JC, and CC conducted the phenotyping. LC performed the molecular subtyping. IP wrote the first draft of the manuscript. All authors contributed to the revision and approval of the manuscript.

## Declarations of interest

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to *Transfusion and Apheresis Science*.

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