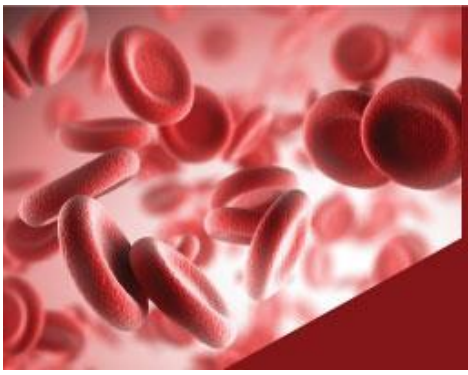

2020 South Central Association of Blood Banks ABSTRACT JOURNAL

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Investigation of incidence and transfusion implications of the (C)ce^{s} type 1 haplotype

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Abstract:
Genotype matching for Sickle Cell Disease (SCD) patients is commonly recommended to avoid transfusion complications and alloimmunization. The (C)ce^{s} haplotype is one of the most common variants seen in the African American (AA) population. This haplotype is not detected by serological phenotyping methods so alloimmunization is common. This adds importance and utility to genotype matching.

Commercially available molecular methods can be helpful in screening donors at a high volume. The C (+)* call on the PreciseType HEA assay indicates a possible (C)ce^{s} haplotype. This call may present as an advantage in helping with the selection of units that could be further characterized for possible genotype matching in the SCD population.

The intentions of this retroactive study were to compare published frequencies of the (C)ce^{s} haplotype with a Southeast AA donor population and to investigate the best way to utilize molecular testing for these donors. The advantages of different testing methodologies and strategies to increase utility of AA inventory are also discussed.

Introduction:
Transfusion concerns: Phenotype matching is recommended for SCD patients. Matching for C, E, and K has been reported to decrease alloimmunization by 18%-75%.[1] Due to the availability of AA units, these patients are commonly transfused with RhD negative units from white donors. This can lead to the depletion of the RhD negative blood supply and also pose greater risk of alloimmunization because of the genetic diversity between these populations. Reports state that in about 85% of SCD patients, at least one RH allele differs between the alleles commonly found in the white donor population.[2]
To add to this concern, one study of 550 patients found that when transfused with Rh matched AA donors, 175 Rh antibodies in 105 individuals were formed.[3] Another report found 82% of SCD patients with Rh antibodies presented serologically positive for the corresponding antigen.[3] A large collection of SCD patients demonstrated that 50 of 242 C+ patients have partial C antigen, making them at risk for forming anti-C if routine Rh phenotype matching is employed. The same study showed 21% of patients were homozygous for altered Rhce antigens. 53% of RHCE alleles were altered in a large group of both patients and AA donors.[3] Analysis of antibody frequency in a group of patients with a partial C phenotype showed 30% produced anti-C when with transfused C positive red blood cells.[5] Patients with homozygous RH variants can also be at risk for alloimmunization to high frequency antigens that these populations lack such as hr^{B} , hr^{S} , and Hr^{B}. [6]

These issues highlight the need for genotyping both AA patients and donors. It has been recommended to genotype all SCD patients that present C positive by serology and transfuse with C negative units if a partial C is predicted. Utilizing RH genotyping when Rh antibodies are present has also been suggested.[1]

(C)ce^{s} haplotype: The (C)ce^{s} haplotype (also called r'^{s}) is a result of the genetic exchange of RHCE exons 4 to 7 into the RHD locus. It consists of a hybrid D-CE-D^{s} allele, as well as the ce^{s} RHCE allele. The hybrid gene consists of Exons 1, 2, 3, 8, 9, and 10 from RHD and Exons 4 through 7 from RHCE. The following Single Nucleotide Variants (SNV) are present- 186G>T, 410C>T, 455A>C, 733C>G, 1006G>T. The RHCE ce^{s} allele consists of 48G>C, 733C>G, 1006G>T SNVs. This haplotype yields a weak, partial C (RH2), weak e (RH5), partial c (RH4), VS (RH20) and Rh42 (RH42), and is negative for D (RH1), hr^{b} (RH31), Hr^{b} (RH34), and V (RH10).[7,8,9]

While studying D negative, C positive, and VS positive donors, Blunt et al. suggested this phenotype may be due to an RHD/RHCE recombinant gene.[10] Faas et al. described the hybrid allele when investigating VS positive donors with weak C expression. Faas postulated that the VS expression and the weak e expression was due to the 733C>G mutation is Exon 5.[11] It was hypothesized that the weak C expression was due to the presence of 307T near RHCE sequences in combination with 48G on the hybrid allele and the 186T affecting expression as it is close to sites associated with C expression.[11,12] In 1998, the occurrence of 1006T SNV in both the hybrid allele and the ce^{s} allele was identified. It was also postulated that the molecular background of the V antigen may be linked to the expression of VS due to the presence or absence of the 1006T SNV in combination with the presence of 733G. [12]

A second (C)ce^{s} haplotype which was termed Type 2 was described. It was defined as a hybrid RHD*D-CE(4-7)-D allele (Exons 1, 2, 3, 8, 9, and 10 from RHD and Exons 4 through 7 from RHCE) along with the ce^{s} RHCE allele. It also encodes a weak partial C, partial e, partial c, and VS, but does not produce D, V, or Rh42. The conventional RHD background and differing 5' breakpoints in the hybrid allele are the two discriminating features in this haplotype.[7]

It was reported that the ce^{s} allele also frequently occurs with a non-hybrid DIII type 5 gene. This haplotype does not produce the weak partial C expression or Rh42 and has partial D expression.[7] Later, Westhoff and colleagues explained the 819G>A in the DIII type 5 is silent and does not change amino acid expression thus it is phenotypically identical to the DIIIa allele. They suggested making the DIII type 5 allele designation obsolete to avoid redundant nomenclature. It was also proposed to designate the common hybrid gene RHD*DIIIa-CE(4-7)-D to avoid confusion with the other hybrid. This analysis also verified that the is often associated with the ce^{s} allele.[13]

Published frequencies show the (C)ce^{s} type 1 haplotype occurs in 7–15% of black individuals[14] and 7.5% in SCD patients in Afro-Caribbean populations.[15] The ce^{s} allele has been reported at 9% in South African populations, 12% in a black Dutch population.[12] More recently, it has been found in 4.2% in SCD patients and 3.3% in AA donors , while the RHD*DIIIa-CE(4-7)-D allele was present in 3.1% in SCD patients and 2.6% in AA donors.[3]

Materials and Methods:
Sample selection: Donors who self-identified as black/African American in Florida, Alabama, or Georgia were selected for testing. Informed consent was acquired from all donors. Data was acquired and analyzed in two sets.

The first data set was to establish the incidence of the (C)ce^{s} type 1 haplotype in this AA donor population and the percentage of C positive AA donors with this haplotype present. 1055 African American donors were tested by PreciseType HEA BeadChip assay (Bioarray Solutions-Immucor, Inc., Warren, NJ). Presence of C antigen was serologically tested. Donors identified by HEA to be possible (C)ce^{s} haplotype were reflexed to IDCORE XT (Progenika Biopharma, Bizkaia, Spain/ Grifols, Emeryville, CA).

The second data set was to further characterize the frequencies of other genotypes identified from the (C)ce^{s} call by HEA and determine the number of actual C negative donors in this group. Over a two year period, donors yielding a C (+)* call were tested by serology and reflexed to IDCORE XT. Numbers from data set one were included in data set two.

Molecular Methods: DNA extraction- Genomic DNA was isolated from peripheral blood collected in EDTA by manual or automated methods (QIAsymphony DNA Mini Kit; QIAGEN, Valencia, CA).

Molecular assays: PreciseType HEA Molecular BeadChip Test and the IDCORE XT assay were performed according to manufacturer's recommendations.

Serological methods: Serological testing was performed by one or more of the following methods: automated methods with monoclonal anti-C (Clone MS24) gel cards (Micro Typing Systems, Pompano Beach, Florida) on Ortho Vision (Ortho Clinical Diagnostics, Raritan, NJ), monoclonal anti-C reagent (Clone MS24/P3X25513G8) on the PK7300 system (Beckman Coulter, Brea, CA) or manually by tube method with monoclonal anti-C reagent (Gamma-clone MS24, Immucor, Inc., Norcross, GA).

Testing for (C)ce^{s} haplotype: PreciseType HEA BeadChip assay may cause C typing discrepancies due to the C (+)* on units that had previously been typed by serology as C negative. It can also lead to the demand for C negative units to be placed on D negative supply unnecessarily. This test displays a (+)* for the predicted C phenotype result when 1006G>T and 733C>G variants are detected, as these are two of the SNV present in the ce^{s} allele. This allele commonly is linked to the hybrid RHD*DIIIa-CE(4-7)-D allele that produces a partial C antigen. The assay lacks a probe to verify presence of this haplotype.[16]

Multiple molecular methods for detection of the haplotype have been described. The first uses the 5' breakpoint in RHD intron 3 (IVS3+3100a>g).[17] In 2004, Flegel described an assay that could be used to interrogate the 3' breakpoint found within RHD intron 7.[18]

IDCORE XT uses the 5' breakpoint to predict this haplotype.[19] Because the breakpoint is not shared, IDCORE XT will not detect type 2 haplotypes. It may be worth noting that because IDCORE XT does not interrogate the RHCE c.48C nucleotide, it is unable to distinguish the RHCE*ceVS.03 (ce^{s}) allele from the RHCE*ceVS.05 allele, which possesses the 733C>G and 1006G>T SNVs but not 48G>C. The two are phenotypically similar, the difference being the partial c in the ce^{s} allele.[6] Studies have shown the former to be much more common in AA donors.[3]

Results:
Data set one: Of the 1055 AA donors tested, 48 (4.5%) resulted as C (+)* by HEA, while 1007 (95.5%) did not have the C (+)* call. When tested with the IDCORE XT assay, 27 samples (2.6% of the total 1055 donors) were positive for the (C)ce^{s} type 1 haplotype. The (C)ce^{s} type 1 haplotype was not detected in 21 samples. The genotypes detected and C typing results are presented in Table I. One sample did not have red cells available for serological typing. Only one donor with the (C)ce^{s} haplotype present (RHCE*ce, RHD*r's-RHCE*ce[733G,1006T]) was found to be negative by serology. Sequencing will be performed to investigate the cause of the phenotypic variance. Of the 332 donors that tested C positive by serology, the (C)ce^{s} type 1 haplotype was present in 25 (7.5%).

Table I. Data Set One- Genotypes and Serologic C phenotype

Genotype	Genotype Incidence	C Negative	C Positive	Serology Not tested
RHCE*ce, RHCE*ce[733G,1006T]	16	16		
RHCE*ce, RHD*r's-RHCE*ce[733G,1006T]	16	1	14	1
RHCE*cE, RHCE*ce[733G,1006T]	1	1		
RHCE*ce[712G], RHCE*ce[733G,1006T]	1	1		
RHCE*ce[733G,1006T], RHD*r's-RHCE*ce[733G,1006T]	1		1	
RHCE*ce[733G], RHCE*ce[733G,1006T]	3	3		
RHCE*ce[733G], RHD*r's-RHCE*ce[733G,1006T]	10		10	

Data set two: Over a two year period, 1342 donors' predicted C phenotype by HEA resulted as (+)*. 821 were reflexed to IDCORE XT. Of the 821 tested by IDCORE XT, 10 were unavailable for serological typing. 572 (69.7%) donors had the (C)ce^{s} haplotype present. 229 of those had a variant present in trans. No donors were homozygous for the (C)ce^{s} haplotype. One sample repeatedly resulted as unknown for all RH antigens. Donor will be retested at next donation and sent for sequencing. 3 of the 56 RHCE*ce[733G], RHCE*ce[733G,1006T] donors tested positive for C serologically. One of the two RHCE*ce [733G, 1006T] donors also tested positive for C serologically. Further serological and molecular testing may be performed in the future to investigate these discrepancies.

Table II. Data Set Two- Genotypes and Serologic C phenotype

Genotype	Genotype Incidence	C Negative	C Positive	Serology Not tested
RHCE*ce, RHCE*ce[733G,1006T]	160	156	3	1
RHCE*cE, RHCE*ce[733G,1006T]	28	27		1
RHCE*ce, RHD*r's-RHCE*ce[733G,1006T]	343	1	337	5
RHCE*cE, RHD*r's-RHCE*ce[733G,1006T]	85		82	3
RHCE*ce[712G], RHCE*ce[733G,1006T]	2	2		
RHCE*ce[712G], RHD*r's-RHCE*ce[733G,1006T]	3		3	
RHCE*ce[733G,1006T], RHD*r's-RHCE*ce[733G,1006T]	18		18	
RHCE*ce[733G], RHCE*ce[733G,1006T]	56	53	3	
RHCE*ce[733G], RHD*r's-RHCE*ce[733G,1006T]	119		119	
RHCE*ce [733G, 1006T]	2	1	1	
RHCE*ceAR, RHD*r's-RHCE*ce[733G,1006T]	4		4	
Unknown	1	1		

Discussion:
Using this algorithm does not allow the possibility to discover those donors that may have the (C)ce^{s} haplotype on one allele when the C gene is present on the other allele as HEA will report the C phenotype as C positive.

It is important to note that it is not possible to differentiate a partial C antigen caused by the (C)ce^{s} haplotypes and a conventional C antigen by serology.[20] This accentuates the importance of genotyping all serologically C positive SCD patients.

Using HEA to discover potential donors to reflex to further RHCE testing to use as genotype matched donors should be considered. The presence of the VS antigen may also be a viable way to screen potential donors.

There are limited commercial options available for RHCE genotyping. The IDCORE XT assay can predict 69 different RHCE genotype combinations and provides that information without additional testing to the RBC genotyping assay. This test is also approved for In Vitro Diagnostics (IVD).[19] The Immucor RHCE Molecular BeadChip Test is another option which uses 25 markers to make allele calls, 17 of which are not included in the IDCORE XT assay. It is currently labeled for Research Use Only (RUO).[21] There are several options for commercial real time polymerase chain reaction RHCE kits available.

The promise of next generation sequencing will no doubt benefit transfusion medicine in many aspects including genotype matching. [22] However, blood centers as well as hospitals will need to address the complication of having an effective way to store and utilize that data.

Summary:
Alloantibody development in patients with the (C)ce^{s} haplotype is a risk that may be avoided with genotyping. Genotyping appropriate donors and being prepared to offer genotyped units may help mitigate transfusion risks in these patients.

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