Decoding RHD Homology in African Descendants for Precision Sickle Cell Disease Management: Validating the Clinical Necessity of Molecular Genotyping in Light of NGS Limitations

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Introduction- Background

- Red cell alloimmunization is a significant complication for individuals with sickle cell disease (SCD), with prevalence rates ranging from 5% to 75%, compared to just 2% to 5% in the general population (Linder & Chou, 2021).
- Chronically transfused SCD patients who type as RhD-positive (D+) may actually possess "partial D" antigens with missing or altered epitopes. When these patients receive conventional D+ red blood cells, their immune systems recognize the donor's fully intact D antigens as foreign and produce anti-D antibodies (Takasaki et al., 2023).
- The increased risk is largely driven by the high Rh diversity shared between SCD patients and donors of African descent, who often possess similar frequencies of variant RH genes (Takasaki et al., 2023).
- In individuals of African descent, including patients with sickle cell disease (SCD) and blood donors, several variant RHD alleles are hybrid genes formed by the exchange of genetic material between the highly homologous RHD and RHCE genes (Gaspardi et al., 2016, Kappler-Gratias et al., 2014)
- Hybrid RHD variants made up of both RHD and RHCE components are a diagnostic limitation of next generation sequencing (NGS). Because NGS requires the genes to be amplified using targeted primers before they can be sequenced, these hybrid genes may simply fail to amplify if the primers do not perfectly match the unexpected hybrid sequence (Madgett et al., 2023).

Introduction- Purpose

This meta-analysis aims to (1) distinguish predominant RHD hybrid variant alleles encoding partial antigens in sickle cell disease (SCD) patients and blood donors of African descent in locations of wide genetic admixture (Brazil and the United States) and (2) provide evidence for the clinical relevance of molecular genotyping arrays although next generation sequencing assays are the latest technological advancement in RHD detection.

Introduction- Research Questions

RQ1: What are the predominant hybrid RHD variant alleles in sickle cell disease (SCD) patients and donors of African descent?

RQ2: How is the RHD BeadChip™ molecular genotyping array still clinically state-of-the-art in light of the limitations of next generation sequencing?

Methodology

- Inclusion Criteria:
  - Article is related to sickle cell disease management.
  - Article mentions RHD molecular genotyping.
  - Article mentions variant RHD hybrid alleles.
  - Article is a published clinical trial.

- Literature Appraisal: The results of a PubMed.gov literature search were collected per groupings defined by the inclusion criteria and selected for review. A total of 8 articles were identified as suitable for inclusion and were each analyzed according to the following criteria presented in the tables below:

Methodology Continued

Level	Weighting of the Clinical Literature
1	Literature for the device itself
2	Literature for key data / very similar devices
3	Literature for similar device

Level	Hierarchy of Clinical Evidence
I	Systematic analyses, Meta-analyses, Randomized controlled trials
II	Observational studies, Comparison study, Cohort studies, Case-controlled studies, Cross-sectional studies
III	Case series, Cross-sectional surveys
IV	Case reports, Clinical guidance

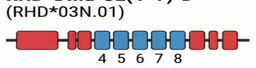
Results

Hybrid RH Alleles in African Descendants: Genetic Architecture & Clinical Risks

CONTEXT SUMMARY: In African descendants, the close proximity of the *RHD* and *RHCE* genes facilitates genetic shuffling, creating hybrid genes with replaced segments. These often result in "partial" antigens, misidentified by standard tests, increasing alloimmunization risk.

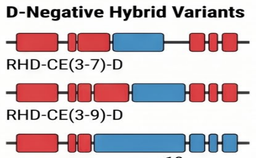
MAJOR *RHD* HYBRID VARIANTS

RHD*DIIIa-CE(4–7)-D (RHD*03N.01)



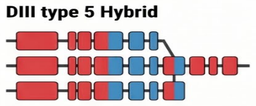
RHCE exons 4–7 replace RHD counterparts; encodes partial C but no D antigen.

D-Negative Hybrid Variants



RHCE(1-3)-D(4-10) result in D-negative phenotypes despite RHD material.

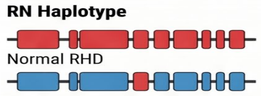
DIII type 5 Hybrid



A recombination between DIVa and weak D type 4 clusters resembling the DIIIa allele.

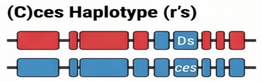
HYBRID *RHCE* ALLELES & HAPLOTYPES

RN Haplotype



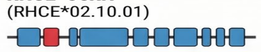
Pairs a normal RHD gene with a hybrid RHCE-D(4)-CE gene.

(C)ces Haplotype (r's)



Features a hybrid RHD-CE-Ds gene paired with an altered *"ces"* allele.

RHCE*CeRN (RHCE*02.10.01)



RHCE exon 2 is identical to RHD; results in clinically significant partial C expression.

D-Ce(3-8)-D Conversion



A hybrid gene resulting from genetic conversion, characterized by the 455A>C mutation.

CLINICAL SIGNIFICANCE & TRANSFUSION RISK

Serological Matching Failure
Hybrids often cause standard blood typing to fail, appearing as discrepant or weak results.

Increased Alloimmunization Risk
Partial antigens allow patients to form antibodies (e.g., anti-C or anti-D) against conventional donor blood.

Critical for Sickle Cell Disease (SCD)
Precise molecular matching is required for SCD patients to prevent life-life-threatening transfusion reactions.

RHD exon
RHCE exon

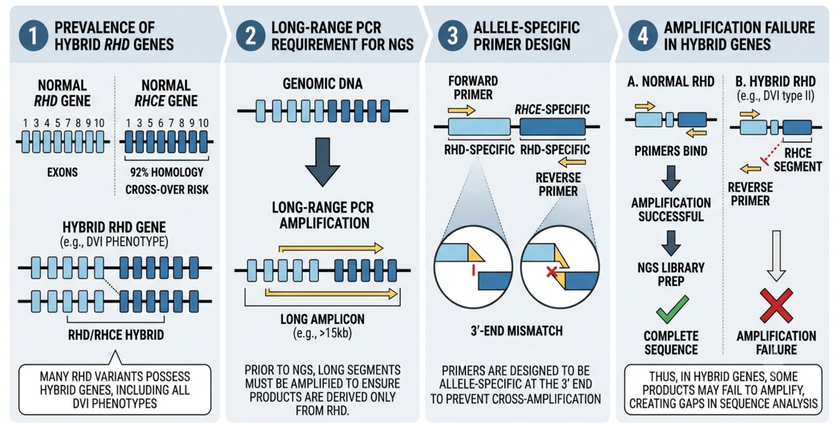
STRUCTURAL CHANGES & SEROLOGICAL OUTCOMES SUMMARY

Allele/Haplotype	Genetic Mechanism	Resulting Phenotype
RHD*DIIIa-CE(4-7)-D	Exon 4–7 Replacement	Partial C+ / D-negative
RHD*DIIIa-CE(4-7)-D	Exon 4–7 Replacement	Partial C+ / D-negative
RN Haplotype	RHD Exon 4 into RHCE	Partial C expression
RHCE*CeRN	Exon 2 Conversion	Partial C expression

NotebookLM

Results Continued

CHALLENGES IN SEQUENCING HYBRID *RHD/RHCE* GENES WITH CURRENT NGS TECHNOLOGY



Discussion- Clinical Significance

- This research establishes prophylactic RHD molecular genotyping as a critical strategy for blood inventory conservation and the optimized management of sensitized patients with sickle cell disease (SCD) for the prevention of delayed hemolytic transfusion reactions.
- Hybrid RHD variants are made up of both RHD and RHCE components. Because NGS requires the genes to be amplified using targeted primers before they can be sequenced, these hybrid genes may simply fail to amplify if the primers do not perfectly match the unexpected hybrid sequence. (Madgett et al., 2023).
- RHD DIIIa-CE(4–7)-D (RHD03N.01): This common hybrid involves RHCE exons 4 through 7 replacing the corresponding RHD exons (Takasaki et al., 2023; Linder & Chou, 2021;). It encodes a partial C antigen but no D antigen, leading these individuals to type serologically as C-positive while remaining at risk for developing allo-anti-C (Israelyan et al., 2024; Linder & Chou, 2021).
- RN Haplotype: This features a normal RHD gene paired with a hybrid RHCE-D(4)-CE gene (where RHD exon 4 replaces the RHCE sequence) (Kappler-Gratias et al., 2014). It is one of the most frequent variant haplotypes in individuals of African origin (Kappler-Gratias et al., 2014).
- (C)ces Haplotype (r's): A complex hybrid involving an RHD-CE-Ds hybrid gene paired with an altered ces allele of RHCE (Kappler-Gratias et al., 2014).
- A hybrid allele resembling DIIIa that evolved through recombination between the DIVa and weak D type 4 clusters (Wagner et al., 2002).
- A hybrid gene resulting from a genetic conversion characterized by the 455A>C mutation (Kappler-Gratias et al., 2014).
- D-Negative Hybrid Variants: Several hybrids result in a D-negative phenotype despite containing RHD genetic material, including: RHD-CE(3-7)-D, RHD-CE(3-9)-D, and RHCE(1-3)-D(4-10) (Kappler-Gratias et al., 2014).

References

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