ORIGINAL REPORT

Concordance of two polymerase chain reaction–based blood group genotyping platforms for patients with sickle cell disease

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In recent years, polymerase chain reaction–based genotyping platforms, which provide a predicted phenotype, have increased in both patient and high-throughput donor testing, especially in situations where serologic methods or reagents are limited. This study looks at the concordance rate between two platforms commercially available in the United States when used for testing samples from patients with sickle cell disease (SCD), a group particularly vulnerable to alloimmunization. DNA extracted from samples from 138 patients with SCD was tested by human erythrocyte antigen (HEA) BeadChip (Immucor, Norcross, GA) and by ID CORE XT (Progenika-Grifols, Barcelona, Spain). Predicted phenotype results were compared, and a concordance rate was calculated. Discrepancies were resolved by Sanger sequencing. All testing was done under an institutional review board–approved protocol. A concordance rate of 99.9 percent was obtained. Sanger sequencing was performed on four samples with discrepancies in the Rh blood group system. Three samples had a similar allelic variant detected by ID CORE XT. Two of the three discrepant samples were correctly identified as V+^{w}, VS– by ID CORE XT but not by HEA BeadChip. The third sample, predicted to have a phenotype of V+, VS+ by sequencing, was called correctly by HEA BeadChip but not by ID CORE XT, which had predicted V+^{w}, VS–. The fourth discrepancy was identified in a sample that ID CORE XT accurately identified as *RHCE*ce[712G]* and predicted a partial c phenotype. This result was confirmed by Sanger sequencing, whereas HEA BeadChip found no variants and predicted a c+ phenotype. The high concordance rate of the two methods, along with the known limitations of serology, warrant further discussion regarding the practice of serologic confirmation of extended phenotypes. Clinical significance of the identified discrepancies remains to be determined. ***Immunohematology* 2020;36:123–128.**

Key Words: blood group genotyping, sickle cell disease, ID CORE XT, HEA BeadChip

Alloimmunization is a known complication in which patients develop antibodies to non-self, red blood cell (RBC) antigens after exposure through transfusion, transplant, or pregnancy. Patients with sickle cell disease (SCD) are especially vulnerable, with up to 47 percent of transfused patients reported to be alloimmunized.[1–8] A majority of these alloimmunization events occur within the Rh and Kell blood group systems. Providing antigen-matched blood for antigens in these two blood group systems has been shown to reduce alloimmunization rates in patients with SCD.[5,9] Extended matching to include antigens in the Kidd, Duffy, and MNS blood group systems has further reduced alloimmunization rates.[6,10] Alloimmunization mitigation is extremely important in decreasing delayed hemolytic transfusion reactions, which occur in 4–11 percent of patients with SCD and can be life threatening.[11–13] Additionally, recent studies have shown that alloimmunization may contribute to increased pain episodes and decreased patient survival by delaying transfusion while searching for compatible RBC units.[8,14]

Although providing antigen-matched RBCs based on serologic phenotyping before transfusion of patients with SCD has decreased alloimmunization rates, it has not eliminated alloimmunization, primarily due to Rh variation, as shown by Chou et al.[15] Serology is limited by its inability to detect variant antigens, the unavailability of antisera to rare or low-prevalence antigens, and its inability to provide valid results in recently transfused patients.

Polymerase chain reaction (PCR)-based genotyping platforms have been increasingly used to provide extended antigen-matched blood for patients requiring chronic transfusion[16–18] (e.g., patients with SCD or warm autoimmune hemolytic anemia), for recently transfused patients, or for patients with complex serologic workups. Genotyping can assist in identifying antigen-negative units when commercial antisera are unavailable or when the assistance of rare donor registries is required to obtain high-prevalence antigen-negative blood for transfusion. Several studies have compared molecular genotyping platforms with serologic phenotyping in patients and donors, with excellent concordance rates.[16,19–21]

Two PCR-based molecular genotyping platforms commercially available in the United States for blood group genotyping are the human erythrocyte antigen (HEA) PreciseType (formerly HEA BeadChip; Immucor, Norcross, GA) and ID CORE XT (Progenika-Grifols, Barcelona, Spain). (Note: This study was performed before release of the U.S.

Food and Drug Administration–approved HEA PreciseType Molecular BeadChip reagents and used the research-use-only HEA BeadChip reagents. Both kit versions interrogate the same genetic variants and predict the same antigens.) The HEA BeadChip and ID CORE XT assays are very similar, although the differences could result in discordant findings and/or provide information unique to one assay. For instance, HEA BeadChip includes assays for antigens in the LW and Scianna blood group systems, whereas ID CORE XT does not. Similarly, ID CORE XT includes an assay for antigens in the Cartwright blood group system, whereas HEA BeadChip does not. More importantly, the assays interrogate some different single nucleotide variants (SNVs), especially in the Rh blood group system. The predicted phenotype result takes into account the presence or absence of variant sequences at polymorphic positions as well as population frequency of alleles when reported in the literature. These assays may differ in the genotype call made by the software algorithm, which may result in a discordant predicted phenotype for the sample. This study compared samples tested by both assays to determine the resulting concordance rate in patients with SCD.

Materials and Methods

A total of 138 samples from patients with SCD were sent to LifeShare Blood Center (Shreveport, LA) for molecular genotyping between 2013 and 2016. DNA was extracted using automated QiaCube (Qiagen, Hilden, Germany) or manual validated procedure (Puregene; Qiagen). Qualitative and quantitative assessment of the DNA samples was performed by spectrophotometry (NanoDrop; Thermofisher, Waltham, MA) before their use in the molecular genotyping assay. Molecular genotyping was performed using HEA BeadChip at LifeShare Blood Center. The extracted DNA aliquots were stored at –20°C.

The de-identified frozen DNA samples were transported to the ITxM Immunohematology Reference Laboratory (Virginia Blood Services, Richmond, VA, now an affiliate of Blood Systems, Inc.) and tested using the ID CORE XT platform. ID CORE XT is an automated testing platform that analyzes 29 polymorphisms predicting 37 antigens in the Rh, Kell, Kidd, Duffy, MNS, Diego, Dombrock, Colton, Cartwright, and Lutheran blood group systems.

The HEA BeadChip data were compared with the ID CORE XT data after all testing was completed. Discrepancies were defined as differences in antigens common to both platforms. Samples with phenotype discrepancies were sent to Grifols IHC (San Marcos, TX) for resolution by Sanger sequencing. The compiled data were then used to calculate the concordance rate and to evaluate the discrepancies. The study was performed under an institutional review board–approved protocol.

The possible predicted phenotype results of each of the antigens determined by ID CORE XT were as follows: positive; negative; no call (NC): if the genotyping platform is unable to assign a phenotype; and unknown (UN): if the particular combination has not been previously described as associated with a phenotype. If a sample generated an NC or UN result, the DNA quality and quantity were verified by spectrophotometry before including the data in the tally.

Results

Predicted phenotype results were comparable across platforms for 31 antigens. Thus, for the 138 patient samples, there was a total of 4278 comparable calls. Three antigens were NC or UN by ID CORE XT and were therefore excluded from the concordance tally. HEA BeadChip does not interrogate an SNV associated with expression of the Mi^{a} antigen; therefore, Mi^{a} was not included in the comparison. Likewise, LW, Scianna, and Cartwright antigens were not included in the analysis for discrepancies or in our concordance calculation. We found four discrepant results. Thus, the concordance rate was 99.9 percent (100 × [4275 – 4]/4275).

All four discrepancies were in the Rh blood group system (Table 1). ID CORE XT identified the *RHCE*ceAR* allele in three samples. This allele encodes a partial c, partial e, V+^{w}, VS– phenotype. Two of the three samples were confirmed to have an *RHCE*ceAR, RHCE*ce* genotype by sequencing. The third sample was found to have an *RHCE*ceVS.01, RHCE*ceBI* genotype for a predicted V+, VS+ phenotype. HEA BeadChip predicted all three samples to be V+, VS+.

ID CORE XT accurately identified *RHCE*ce(712G)* in two samples. One sample was *RHCE*Ce, RHCE*ce(712G),* for which ID CORE XT predicted a partial c phenotype that was confirmed by Sanger sequencing. HEA BeadChip did not find a variant allele in this sample. The other sample was *RHCE*ce, RHCE*ce(712G).* The *RHCE*ce* allele encodes a normal c antigen. Therefore, the predicted phenotypes were in agreement for the two platforms (data not shown). HEA BeadChip predicted all three samples to be V+, VS+ because of interrogation of only c.733 and not c.712 by this platform.

Two samples were NC for Rh and Dombrock blood group systems on ID CORE XT because of insufficient DNA quality

Table 1. Comparison of samples with discordant results

Number of samples	ID CORE XT			HEA BeadChip		Sequencing (gDNA or cloned gDNA)			
	Genotype	Alleles	Predicted phenotype	Genotype	Predicted phenotype	Targeted regions	Genotype	Alleles	Predicted phenotype
2	c.122A c.307C NO c.335+3039ins109 c.676G c.712A/G c.733C/G c.1006G	*RHCE*ce* *RHCE*ceAR*	V+^{w}, VS–[†]	RHCE-P103S(Ax) 109Ins(AA) A226P(AA) L245V(AB) G336C(AA)	V+, VS+	Exon 5	c.712A/G c.733C/G c.787A/G c.800T/A	*RHCE*ce* *RHCE*ceAR*	V+^{w}, VS–
1	c.122A c.307C NO c.335+3039ins109 c.676G c.712A/G c.733C/G c.1006G	*RHCE*ce* *RHCE*ceAR*	V+^{w}, VS–[†]	RHCE-P103S(Ax) 109Ins(AA) A226P(AA) L245V(AB) G336C(AA)	V+, VS+	Exons 1–10	c.48G/C c.712A/G c.733C/G c.818C/T c.1132C/G	*RHCE*ceVS.01* *RHCE*ceBI*	V+, VS+
1	c.122A c.307C c.335+3039ins109 c.676G c.712A/G c.733C c.1006G	*RHCE*Ce* *RHCE*ce[712G]*[‡]	C+, partial c, e+	RHCE-P103S(Ax) 109Ins(AB) A226P(AA) L245V(AA) G336C(AA)	C+, c+, e+	Exon 5	c.712A/G c.787A/G c.800T/A	*RHCE*Ce* *RHCE*ceEK*	C+, partial c, e+

HEA = human erythrocyte antigen; gDNA = genomic DNA.

[†]The ID CORE XT report includes the following description: Also possible although less likely: *RHCE*ce[712G], RHCE*ce[733G].* Possible phenotype: c+ weak or partial, e+ weak or partial, VS+, V+.

‡The bracket in allele descriptions refer to the fact that the analysis of the gene has been limited (targeted) to certain key positions.

and/or quantity as determined by spectrophotometry. In both cases, HEA BeadChip predicted common phenotypes. The antigens in these two blood group systems were not investigated further by sequencing and, therefore, were not tallied in the concordance rate. Nonetheless, these samples are included in Tables 2 and 3 because valid phenotype predictions were obtained for other antigens. One other sample was UN for S on ID CORE XT. Sequencing detected the presence of Mi^{a} encoding variant c.140A on a *GYPB*S* background, whereas the ID CORE XT software only recognizes it on a *GYPB*s* background. Thus, this finding was not included as a discrepancy in the calculation of the concordance rate. The

Table 2. Percent of samples in this study with genotypes predicted to encode an hr^{B}–, Fy(b–), or Fy(b+) phenotype

			Percent (*n*); *N* = 138	
Predicted phenotype	Genotype ID Core XT	Genotype HEA BeadChip	Found in this study	Total
hr^{B}–, V+, VS+[†]	*RHCE*ce[733G]*	L245V (BB), G336C (AA)	4.3 (6)	10.9 (15)
	*RHCE*ce[733G], RHCE*ce[733G, 1006T]*	L245V (BB), G336C (AB)	0.7 (1)	
	*RHCE*cE, RHCE*ce[733G]*	L245V (AB), G336C (AA)	4.3 (6)	
hr^{B}–, V–, VS+	*RHCE*cE, RHD*r^{IS}–RHCE*ce[733G, 1006T]*	L245V (AB), G336C (AB)	1.4 (2)	
Fy(b–) (GATA)	*FY*A, FY*B_GATA*	FYA/FYB (AB), GATA (AB)	10.1 (14)	79.7 (110)
	*FY*B_GATA*	FYB/FYB (BB), GATA (BB)	69.6 (96)	
Fy(b+)	*FY*B, FY*B_GATA*	FYB/FYB (BB), GATA (AB)	15.2 (21)	18.8 (26)
	*FY*B*	FYB/FYB (BB), GATA (AA)	2.2 (3)	
	*FY*A, FY*B*	FYA/FYB (AB), GATA (AA)	1.4 (2)	
Fy(b–)	*FY*A*	FYA/FYA (AA), GATA (AA)	1.4 (2)	1.4 (2)

HEA = human erythrocyte antigen.

[†]*RHCE*ce[733G], RHCE*ce[733G, 1006T],* and *RHCE*cE, RHCE*ce[733G]* genotypes are recently reported to predict an hr^{B}+^{vw}/– phenotype. http://www.isbtweb.org/fileadmin/user_upload/ISBT004-RHCE-15th_July_2019.pdf.[22]

Table 3. Prevalence of antigens not typically analyzed by serologic methods in the cohort of patients with sickle cell disease (*N* = 138)

Blood group system	Antigen	Positive, *n*	Negative, *n*	Prevalence
Rh	hr^B	123	15	0.891
Kell	Js^a	14	124	0.101
	Js^b	137	1	0.993
	k	138	0	1
	Kp^a	0	138	0
	Kp^b	138	0	1
MNS	U	137	1	0.993
Lutheran	Lu^a	4	134	0.029
	Lu^b	138	0	1
Diego	Di^a	0	138	0
	Di^b	138	0	1
Colton	Co^a	138	0	1
	Co^b	5	133	0.036
Dombrock	Do^a	67	71	0.486
	Do^b	128	10	0.928
	Jo^a	137	1	0.993
	Hy	137	1	0.993
Landsteiner-Wiener	LW^a	138	0	1
	LW^b	0	138	0
Scianna	Sc1	138	0	1
	Sc2	0	138	0
Cartwright	Yt^a	138	0	1
	Yt^b	0	138	0

predicted phenotypes for all other antigens common to both platforms were non-discrepant.

Other phenotype information obtained by genotyping (without serology) is also reported. Table 2 shows the predicted phenotype for the Duffy blood group system based on the presence or absence of the GATA mutation in the promoter region of the gene. A GATA mutation affecting Fy^b expression was found in 110 of the 138 samples (79.7%).

We found a relatively large number of samples that lacked or had altered high-prevalence antigens not routinely detected by serology. In particular, 15 hr^B–, 1 U–, 1 Jo(a–), and 1 Hy– sample were identified (Table 3).

Discussion

Blood group genotyping is particularly useful in chronically transfused patients with SCD or other patient populations with a high risk of alloimmunization. In this study, we compared two blood group typing assays in chronically transfused patients with SCD. Results showed a high concordance rate between the two genotyping platforms.

The findings of the current study were consistent with those of Casas et al.,[16] who demonstrated high concordance (99.9%) between serologic phenotyping and PCR-based genotyping platforms when antigens that were common to both methods were compared. Additional information, however, was obtained via genotyping that was not identified using serologic methods. That study found that 66 of 494 subjects either lacked or possessed a variant high-prevalence antigen detected by genotyping. Additionally, 404 of 410 patients serologically determined to be Fy(b–) were found by genotyping to have the GATA mutation. Finally, 23 patients had a partial C (serologically typed as C+) and are at risk for producing alloanti-C. Meyer et al.[19] also found high concordance between serotyping and a high-throughput matrix-assisted laser desorption/ionization, time-of-flight mass spectrometry assay for Kell, Kidd, and Duffy antigens (the lowest concordance reported was 99.2%). They detected new alleles using their molecular platform.

Our study showed that identified discrepancies between the two assays primarily occurred in the highly variable Rh blood group system. In some cases, the two assays interrogated different positions in the gene, and the software made different assumptions based on the population frequency of the alleles. For example, ID CORE XT interrogated position c.712; *RHCE*ceAR* includes variants c.712G and c.733G. It is noteworthy that these two variants can also be found *in trans*. The algorithm in the ID CORE XT software was designed to report *RHCE*ceAR* whenever c.712G and c.733G occurred in heterozygosity, based on the manufacturer's experience that these variants were found together significantly more often than *in trans*. Thus, ID CORE XT predicted the phenotype based on this assumption and included a note indicating that an alternative genotype was possible but less likely. This assumption led to the reported *RHCE*ceAR* allele as the most likely result in three samples. The assumption proved true for two of the samples. The sample for which ID CORE XT was wrong had c.712G as part of *RHCE*ceBI* and c.733G as part of *RHCE*ceVS.01*. Therefore, ID CORE XT correctly predicted the phenotype in two of the three samples. HEA BeadChip correctly predicted the phenotype in one of the three samples because of its lack of interrogation of c.712. It should be noted that these assays are of medium resolution, and variants may be missed by assays of this type.

These differences account for the discrepancies seen in the predicted phenotype results of the two assays. The clinical significance of these discrepancies is unknown. However, in one of the samples reported here, ID CORE XT correctly

predicted a partial c expression rather than a conventional c+ expression. This finding theoretically could have clinical implications. A donor sample could be inappropriately serotyped as c– and expose a patient requiring c– blood to a potentially immunizing partial c or result in potential increased clearance of the donor RBCs in a patient with preformed anti-c. If the patient's sample was inappropriately serotyped as c+, the patient would not be recognized as requiring c– blood to prevent alloimmunization.

Importantly, as reported by Casas et al.,[16] genotyping platforms can provide additional information about the lack of or presence of altered high-prevalence antigens not available through serology alone, making these platforms of benefit to transfused patients. Table 3 is a modification of the table created by Casas et al.[16] using the prevalence rates of our cohort. Antigen prevalence found in our current study were consistent with those reported by Casas et al.[16] and Reid et al.[23]

Finally, the number of patients in our study with a predicted phenotype of Fy(b–) and Fy(b–) with the GATA mutation were similar to those reported by Casas et al.[16] Wilkinson et al.[24] reported similar rates of the GATA mutation in patients with SCD who they genotyped.

One important limitation of this study was the sample size, which affects the validity of the concordance rate. Additionally, these data must be interpreted with caution because the special cohort of patients with SCD may yield different concordance rates than those found when analyzing other populations, such as donors and patients with warm autoimmune hemolytic anemia who may have a less homologous ethnic background. Patients with SCD are largely of African ancestry, a population characterized by a higher frequency of variant *RH* alleles. Therefore, concordance rate in other populations remains to be elucidated. Nevertheless, a similar study using 1000 samples from random donors, patients, and neonates showed a high concordance rate similar to that seen in this population.[25] Additionally, the serology data would have made a good complement to the resolution of discrepancies by DNA sequencing.

Conclusions

Genotyping provides valuable information not available by serologic methods, such as silencing of Fy^b expression by the GATA mutation in the *FY*B* promoter and missing or variant high-prevalence antigens. Results from samples tested on both the HEA BeadChip and ID CORE XT genotyping platforms were found highly concordant. Discordance in the predicted phenotype most commonly occurs in the Rh blood group system because of differences in the interrogated SNVs and in the prediction algorithms. The clinical significance of the discordant results remains to be determined.

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Author Contributions

CAS and MK were co-principal investigators and designed the protocol. NLB and GO-G contributed to the interpretation of the results. MK provided DNA samples and genotyping results. GM wrote the manuscript with the support of CAS and MK. All authors revised the final manuscript and agreed to submission.

Disclosure of Conflict of Interest

GM and GO-G are employees of Grifols. The other authors declare no conflict of interest.

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