Serology and *In Silico* Predictions of Novel *ABO*, *KEL* and *GYPB* Alleles with Non-coding Variants

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INTRODUCTION

Variants in non-coding regions of blood group genes can cause altered, weakened or null phenotypes.

This study presents five novel alleles with non-coding variants in *ABO*, *KEL* and *GYPB* and reports the serologic findings and *in silico* predictions for each.

MATERIALS AND METHODS

- Serologic testing was performed using reagents and methods indicated in Tables 1a-5a.
  - IS = Immediate Spin; RT = 5-15min Room Temp Incubation; IAT = Indirect Antiglobulin Test; PEG = Polyethylene glycol; mi = microscopic; vw = very weak
- Genomic testing was performed on DNA isolated from WBCs (Qiagen).
- Genotyping included:
  - Sanger sequencing of *ABO*, *KEL* and *GYPB* exons, flanking introns and *ABO* promoter/enhancer.
  - Long-range PCR (LR-PCR) and long-read sequencing (LRS; Oxford Nanopore).
- SpliceAI, PromoterAI and AlphaGenome(AG) models to predict variant impact (Tables 1b-5b).

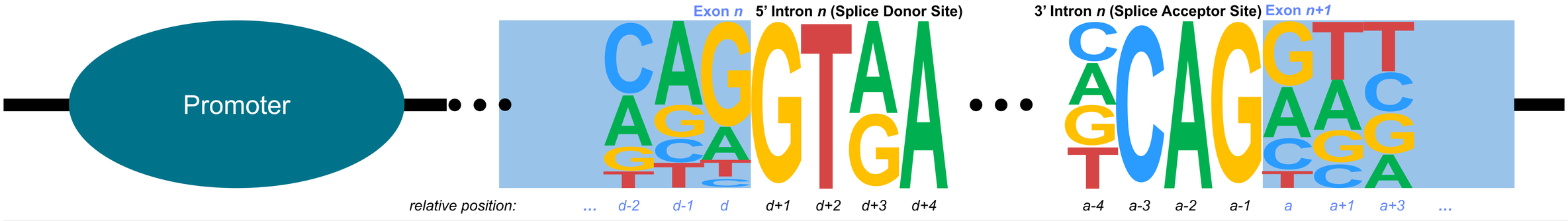


Fig1. Reference gene map indicating promoter region followed by two adjacent exons (exons *n* and *n+1*) and flanking intron regions. Pictographs are of the consensus sequence of canonical human splice donor and acceptor sites.[2]

RESULTS

- Refer to Tables for serology and *in silico* prediction results for samples 1-5 (S1-S5).

- **S1:** 34yo female.
  - Serology: weak A
  - Sequencing:
    - *ABO*A2.01/O.01.09* and c.98+3A>G;
    - LR-PCR: c.98+3A linked to *A2.01*.

ABO*A2.01(98+3G)

```
Position:  95    98   +1   +3
Reference: T T G G | G T A A
Variant:   T T G G | G T G A
```

Table 1a – Serology for S1

Immucor Anti-A (IS)	1+
Immucor Anti-A,B (IS)	2+
Immucor Anti-B (IS)	0
Immucor Anti-A1 lectin (IS)	mi

Table 1b – *In Silico* Predictions

Model	Score	Impact
SpliceAI	0.37	Slight
AlphaGenome	1.80	Strong

- **S2:** male neonate
  - Serology:
    - Possible A_2 phenotype; also mixed field agglutination
  - Sequencing:
    - *ABO*A1.01/O.01.01* and promoter variant c.-67C>T;
    - LR-PCR: c.-67T linked to *A1.01*.

ABO*A1.01(-67T)

```
Position:  -80      -75      -70    -67  -65      -60
Reference: G G C C G G G A G G G G C G C C T C G G
Variant:   G G C C G G G A G G G G T G C C T C G G
```

Nucleotides in red indicate positions of known variants in A_3 and B_3 alleles [1]

Table 2a – Serology for S2*

Immucor Anti-A (IS)	4+ mf
Immucor Anti-B (IS)	0
Immucor Anti-A1 lectin (IS)	mi

*testing performed by referring facility

Table 2b – *In Silico* Predictions

Model	Score	Impact
PromoterAI	-0.48	Moderate
AlphaGenome	-1.74	Strong

- **S3:** 36yo Black male
  - Discrepancy: Js(a+b+) by ID Core XT but Js(b-) serology
    - Anti-Jsb detected in patient's plasma
  - Sanger and LRS: detected *KEL*02(1899G)/02.06(1680C)* with critical 8-bp deletion on *KEL*02.06(1680C)*

KEL*02(1680C,1941_1941+7del)

```
Position:          1941  +1      +5  +7     +10
Reference: G C T G C A G | G T A T G C A A G T G T
Variant:   G C T G C A A ? G T G T C A A G G G C C
```

Table 3a – Serology for S3*

Unlicensed Anti-Js[a] (PEG)	2+
Unlicensed Anti-Js[b] (PEG/gel)	0/vw
Grifols Anti-K (RT)	0
Immucor Anti-Kp[a] (IAT)	0
Immucor Anti-Kp[b] (IAT)	2+
Immucor Anti-k (IAT)	4+

Table 3b – *In Silico* Predictions

Model	Score	Impact
SpliceAI	1.0	Strong
AlphaGenome	2.79	Strong

*testing performed by referring facility

- **S4:** 21yo Black male
  - PreciseType HEA predicted s+
  - Serology: variable reactivity (0 to 2+) with anti-s reagents
  - LRS: *GYPB*S/s* and placed c.38-3C>T on *GYPB*s*.

GYPB*s(38-3T)

```
Position:   -3  -1   38
Reference: A C A G | A A A T
Variant:   A T A G | A A A T
```

Table 4a – Serology for S4

Immucor Anti-s (RT)	0*/2+
Alba Anti-s (IAT)	0
BioRad Anti-s (IAT)	0
Immucor Anti-S (RT)	4+

*testing performed by referring facility

Table 4b – *In Silico* Predictions

Model	Score	Impact
SpliceAI	0.31	Slight
AlphaGenome	1.01	Strong

- **S5:** 64yo Black male
  - Versiti Red Cell Genotyping Panel: intermediate results for S/s polymorphism, prompting sequencing
  - LRS: detected *GYPB*S* and two variants on *GYPB*s*: c.149A>G p.(Gln50Arg) and c.137-3T>C.

GYPB*s(137-3C,149G)

```
Position:   -3  -1  137                 145      149
Reference: A T A G | G A G A A A C G G G A C A A
Variant:   A C A G | G A G A A A C G G G A C G A
```

Table 5a – Serology for S5

BioRad Anti-s (RT)	3+
Immucor Anti-S (RT)	4+
Alba Anti-S (IAT)	3+
BioRad Anti-S (RT)	2+

Table 5b – *In Silico* Predictions

Model	Score	Impact
SpliceAI	0.01	None
AlphaGenome	0.15	None

CONCLUSIONS

We report 5 novel alleles with non-coding variants:

1. **S1 – *ABO*A2.01(98+3G)*:** Intron 2 splice donor variant in a sample with weak A antigen expression.
2. **S2 – *ABO*A1.01(-67T):*** promoter region variant in neonate with mf; other reported variants near this region cause A_3/B_3.
3. **S3 – *KEL*02(1680C,1941_1941+7del)*:** 8-bp deletion affecting Intron 17 splice donor site; RBCs typed Js(b-) and anti-Js[b] detected in patient's plasma.
4. **S4 – *GYPB*s(38-3T):*** Intron 1 splice acceptor variant weakens s antigen expression.
5. **S5 – *GYPB*s(137-3C,149G)*:** Intron 3 splice acceptor variant **not** effecting s antigen expression.

In silico modeling predictions of all non-coding variants were concordant with serology. Of note, AlphaGenome predicted c.38-3T on *GYPB*s* (S4) to have a stronger impact than SpliceAI which was serologically observed – likely reflecting the multiomics integration of this model.

Non-coding variants have variable effects on antigen expression; *in silico* modeling can predict these effects and be a valuable tool to complement serology.

REFERENCES

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