YTGT: A New High-Prevalence Antigen in the Yt Blood Group System in Two Unrelated Native American Patients

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Background: the patients

- We describe a serological and molecular investigation that identified a new high-prevalence Yt antigen.
- A Native American (NA) female (patient 1) with severe post-operative anemia required urgent transfusion with incompatible blood due to an unidentified antibody.
- The plasma reactivity was reminiscent of that seen in a NA male (patient 2) with an unidentified antibody and a history of GI bleeding and kidney failure who was investigated in 2010 and 2019.
For decades the YT system consisted of 2 antithetical antigens:
- $Yt^a$ (YT1), of high-prevalence, found in 1956
- $Yt^b$ (YT2), of lower prevalence (8 – 26%), found in 1964

- Carried on the GPI-linked glycoprotein Acetylcholinesterase ($AChE$) encoded by $ACHE$
- Use of soluble recombinant $Yt$ (srYT) protein and $AChE$ sequencing led to the recent discovery of high-prevalence antigens $YTEG$ (YT3), $YTLI$ (YT4) and $YTOT$ (YT5)
- YT antigens are each the result of a single nucleotide change in $ACHE$
- YT antibodies typically are not clinically significant but some DHTR due to anti-$Yt^a$ have been reported

\begin{align*}
YTOT & \text{Arg34Gln} \\
YTLI & \text{Gly57Arg} \\
YTEG & \text{Gly89Glu}
\end{align*}
Materials and Methods

Serology testing:
- Standard tube and column agglutination techniques (CAT) were used
- RBCs were treated with papain, trypsin, α-chymotrypsin and dithiothreitol (DTT)
- So that ABO-incompatible rare RBC samples could be tested, acid eluates using patient plasma were prepared from phenotypically similar RBCs using Gamma ELU-KIT II (Immucor)
- Rare reagents and RBCs with rare phenotypes were from our in-house collection
- Inhibition studies used soluble recombinant Yt (srYt, imusyn, Hannover, Germany).

DNA testing:
- Genomic DNA was isolated from WBCs by standard methods (QIAamp, QIAGEN, Inc., Valencia, CA)
- The coding exons (2 to 5) and surrounding intronic regions of ACHE were Sanger sequenced and analyzed with ClustalX
- ID CORE XT (Grifols, Barcelona, Spain) was used to determine the patients’ extended antigen types and the profiles were unremarkable
Results: serology

Patient 1 & 2 RBCs:
- Group O, Rh-positive
- DAT negative
- Positive with multiple antibodies to high-prevalence antigens including anti-Yt\textsuperscript{a}

Patient 1 & 2 plasma
- Reacted by LISS IAT, PEG IAT and CAT; autologous RBCs were non-reactive
- Underlying alloantibodies were ruled out by adsorption using phenotypically similar RBC samples
- Reactivity was not inhibited by normal pooled donor plasma
- Antigen being detected was:
  - Resistant to treatment with papain, ficin, trypsin or DTT
  - Sensitive to $\alpha$-chymotrypsin
  - Pattern fits with Cromer, Yt and some Diego antigens
Results: serology (continued)

Patient 1 & 2 plasma/eluate testing
- Positive with the following rare phenotypes:
  - AnWj–, At(a–), Co(a–), Cromer null, Di(b–), DISK–, En(a–), Er(a–), GE:–2,–3, Gy(a–),
    In(Lu) (Lu(a–b–), JMH–, Jr(a–), Ko, Lan–, LW(a–), PEL–, Rhnull, SC:–1,–2,–3, Vel–, Yt(a–),
    serological Knops-null
- Plasma from patient 1 was compatible with stored RBCs from patient 2; no plasma
  available from patient 2 for cross-testing
- Non-reactive RBCs:
  - PNH-III RBCs
    - PNH-III RBCs lack GPI-linked proteins and do not express DO, Yt, CROM, JMH, or
      EMM antigens
    - Antibodies to the known antigens of these systems, including EMM, were ruled out
      either by testing the patients’ RBCs or the plasma
- Testing with soluble recombinant (sr) blood group proteins showed that the plasma
  reactivity was neutralized by srYt
ACHE sequencing

- Exon 2: homozygous for **novel change c.290A>G encoding p.Gln97Arg**
  - c.1057C/C (p.353His) associated with YT*A/A (i.e., YT*01/01)
- Exon 3, 4, and 5: no changes

Genotype: **novel YT*01(290G)/01(290G)**

- Exon 2: c.290A>G (p.Gln97Arg)
Patient 1: clinical events

- 47-year-old NA female, G3P3 with no history of transfusions or antibodies admitted for resection of her rectal mass
- Her hemoglobin (Hb) fell from 8.8 g/dL to 5.9 g/dL and was 3.7 g/dL on post-op day 1, requiring transfusion
- Type and screen ordered at this time was positive; reactions with all panel cells observed but with a negative autologous control
- After receiving ~150 mL of “least incompatible” K– blood, the patient developed chills, tachycardia, confusion, hypotension and mild temperature elevation (36.9 to 37.3°C)
- Post-transfusion: Hb 4.1 g/dL, with slight elevation of LDH (286 IU/L), low normal haptoglobin (51 mg/mL), and slightly increased bilirubin (Total 3.8; Direct 2.8)
- DAT was 1+ with anti-IgG
- Serum was red-tinged, but free Hb was not tested; orange urine interfered with UA testing
Patient 1: clinical events (continued)

- Patient transferred to regional medical center for specialized ICU care, requiring 3L of oxygen and treatment in hyperbaric chamber for hypoxemia, lethargy and confusion, as well as vasopressors support for hypotension
- ICU lab results: Hb 4.0 g/dL, LDH increase to 340 IU/L, increased bilirubin (Total 6.2; Direct 4.5), decreased haptoglobin (15 mg/dL)
- Transfusion reaction investigation and antibody identification was initiated
- Crossmatch compatible blood was not available; she was started on erythropoietin, Venofer, and vitamin B12
- She received one RBC unit on hospital day two and another on day three resulting in a final Hb increase from 3.6 to 6.1 g/dL
- To mitigate the risk of a hemolytic transfusion reaction methylprednisolone 500 mg was given immediately prior to transfusion and IVIG 0.4 g/kg following the transfusion
- The patient clinically stabilized and no longer required vasopressor support or supplemental oxygen
Summary: anti-YTGT

- Anti-YTGT, detecting a new high prevalence Yt antigen preliminarily designated YTGT (YT6), was found in two unrelated Native American patients
  - A female, was G3P3 with no prior transfusion
  - A male, previously transfused, but no record regarding negative sequelae after transfusion were found
  - Antibody from the female patient was compatible with RBCs from the male patient
  - The female is a member of the Bois Forte tribe, band of the Ojibwe/Chippewa; the male was from South Dakota but no tribal information is available

- Identification of the specificity was aided by:
  - Enzyme sensitivity
  - Use of PNH-III RBCs
  - Neutralization using soluble recombinant Yt protein
  - DNA analysis

- Anti-YTGT demonstrated clinical relevance based on the acute hemolytic transfusion reaction experienced by the female patient
Summary: YTGT antigen

- The YTGT antigen is encoded by c.290A (p.Gln97) but lost by the change from Glutamine to Arginine.
- This increases the number of YT antigens to 6, with 5 being of high prevalence.
- The c.290A>G change has not been previously reported but is present in the gnomAD database (rs768165907) that shows it as being very rare with a current frequency of 0.00003007 (Latinos).
- Genotype: **YT*01(290G)/01(290G)**
YT blood group system currently

Amino acids 1-31 are cleaved

YTOT Arg34Gln
YTLI Gly57Arg
YTEG Gly89Glu

Gln97Arg YTGT

Yt^a (YT1)

Yt^b (YT2)

YTEG (YT3)

YTLI (YT4)

YTOT (YT5)

YTGT (YT6)*

Given the possible proximity of YTGT to YTEG, YTLI and YTOT on the YT glycoprotein, cross-testing with anti-YTEG, YTLI and YTOT is of interest but was not possible
Conclusion

- We have shown that lack of YTGT antigen can result in immunization to YTGT and destruction of transfused YTGT+ RBCs.

- This case demonstrates the successful use of IVIG and steroids for ameliorating the immunological response to serologically incompatible RBCs.
Thank you for your attention