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Unmasking A Rare RH Variant: Integrating Serology, Molecular Testing, and Family Studies In A Pregnant Congolese Patient

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Objectives

- Apply serologic and molecular methods to identify complex antibodies.
- Utilize family studies to understand inheritance patterns and confirm rare haplotypes.
- Evaluate clinical implications for transfusion and pregnancy.

Patient Presentation

- Aug 2021- a 31-year-old G5P4 patient presented to UH at 8 months of gestation with medical needs unrelated to pregnancy. Delivery of infant followed a short inpatient stay.
  - O+, negative antibody screen; transfused 2u pRBC crossed electronically, post-delivery.
  - Totally normal, routine-ish situation.

- Spring 2023 - now 33, G6P5 presents to UH as transfer of care from Samuel U Rodgers Clinic for 3rd trimester care/delivery planning in high-risk pregnancy setting.
  - O+, antibody screen is now pos.
  - UH results:

Interp.	Flags	Screen 1	Screen 2	Screen 3	Pos Ctrl
Positive		3+	4+	3+	4+

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Patient Presentation

- Causes of pan-reactivity include multiple RBC antibodies, warm/cold autoantibody(ies), antibody to high-incidence antigen (rare), pre-existing patient allergy/antibody to something in the assay (antibiotic, latex substrate, silica, porcine gelatin, etc.), or medication-induced (anti-CD38)

Interp.	Flags	E-I 1	E-I 2	E-I 3	E-I 4	E-I 5	E-I 6	E-I 7	E-I 8
Complete		2+	1+	3+	2+	1+	2+	1+	4+
									
Interp.	**Flags**	**E-I 9**	**E-I 10**	**E-I 11**	**E-I 12**	**E-I 13**	**E-I 14**	**Pos Ctrl**	**Neg Ctrl**
Complete		3+	4+	4+	4+	4+	4+	4+	0
									

Interp.	Flags	DAT Rxn	Ind Ctrl
Negative		0	4+
			

Comment

The patient's serum reacted with all reagent red cells in the antiglobulin phase of testing. No specific antibody pattern was evident. A direct antiglobulin test was performed and is negative.
We suspect either the presence of multiple red cell antibodies or a single red cell antibody directed against a high frequency antigen.
We suggest that additional specimens be referred to the facility at which the patient will receive medical care to ensure the availability of compatible blood. If transfusions become necessary, difficulty in crossmatching should be anticipated.

Initial sample collection was QNS to send out to IRL, so recollection was necessary.
C-section planned for date 4 weeks away.

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IRL Results

Serology

- Her plasma was strongly reactive with all cells tested, except the autocontrol
- Positive cold autoantibody screen at 4C
- Reactivity remained with Ficin and DTT-treated cells
- Tested cells negative for high prevalence antigens: Js(b-), U-negative, Fy(a-b-), Ge-negative Vel-negative → all were positive.
- Decided to wait for HEA results to see if that gave us some clues.

Patient Test Results

			Rh-hr								MNSs				P1	Lewis		Lutheran		Kell						Duffy		Kidd		Sex	Phase Data							
	Lot - Vial # OutDate	Donor ID	D	C	E	c	e	f	Cw	V	M	N	S	s	P1	LeA	LeB	LuA	LuB	K	k	KpA	KpB	JsA	JsB	FyA	FyB	JkA	JkB	XgA	5' RT	PEG IAT	3	Ficin RT	Papain IAT	6	30' RT	30' 4°
1	09461 - 03 - 5/5/2023	C4883	+	0	+	+	0		0	0	+	0	0	+	+	+	0	0	+	0	+	0	+	0	+	+	+	0	+	0	+	0	0		1+	2			
2	09461 - 04 - 5/5/2023	D2497	+	0	0	+	+		0	0	+	0	0	+	+	0	0	0	+	0	+	0	+	0	+	0	0	+	+	+	0	3+		1+	2			
3	09461 - 07 - 5/5/2023	G1737	0	0	0	+	+		0	0	+	0	+	0	0	0	+	0	+	+	+	0	+	0	+	0	+	0	+	+	0	2		1+	2			
4	09461 - 10 - 5/5/2023	B9195	+	+	0	0	+		0	0	+	+	0	+	+	0	+	0	+	+	+	0	+	0	+	+	0	+	0	+	0	3+		1+	2			
5	644023005 - I - 4/15/2023	M5160HM	+	+	0	0	+		0		+	0	+	0	+	0	+	0	+	+	+	0	+	0	+	+	+	+	+	+							1+	2+
6	644023005 - II - 4/15/2023	M6977AM	+	0	+	+	0		0		+	+	+	+	+	0	+	0	+	0	+	0	+	0	+	+	0	0	+	+							1+	3+
7	644023005 - III - 4/15/2023	M6227AM	0	0	0	+	+	+	0		0	+	0	+	W	+	0	0	+	0	+	0	+	0	+	0	+	+	0	0							2+	4+
8	04423 - 04 - 3/31/2023 W-	D1354	+	0	0	+	+		0	+	+	+	0	W	+	0	+	0	+	0	+	0	+	+	+	0	0	+	+	0	0	2						
9	02413 - 20 - 3/17/2023 W-	D1405	+	0	0	+	+		0	0	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	0	2						
10	Patient Cell - N/A																													0	(0)						

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Patient HEA Results

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	0	
	e	+	
	E	0	
	V	0	
	VS	0	
Kell	K	0	
	k	+	
	Kpa	0	
	Kpb	+	
	Jsa	0	
	Jsb	+	
Duffy	Fya	0	
	Fyb	(0)*	Not at risk for anti-Fy^{b}
Kidd	Jka	+	
	Jkb	0	
MNS	M	+	
	N	+	
	S	0	
	s	+	
	U	+	
Lutheran	Lua	0	
	Lub	+	
Diego	Dia	0	
	Dib	+	
Colton	Coa	+	
	Cob	0	
Dombrock	Doa	0	
	Dob	+	
	Hy	+	
	Joa	+	
Landsteiner--Wiener	LWa	+	
	LWb	0	
Scianna	Sc1	+	
	Sc2	0	

Results

Serology continued..

- Negative reactions were obtained only when Rh_{null} ,D- -, and Rh-46 cells were tested

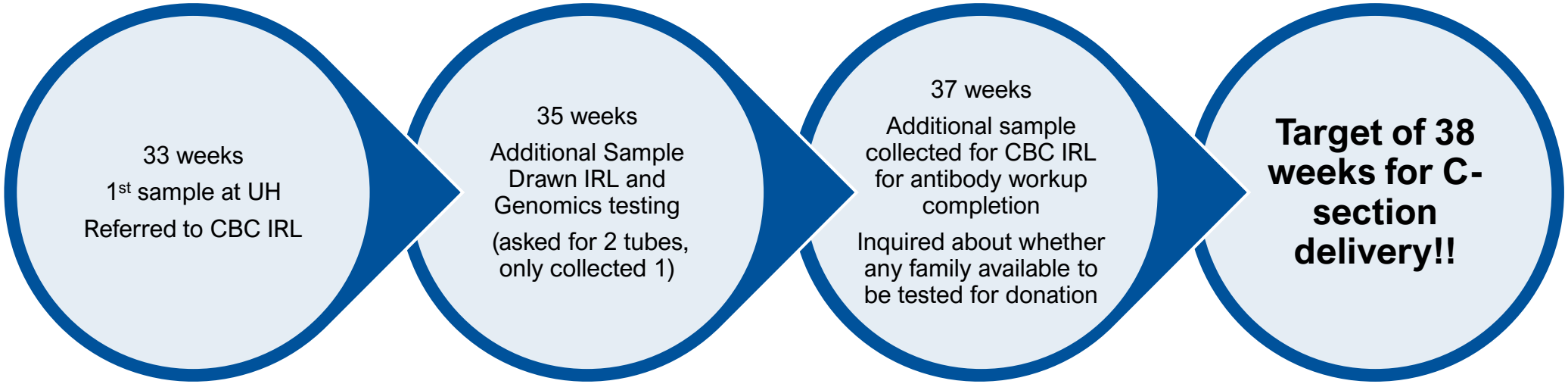
- Requested RH genomic testing.
  - Patient was not likely to be Rh-46
  - Rh-46 is located on a C background; our patient is C-negative

- The patient was predicted $hr^S–$ (from preliminary *RHCE* DNA results indicating *RHCE*ceEK / ceEK*)
  - Her RBCs serologically typed: D+, C–E–c+e+, $hr^S–$.
  - Plasma was still reactive with donor C–E–$hr^S–$ cells

	5' RT	PEG IAT
Rh NULL R5-3	0	(0)
A+(a-) zz-1-11	r	@
JR(a-) NNZ-8	1+w	@
Anwj- YY6-12	0	@
Rh NULL MM6-2	0	(0)
Rh-46 AA7-2	0	(0)

r = rough

Timeline



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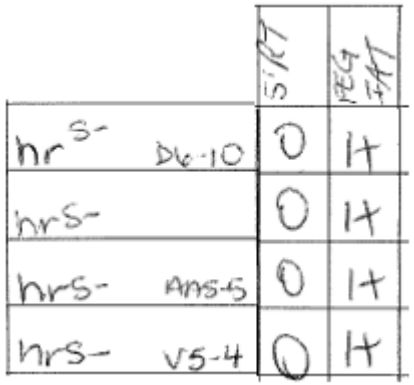
Coordination

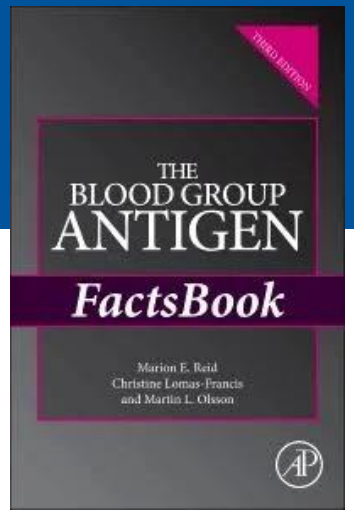
- CBC IRL
  - Prompted IRL to contact UH and see if any family members are available for testing and possible donation
  - Placed order for D-- or Rh null units with ARDP
    - Extremely rare even for ARDP
- UH
  - All communications delayed significantly due to the “phone tag” nature of interpreting services.
  - Reference Labs have no means to contact patients, but CBC provided a letter in easy-to-understand terms.
  - Policy limitations on who can use interpretation services and what can/should be communicated by whom.
  - Direct phone calls to family members who were fluent in both languages were allowed with explicit patient permission only.

What Now?

- Patient doesn't react with Rhnull and D-- red cells
- Patient is predicted to be D+, C–E–c+e+, hr^S–.
  - R_2R_2 (D+C-e-) cells predicted to be hr^S-
- Plasma was still reactive with donor C–E–hr^S– cells
  - Observed weaker results
- The Blood Group Antigen Factsbook mentions anti-Hr in the presence of anti-hr^S



Anti-Hr



- Most populations,100%
- Hr- only found in African Americans
- Transfusion reaction: No to fatal
- HDFN: Moderate
- Anti-Hr is made by hr^S- people
- Hr antigen is present on all RBCs except hr^S-, Rhnull, and RhCE-depleted phenotypes
  - Our patient still reacted with C-E- hr^S- red cells????
    - Historically, the literature is confusing because the RH genotypes of the antibody makers and the RH genotypes of hr^S and Hr cells were unknown so not consistent.
    - To sum it up…the hr^S- cells we tested may have Hr antigen present on them

Still Testing….But Then A Finale!

- Plasma alloadsorbed with R_2R_2 RBCs (D+C-e-E+e+ hr^S-):
- Alloadsorbed plasma
  - Contains: anti-C and anti-hr^S
- Prepared an eluate from the adsorbing cells
  - Contains: anti-E and anti-Hr

- Finally able to report to UH
  - ABORH: O Positive
  - DAT: Negative
  - Antibody identification:

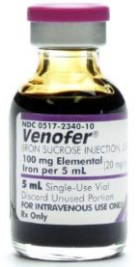
Antibody	Clinical Significance	HDFN Risk
Anti-Hr (Rh18)	No to fatal	Moderate
Anti-hrS (Rh19)	No to fatal if with anti-Rh18	Little evidence to indicate it causes HDFN
Anti-E	Significant	Yes
Anti-C	Significant	Yes
Cold autoantibody	Not usually significant	No evidence

What To Transfuse?

- ARDP
  - No luck with D-- or Rh null units
  - Could not provide genotype match
    - Patient had a new allele/haplotype
- Rare Unit
  - Able to locate a frozen D-- unit at our partner facility in MN
- Family
  - Father of patient was ABO incompatible (A+)
  - No other family samples obtained

Antepartum Prep and Postpartum Care

- Venofer infusions x3 were conducted to reduce potential chance of transfusion.
- Scheduled for autologous blood salvage with third party for C-Section.
- Family studies conducted to identify a suitable directed donor.
- Boosted internal communications with group chats (brought Pathology, Blood Bank, Medical Team in close communication)
- An EXTREME stroke of good luck finding of a D - - unit in the Supplier Enterprise
  - Recall of the 8.3B of us, fewer than 50 worldwide are Rh Null.
  - D- - is basically D+ but all Cc and Ee genes cannot produce an antigen, <0.23%



- Uncomplicated delivery of healthy baby boy 4/2023.
  - O Pos (RHD and RHCE as described before) negative DAT.
  - Day of life 2 total bilirubin was 6.4
  - Discharged home on day 3.

- Maternal course of care also uncomplicated.

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Here we go again…

- December 24, 2024 – guess who showed up at the outpatient lab for transfer of care for delivery at 36w gestation?
- This time, we had the benefit of both education and experience. Communication went much smoother.
- With pregnancy #7, autologous donation was planned, but unable to be conducted.
- Shorter timeframe of presentation to delivery; due date 01/21/2025.
- Able to connect with family through Genetic Counseling in person and complete additional family studies and conduct 2 Venofer infusions.

2024 Pregnancy

CBC IRL Workup Results

- ABORH: O Positive
- DAT: Negative
- Antibody identification:
  - Identified:
    - Anti-hr^{S}
    - Anti-E
  - No new alloantibodies
  - Did not detect:
    - anti-Hr
    - Cold autoantibody
    - anti-C

Family Samples

- Testing of cord blood after the birth of 6th child (tested in 2023)
  - ABO compatible (O+)
  - 2+ incompatible with patient plasma
- Oldest daughter of the patient
  - ABO incompatible (B+)
- Father of 6th and 7th children
  - ABO compatible (O+)
  - 4+ incompatible with patient plasma
- Brother of patient
  - ABO compatible (O+)
  - 3+ incompatible with patient plasma

Still waiting on these STAT results…

13498372 STAT	Service Order	In Process on 05/16/2023 07:58 CDT	25178h 42m

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Again..What To Transfuse?

- ARDP
  - No luck with D-- or Rh null units
  - Could not provide genotype match
    - Patient had a new allele/haplotype

- Rare Unit
  - Still had the frozen D-- unit we obtained in 2023

- Family
  - No family member was compatible

Baby #7 – Clinical Outcome

- Blood Type B Pos, DAT strongly positive.

- Bilirubin Trend:
  - 5.4 at birth
  - 9.9 at 26 hours
  - Peak at 17.3 on at 120 hours, resulting in Ivlg infusion.
  - Trended slowly down to 13.7 on discharge.

- Phototherapy started within 24 hours of birth and was maintained until discharge.

- About 10% weight loss over a 6-day NICU stay.

- Meeting developmental goals at discharge.

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Now Off To The DNA Lab!

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Methods

- DNA testing consisted of:
  - PreciseType HEA, RHD and RHCE BeadChips (Werfen)
  - Sanger sequencing:
    - *RHD* exon 8 for c.1136C>T (*DAU*)
    - *RHCE* exon 5 for confirmation of variant identified by NGS.

- Targeted Next-Generation Sequencing (NGS) of the *RH* locus was done on the proband, her father, and two children.

Family Samples

- Spring 2023 pregnancy
  - Father of proband available
  - Cord blood after delivery of 6th child
- Subsequent pregnancy end of 2024
  - Eldest child of proband (17 years of age)

	ABO	RH	hrS	XM
Proband	O	Pos	Neg	N/A
Father of Proband	A	Pos	Pos	Not Tested
Child #6	O	Pos	Pos	Incompatible
Eldest Child	B	Pos	Neg	Not Tested

Father of Proband

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	0	
	e	+	
	E	0	
	V	0	
	VS	0	

Child #6

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	0	
	e	+	
	E	0	
	V	+	
	VS	+	

Eldest Child

Blood Group	Antigen	Result	Comments
Rh	c	+	
	C	0	
	e	+	
	E	0	
	V	+	
	VS	+	

Results

DNA

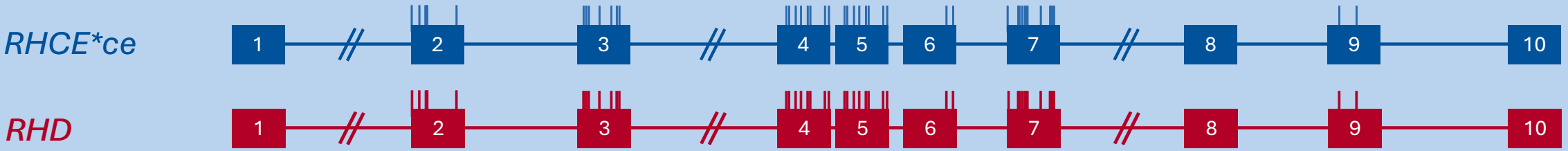
- RHCE BeadChip found *RHCE*ceEK/ceEK;* this genotype is predicted to encode a C–E–c+(partial), e+(partial), hr^S–Hr– phenotype.

- RHD BeadChip reported *RHD/DIIIa-CE(4-7)-D*
  - *DIIIa-CE(4-7)-D* predicts partial C **but** this result was not supported by C– type.

- *RHD* exon 8: c.1136C/T (*RHD*DAU)*

- Of note:
  - *RHCE*ceEK* is often linked with *RHD*DAR* or *RHD*; neither of which are present in this sample

- All information compiled---prompted *RH* Next Generation Sequencing (NGS)



NGS copy number analysis of *RHD* : *RHCE* alleles

1 : 2

Total # of *RH* alleles:
3

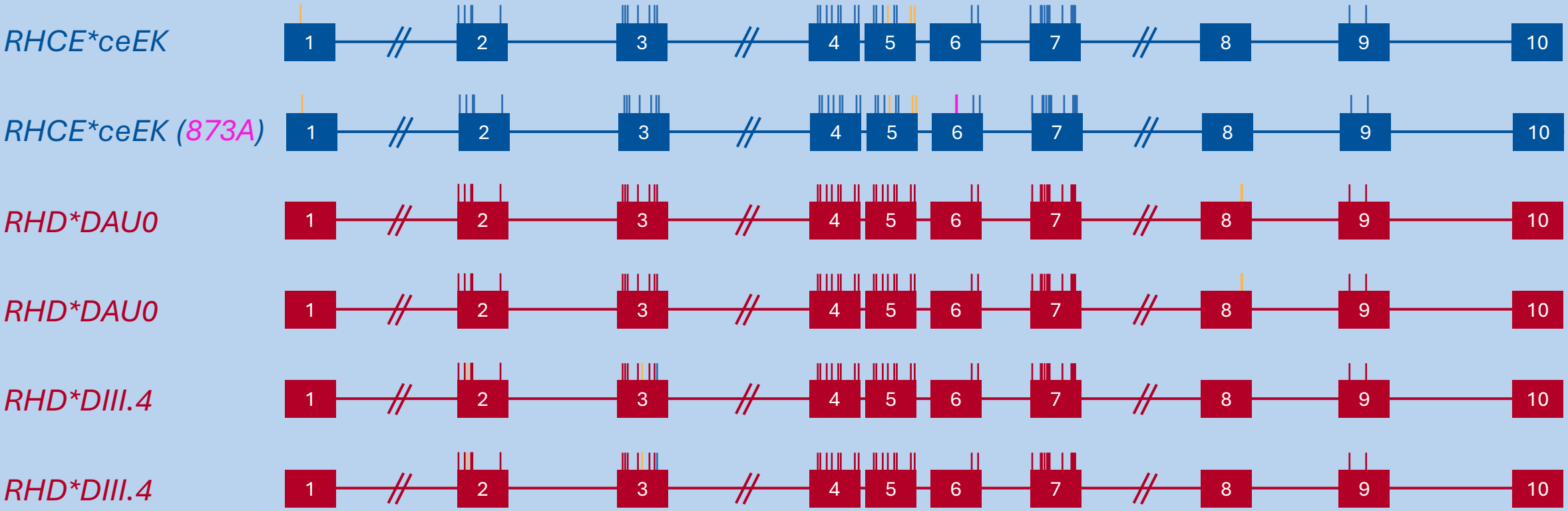
Results

RH NGS

- Targeted RH NGS revealed two unique haplotypes:
  - Hap1: *DIII.4 – DAU0 – ceEK*
  - Hap2: *DIII.4 – DAU0 – ceEK(873A).*

- c.873G>A is synonymous variant (p.Pro291=) that was confirmed by Sanger sequencing.
  - May not lead to a change in the protein
  - Helped determine the copy number

- Family helped support the proband's genotype



NGS copy number analysis of alleles with 873A : 873G

1 : 5

Total # of *RH* alleles: 6

NGS copy number analysis of RHD : RHCE alleles

4 : 2

Total # of *RH* alleles: 6

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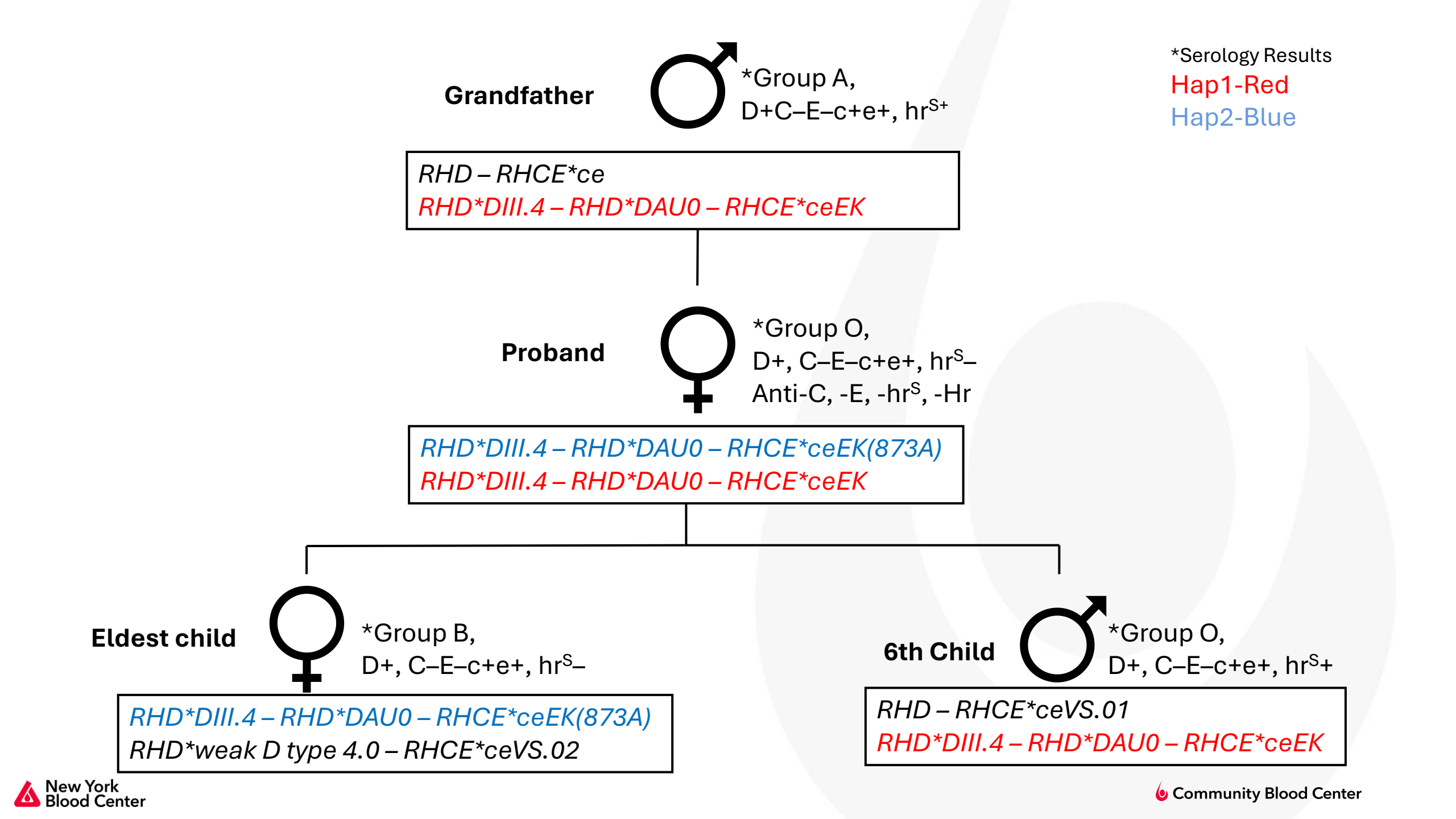
*Serology Results
Hap1-Red
Hap2-Blue
Grandfather
*Group A,
D+C–E–c+e+, hrS+
RHD – RHCE*ce
RHD*DIII.4 – RHD*DAU0 – RHCE*ceEK
Proband
*Group O,
D+, C–E–c+e+, hrS–
Anti-C, -E, -hrS, -Hr
RHD*DIII.4 – RHD*DAU0 – RHCE*ceEK(873A)
RHD*DIII.4 – RHD*DAU0 – RHCE*ceEK
Eldest child
*Group B,
D+, C–E–c+e+, hrS–
RHD*DIII.4 – RHD*DAU0 – RHCE*ceEK(873A)
RHD*weak D type 4.0 – RHCE*ceVS.02
6th Child
*Group O,
D+, C–E–c+e+, hrS+
RHD – RHCE*ceVS.01
RHD*DIII.4 – RHD*DAU0 – RHCE*ceEK
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Conclusion

- We identified two novel *RH* haplotypes, each inferred to consist of two *RHD* genes and one *RHCE* gene: *DIII.4 - DAU0 - ceEK* and *DIII.4 - DAU0 - ceEK(873A)*.

- The proband is a Congolese woman with anti-C, -E, -hr^S and –Hr in her plasma, likely stimulated by transfusion.

- Samples from her father and two of her children allowed verification of the novel *RH* haplotypes.
  - mRNA analysis may determine whether both *RHD* alleles (*DIII.4*, *DAU0)* are expressed.

- This case highlights the importance of quantitative assays and family studies to elucidate and confirm rare structural variants.

- Bridging the gap between a rare match and a safe delivery/post-partum care is built on multidisciplinary communication.
  - Consider how one may summarize the case just presented to a clinical team…
  - Our work is *also* about refusing to see a “matchless” situation as a lost cause.

Resources

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Thank you!

All the staff at CBC IRL, UH, and NYBC Genomics Laboratory

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