Deletion in the *A4GALT* Gene Associated with Rare “P null” Phenotype: The First Report from India

Shamee Shastry^a Kapaettu Satyamoorthy^b Kiran V. Acharya^c
Vijay Ram Reddy^a Ganesh Mohan^a Chenna Deepika^a Dinesh Reghunathan^b
Manjunath B. Joshi^b

^aDepartment of Immunohematology and Blood Transfusion, Kasturba Medical College, Manipal, India, Manipal Academy of Higher Education, Manipal, India; ^bManipal School of Life Sciences, Manipal Academy of Higher Education, Manipal, India; ^cDepartment of Orthopedics, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, India

Keywords

Null phenotype · *A4GALT* gene · Large deletion · Rare blood group · Anti-PP1P^k · Blood transfusion · Patient blood management

Abstract

Background: The present report illustrates a case with rare “P null” phenotype due to a large deletion in chromosome 22q13.2 and with clinically significant anti-PP1P^k antibody. Patient blood management in such cases is challenging. **Case Report:** The transfusion center supporting the tertiary care referral center in the southern part of India received a blood sample from a trauma case for pre-transfusion testing. An antibody to a high-frequency blood group antigen was initially suspected. Following extensive immune-hematological workup, the patient was diagnosed to have naturally occurring anti-PP1P^k antibody and a rare “P null” phenotype. The genomic DNA of the patient was analyzed by exome sequencing followed by Sanger’s sequencing. Molecular diagnostics revealed a large 21-bp deletion in chromosome 22q13.2 which encodes the *A4GALT* gene, resulting in truncation of seven amino acids I245–251P and resulted in rare “P null” phenotype. Patient blood management strategies were adopted to manage the patient conservatively without

blood transfusion. **Conclusion:** A large deletion in chromosome 22q13.2 had resulted in a rare “P null” phenotype in the present case. The patient was a victim of a road traffic accident, required emergency hospitalization, as well as surgical intervention, and his plasma had antibodies to high-frequency antigens. A rare donor registry plays a major role in providing transfusion support to such cases.

© 2019 S. Karger AG, Basel

Introduction

The molecular and biochemical aspects of the P, P1PK histo-blood group system is very complex. Of these, P^k, P, PX2, and LKE are high-frequency antigens. More than 99.9% of the Asian population has P1 and/or P2 phenotypes [1]. P1 and Pk antigens are synthesized by 4- α -galactosyltransferase, encoded by the *A4GALT* gene located at 22q13.2. The antigens of the P1PK system have been identified as glycosphingolipids on the RBC membrane. However, the study by Stenfelt et al. [2] indicated that the P1 antigen resides on human RBC glycoproteins, and further glycosidase studies suggested that at least part of the epitopes occurred on N-glycans. Single nucleotide polymorphisms (SNPs) and other alterations of the cod-

Fig. 1. Schematic representation of the deletion in the *A4GALT* gene. The deletion of 21 bp in exon 3 resulting in truncation of seven amino acids in the luminal domain of the α 1,4Gal-T protein is presented. Yellow and blue boxes represent noncoding exons and open reading frame, respectively.

ing region of *A4GALT* are a known result in “p” phenotype. In the present study, we report a rare case of “P null” phenotype because of a 21-bp deletion in chromosome 22q13.2 and the patient blood management. This is the first report of its kind from India.

Case Report

A 45-year-old male patient was admitted to the trauma center of the Kasturba Medical College, Manipal, India, with a fractured shaft of the right femur as diagnosed by X-ray and multiple crush injury to the toes following a road traffic accident. On admission, he was conscious and oriented with stable vital signs. His hemoglobin was 8.5 g/dL, with normal platelet count and coagulation profile. There was no history of previous transfusion nor had his blood grouping been determined before.

On blood grouping, the patient was typed as B, RhD positive. However, serum grouping showed pan-reactivity with all the reagent red cell used (ORTHO Auto-Vue; Innova System, Ortho Clinical Diagnostics, USA). Antibody screening and identification tests using commercially available 3-cell and 11-cell panels (BioRad, Switzerland) showed pan agglutination with negative auto control and Direct Antiglobulin Testing. The antibody reacted at 4°C as well as at 37°C, and before and after the treatment with dithiothreitol. The antibody reacted equally with red cells untreated and treated with a proteolytic enzyme. Agglutination reaction persisted even after treating the serum with hydatid cyst fluid (P neutralizing substance). Meanwhile, we cross-matched 80 red blood cell units of the B and O group randomly, but yielded no compatible unit. Also, his younger sister was tested and found to be incompatible with his antibody. The patient’s blood samples were referred to the International Blood Group Reference Laboratory (IBGRL), Bristol, UK, for further analysis. They identified the antibody as anti-PP1P^k. Three

examples of red cells typed “pp” were compatible with the patient’s plasma and no additional antibodies were detected. His red cells failed to react with anti-P made in P^k individuals, anti-PP1P^k made in “pp” individuals, and monoclonal anti-P^k, thereby confirming his blood group as a rare “pp” or “P null” phenotype.

Molecular Diagnostics

Genomic DNA was isolated from the blood of the patient and checked for purity. Subsequently, Exome library was prepared using Exome RDY panel (ThermoFisher Scientific, Waltham, MA, USA) and further adaptors were ligated using Ion AmpliSeq DNA library kit (ThermoFisher Scientific). Approximately 100 picomoles of the library was used for emulsion PCR to prepare template positive ion sphere particles (ISPs) using Ion PI Template OT2 kit v2 (ThermoFisher Scientific), following the manufacturer’s instruction. Subsequently, template-positive ISPs were enriched using streptavidin C1 beads in the Ion PI enrichment system (ThermoFisher Scientific) and loaded onto Ion PI v3 Chip (ThermoFisher Scientific). Sequencing reads from Ion Proton were aligned to the human reference sequence (human genome build-19), analyzed using Ion Torrent Variant Caller v5.0 (TVC) and viewed using Integrative Genomic Viewer software and check for strand biases, homopolymer length, and sequencing errors.

In order to validate the results of the exome sequencing, a set of primers were designed for *A4GALT* gene at chromosome 22. A 328-bp region encompassing the Chr 22:43089056–43089383 region was amplified using the following set of primers P1_A4GALT-F: 5′-GGGTTGATGTCCTCAAAGTA-3′; P2_A4GALT-R: 5′-ACACGGAAGTTCATTGTTCTC-3′ in Veriti 96 well Thermal cycler (ThermoFisher Scientific). Subsequently, amplicons were purified and sequenced in both directions using the Sanger’s sequencing method ABI 3130 (ThermoFisher Scientific).

Results of exome sequencing were aligned with the hg19 reference sequence. The exome data analysis using IGV (Integrative Genome Viewer) tool indicated a deletion of 21 bp in chromosome 22q13.2 which encodes the *A4GALT* gene. The results of exome

Fig. 2. The patient's response to erythropoietin administration.

sequencing were further validated using Sanger's sequencing (Fig. 1). Further analysis revealed that the deletion of 21 bp in the *A4GALT* gene corresponding to the A995–1015G position, resulted in truncation of seven amino acids I245–251P.

As there was no realistic possibility to get the compatible blood unit of this rare type, we discussed the patient blood management strategies with all stakeholders involved in the patient's treatment. Hence, surgical correction was postponed by 1 week, and the patient was simultaneously started on recombinant human erythropoietin and ferric carboxy maltose (FCM) to accelerate the erythropoiesis and to increase the hemoglobin level to the targeted value of 11 g/dL (Fig. 2). When the hemoglobin level reached 11 g/dL, the surgical procedure was performed. The intraoperative and postoperative period remained uneventful. Postoperative hemoglobin was reduced to 9 g/dL, and hence erythropoietin and iron supplements on alternate days were continued. He recuperated well and was discharged on the 17th day of admission with a hemoglobin level of 10.6 g/dL. He was advised to continue with oral iron supplementation for the next 4 weeks.

Discussion

The rare blood groups reported from India include the Bombay phenotype, $-D-/-D-$, In (a+b-), Co(a-b-), I-i-, Cde/Cde ($r'r'$), Mg, and the weaker variants of ABH antigens [3]. Some of these are innocuous to blood bank practice, others have potential to pose pressure in procuring compatible blood units for the transfusion recipients. P-null is a rare phenotype with an approximate frequency of 1 in 5.8 per million in European populations. In the Indian population, the frequency is not known [4]. This phenotype possesses a naturally occurring antibody termed anti-PP1P^k, and it has the potential to cause intravascular hemolysis of the incompatible transfused red cells. This is the first report of its kind from India. An uncommon large deletion in the *A4GALT* gene had resulted in "P null" phenotype in the present case.

There are few case reports highlighting the clinical significance of anti-PP1P^k in the literature. Reeves et al. [5] reported anti-PP1P^k antibody in a less than 4-month-old child which was not of maternal origin. An association with habitual abortion was established by Iseki et al. [6]. Acute renal failure following a severe hemolytic transfusion reaction was noted in a patient with anti-Tj^a antibody with a titer of 1:16 [7]. A case of hemolytic disease of a newborn born to a mother with anti-Tj^a was treated for exchange transfusion using maternal blood [8].

Studies have demonstrated genetic polymorphisms in the *A4GALT* gene located at chromosome 22q13.2, and mutations in this gene have been attributed to the "P null" phenotype [9]. The P1/P2 polymorphism is connected to the different *A4GALT* gene expression levels. In a systematic analysis among four ethnic groups, screening of 416 SNPs in the *A4GALT* gene indicated that rs2143918 and rs5751348 were significantly associated with the P1/P2 phenotypes [10]. Li et al. [11] reported that a deletion (c.345delA) in the *A4GALT* gene led to a frameshift and introduced a premature translation stop codon. A study in Amish individuals showed that homozygous 299C>T mutation changing serine to leucine residue in *A4GALT* protein was associated with the "P null" phenotype [12]. Kaczmarek et al. [13] found that nucleotide substitution 631C>G in the *A4GALT* gene lead to a replacement of glutamine with glutamic acid (Q211E) in a subject with "P null" phenotype. Further, site-directed mutagenesis followed by transfection of vectors cloned with Gb3/CD77 synthase with substitutions p. Q211D or p. Q211N did not express Pk, P1, and NOR antigens, suggesting a significant loss of enzymatic activity. Wild-type vectors expressing Q211 produced P1 antigens indicating that Q211 was critical for Gb3/CD77 synthase activity [13]. In three individuals with "P null" phenotype, long-range PCR and sequencing revealed large deletions in the pro-

moter and noncoding exons 1 and 2a of the A4GALT gene, and quantitative PCR assays showed an absence of A4GALT transcripts. Although breakpoints varied in between three individuals, these regions harbored Alu or MIRb sequences [14]. This suggested that the loss of the promoter region was crucial and affected the synthesis of P1 and Pk antigens. Xu et al. [15] found a homozygous 26-bp deletion (nucleotide position 972–997) in a coding region of the A4GALT gene and deletion resulted in a shift of the reading frame, which led to a variant polypeptide chain with additional 83 amino acid residues.

The proband in our study showed a deletion of 21 bp from the nucleotide position 995–1,015 in the A4GALT transcript and led to the truncation of seven amino acid residues (I245_251Pdel). The A4GALT protein consists of three domains which are cytoplasmic, transmembrane, and luminal. The conserved DXD (192–194) domain responsible for the enzymatic activity is located in the luminal domain. Interestingly, deletion of seven amino acids as observed in our study was also within the luminal domain and might result in the loss of enzyme activity. However, structural and functional analysis of mutated protein is warranted to confirm biological activity. The literature on transfusion support to patients with rare phenotypes is limited. For elective procedures, all stakeholders must discuss and formulate the strategy well in advance. However, it is not always a success story on every case in an acute emergency. In a survey, it was observed that transfusion support was unsatisfactory in about one-third of the hospitalized patients with antibodies to high-frequency antigens [16]. In such cases, a rare donor registry plays a major role in providing transfusion support. Cryopreservation

of red blood units and development of the National Rare Donor Registry of India is the need of the hour.

Acknowledgement

The authors thank to Dr. Sanmukh R. Joshi of Lok Samarpan Regional Blood Center, Surat, for his valuable guidance while performing immunohematological investigations on the patient. The authors are also in-debt to Ms Nicole Thornton and Shane Grimsley from Red Cell Reference Section, IBGRL, Bristol, UK, for their immense help in identification the antibody in our rare pp phenotype.

Statement of Ethics

The study was approved by the institute's committee on human research. Written informed consent was obtained from the patient. Patient identity is not disclosed in the manuscript.

Disclosure Statement

The authors have no conflicts of interest to disclose.

Author Contributions

Dr. Shamee Shastri, Dr. Vijay Ram Reddy, Dr. Ganesh Mohan, Dr. Kiran Acharya, and Dr. Chenna Deepika were involved in the serological diagnosis of the rare phenotype and the patient blood management.

Dr. Satyamoorthy Kapaettu, Dinesh Reghunathan, and Dr. Manjunath B. Joshi performed the molecular work and interpretation of the data.

References

- Westman JS, Olsson ML. ABO and other carbohydrate blood group systems. In: Fung MK, Eder AF, Spitalnik SL, Westhoff CM, editors. Technical manual. 19th ed. Bethesda: AABB; 2018. p. 265–294.
- Stenfelt L, Westman JS, Hellberg Å, Olsson ML. The P1 histo-blood group antigen is present on human red blood cell glycoproteins. *Transfusion*. 2019 Mar;59(3):1108–1117.
- Joshi SR, Vasanth K. A profile of rare bloods in India and its impact in blood transfusion service. *Asian J Transfus Sci*. 2012 Jan;6(1):42–3.
- Benidt GR, Jaben EA, Winters JL, Stubbs JR. Identification of anti-PP1Pk in a blood donor and her family: a case report following her pregnancy and review. *Transfus Apher Sci*; 2010 Dec;43(3):369–74.
- Reeves HM, Cary V, Mino MA, McGrath C, Westra JA, Piccone C, et al. Unexpected Non-Maternally Derived Anti-PP1Pk in an 11-Week-Old Patient. *J Pediatr*. 2017 Feb;181:302–5.
- Iseki S, Masaki S, Levine P. A remarkable family with the rare human isoantibody anti-Tja in four siblings: anti Tja and habitual abortion. *Nature*. 1954 Jun;173(4416):1193–4.
- Gómez-Pérez C, Mujika-López A, de Manzanos TV, Álvarez-Quintana E, Gainza-de Los Ríos FJ, Gómez-Larrambe N. [Acute renal failure due to haemoglobinuria secondary to P antigen]. *Nefrologia*. 2014;34(2):265–6. Spanish.
- Levene C, Sela R, Rudolphson Y, Nathan I, Karplus M, Dvilansky A. Hemolytic disease of the newborn due to anti-PP1 Pk (anti-Tj a). *Transfusion*. 1977 Nov-Dec;17(6):568–72.
- Hellberg A, Westman JS, Thuresson B, Olsson ML. P1PK: the blood group system that changed its name and expanded. *Immunohematology*. 2013;29(1):25–33.
- Lai YJ, Wu WY, Yang CM, Yang LR, Chu CC, Chan YS, et al. A systematic study of single-nucleotide polymorphisms in the A4GALT gene suggests a molecular genetic basis for the P1/P2 blood groups. *Transfusion*. 2014 Dec;54(12):3222–31.
- Li X, Diao X, Xia X, Hong X, Zhu F. A novel mutation in A4GALT was identified in a Chinese individual with p phenotype. *Transfusion*. 2017 Jan;57(1):215–6.
- Hellberg A, Schmidt-Melbye AC, Reid ME, Olsson ML. Expression of a novel missense mutation found in the A4GALT gene of Amish individuals with the p phenotype. *Transfusion*. 2008 Mar;48(3):479–87.
- Kaczmarek R, Mikolajewicz K, Szymczak K, Duk M, Majorczyk E, Krop-Watorek A, et al. Evaluation of an amino acid residue critical for the specificity and activity of human Gb3/CD77 synthase. *Glycoconj J*. 2016 Dec;33(6):963–73.
- Westman JS, Hellberg A, Peyrard T, Thuresson B, Olsson ML. Large deletions involving the regulatory upstream regions of A4GALT give rise to principally novel P1PK-null alleles. *Transfusion*. 2014 Jul;54(7):1831–5.
- Xu X, Hong X, Ma K, Lan X, Chen S, Liu Y, et al. [A rare p phenotype caused by a 26-bp deletion in a 1,4-galactosyltransferase gene]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*. 2013 Jun;30(3):309–12. Chinese.
- Selstam A, Wagner FF, Salama A, Flegel WA. Antibodies to high-frequency antigens may decrease the quality of transfusion support: an observational study. *Transfusion*. 2003 Nov;43(11):1563–6.