Original article

Single-exon fetal *RHD* genotyping: a 31-month follow up in the obstetric population of Western Sweden

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Background - The Rh blood group system is highly complex, polymorphic, and immunogenic. The presence of *RHD* gene variants in RhD negative pregnant women is a challenge in fetal *RHD* genotyping as it may influence the antenatal management of anti-D prophylaxis. The aim of this study was to determine the efficiency of a non-invasive single-exon approach in the obstetric population of Western Sweden in a 31-month follow up. The frequency and type of maternal *RHD* variants were explored and the relation to the ethnicity was elucidated. Discrepant results between fetal *RHD* genotyping and serological blood group typing of newborns were investigated and clarified.

Materials and methods - *RHD* exon 4 was analysed with quantitative real-time PCR technique in a total of 6,948 blood samples from RhD negative women in early pregnancy. All cases with suspected maternal *RHD* gene and discrepant results observed in newborn samples, were further investigated using both serological and molecular technologies.

Results - A total of 43 samples (0.6%) had inconclusive fetal genotyping result due the presence of a maternal *RHD* gene. These findings were in most cases (>66%) observed in pregnant women of non-European ancestry. Additionally, two novel *RHD* alleles were found. Seven discrepant results between fetal *RHD* genotype and serological RhD type of the newborns, were shown to be related to D antigen variants in newborns. Assay sensitivity was 99.95%, specificity 100%, and accuracy 99.97%.

Discussion - The single-exon approach for fetal *RHD* screening early in pregnancy is an appropriate choice in the population of Western Sweden, with a very low frequency of inconclusive results caused by the presence of maternal *RHD* gene variants. Due to the high sensitivity, specificity, and accuracy of the test, serological typing of neonates born to RhD negative women has no longer been performed at our laboratory since June 2023.

Keywords: *fetal RHD genotyping, RhD serology, RHD molecular typing, RHD variants.*

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INTRODUCTION

The Rh blood group system consists of two genes, *RHD* and *RHCE*, closely linked to each other. The *RHD* gene encodes the RhD protein, carrying the highly immunogenic D antigen, whose clinical significance is well known in transfusion practice and obstetrics.

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The *RHCE* gene encodes the RhCE protein carrying all other antigens included in the Rh system. Approximately 15% of Caucasians, 5% of Africans and <1% of Asians have RhD negative blood group. This phenotype is more commonly determined by a total deletion of the *RHD* gene especially in Caucasians[1]. However, the *RHD* gene is highly polymorphic and, in addition, rearrangements with the *RHCE* gene can occur. Many allelic variants have been detected influencing the expression of D antigen and generating weak, partial, extremely weak (DEL) phenotypes not detectable by serology or, in some cases, no D antigen expression at all. Some weak D, such as type 1, 2 and 3 have a higher frequency in Caucasians, where they represent approximately 90% of all cases giving weaker serological reaction (≤2+) by direct agglutination[2,3]. Other weak or partial D variants such as DAR[4,5], DAU[6] and DEL[7-9] are more often observed in people of African or Asian descent. A non-functional *RHD* gene (*RHDψ*, *RHD*08N.01*) is the most common variant (66%) causing RhD negative phenotype in people of African ancestry[10].

The production of anti-D in individuals lacking the whole D antigen or, in some cases some epitopes, can cause hemolytic transfusion reactions (HTR) in RhD immunized recipients of blood transfusion and possibly hemolytic disease of fetus and newborn (HDFN) in case of RhD negative pregnant women carrying a fetus with RhD positive blood group[11]. Considering the obstetric population, the prevalence of RhD immunization in pregnancy has drastically decreased from about 14% to 1.5% by the introduction of anti-D prophylaxis to RhD negative women after delivery of a RhD positive newborn at the beginning of 1970s. The combination of fetal *RHD* genotyping early in pregnancy and tailored antenatal anti-D prophylaxis in addition to the postnatal treatment, is now a common practice in many countries, contributing to further reduction of RhD immunization to approximately 0.2-0.4%[12-15].

The presence of maternal *RHD* gene variants is a challenge when performing fetal *RHD* genotyping, as it causes inconclusive results. The efficiency of fetal *RHD* testing to generate approved results is related to the population investigated. Therefore, the choice of the technique and the exon(s) to be investigated must take in account the prevalence of maternal *RHD* variants in relation to the local ethnical characteristics.

In Västra Götaland County, Western Sweden, approximately 23,000 pregnancies are yearly registered at the maternal care centres, resulting in about 18,000 deliveries. Blood grouping and antibody screening is performed at the end of the first trimester, according to national recommendations. Fetal *RHD* genotype is simultaneously analysed in women with RhD negative blood group, to administer tailored antenatal anti-D prophylaxis at the beginning of the third trimester, in case of pregnancy with *RHD* positive fetus. Fetal *RHD* genotyping is performed for the whole county at the Department of Transfusion Medicine, Sahlgrenska University Hospital, using a commercially available, CE-IVD marked *RHD* screening kit, using quantitative real-time polymerase chain reaction (qPCR) technique. The assay has previously shown high sensitivity, specificity, and reliability, when comparing the results with the outcome of serological blood group typing of newborns[14,15]. However, in some cases, genotyping results were shown to be inconclusive due to the possible presence of a maternal *RHD* gene.

Here we report a 31-month follow up on fetal *RHD* genotyping in clinical practice, using maternal plasma as a source of fetal DNA. Numerous reports have been published on non-invasive prenatal *RHD* screening in the second trimester. In contrast, almost 7,000 samples were collected in this county between gestation week 10-15. The aims of our study were to evaluate the efficiency of a single-exon assay in the obstetric population of Western Sweden, as part of a quality assurance program, as well as to investigate the frequency of maternal *RHD* variants and the correlation with ethnicity; furthermore, to elucidate the molecular basis of known and novel *RHD* gene variants in the serologically RhD negative obstetric population of Western Sweden.

MATERIALS AND METHODS

Blood samples

Between November 2020 and May 2023, fetal *RHD* screening was performed on a total of 6,948 ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood samples, collected from RhD negative pregnant women as part of clinical routine testing early in pregnancy (gestational week 10-15). A serological and molecular investigation of the maternal samples was performed in

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case of inconclusive fetal *RHD* type given by an apparent discrepancy between maternal phenotype and genotype. Additionally, discrepant results between fetal *RHD* genotype and RhD blood group of newborns performed by direct agglutination, were further investigated using serological and molecular techniques.

Following application, the Swedish Ethical Review Authority decided that no consent or further approval was required for this report (reference No. 2023-02020-01).

Serology

Maternal samples

Blood grouping and antibody screening were performed with gel card technology in an automated system (Bio-Rad IH-system, Europe). RhD phenotype was determined by direct agglutination, using a blend of monoclonal anti-D reagent of IgM class clones within the gel matrix in a single well, LHM59/20 (LDM3), 175-2 (DiaClon ABO/D + Reverse Grouping, Bio-Rad Laboratories, Cressier, Switzerland) in case of previously unknown blood group, or clones TH28/RUM-1/LDM1 (DiaClon ABD-Confirmation for patients, Bio-Rad Laboratories), if the blood group was previously known. None of these reagents detected DVI variant.

Further serological investigation for detection of weak/partial D antigen expression on red blood cells (RBCs) was performed in gel cards (Coombs anti-IgG, Bio-Rad Laboratories) by indirect antiglobulin test (IAT), using monoclonal IgG, clone ESD1 (Bio-Rad Laboratories), Novaclone IgM/IgG monoclonal blend, clones D175-2 and D415 1E4, (Dominion Biological Ltd, Dartmouth, Canada), BioClone Monoclonal IgM, IgG, clones D7B8 H1121G6+LORIFA (Ortho Clinical Diagnostics, Pencoed, UK), Biotest/Biorad Seraclone monoclonal IgM, IgG, BS 232, BS 221+ H41 11B7 (Dreieich, Germany) and ID-Partial RhD Typing Set, clones LHM76/55 IgG, LHM77/64 IgG, LHM70/45 IgG, LHM59/19 IgG, LHM169/80 IgG, LDM1 IgM (Bio-Rad Laboratories).

Newborn samples

The serological RhD blood type of newborns was performed by direct agglutination using reagents from two different gel cards, clones ESD-1M, 175-2, detecting DVI variant and LHM59/20 (LDM3), 175-2, not detecting DVI variant (DiaClon ABO/D, Bio-Rad Laboratories), and clones included in DiaClon ABD-Confirmation for patients, as with maternal samples. Discrepancies were further investigated by IAT as previously described for maternal samples.

Molecular analysis

Fetal RHD screening

Cell-free fetal DNA (cffDNA) was extracted from fresh maternal plasma, prepared from unopened blood samples on day 0-6 after collection, with a DSP Virus/pathogen Kit (Qiagen, Hilden, Germany) on a QIAsymphony instrument (Qiagen). Fetal *RHD* genotyping was assessed by a commercially available CE-IVD marked *RHD* screening kit (Devyser, Hägersten, Sweden), using qPCR analysis detecting exon 4. The samples were analysed in triplicate and *GAPDH* was used as internal endogenous control in each well, according to the manufacturer's instructions. Briefly, every run included one negative control (NTC) and one positive control analysed in duplicate (PC1 and PC2). At first, the *RHD* Ct of the *RHD* positive replicate with the higher cycle value was adjusted to 28.5. Thereafter, the average *GAPDH* Ct value was calculated *([GAPDH Ct in PC1+GAPDH Ct in PC2]/2=X)* and the reference range was set according to the lot-specific batch release certificate. Fetal *RHD* type was considered positive in case of 2/3 or 3/3 *RHD* signals appearing later than *GAPDH* signals. *RHD* type was considered negative when no *RHD* signal was detected, and *GAPDH* signals were approved in all triplicates. Inconclusive results could be due to strong *RHD* signals appearing before *GAPDH*, or 3/3 negative *RHD* signals with *GAPDH* Ct out of the approved interval, or 1/3 positive *RHD* signal later than *GAPDH*. In the latter two cases reanalysis was performed on the same sample, if possible, otherwise a new sample was requested.

Maternal and newborn RHD genotyping

The molecular investigation of discrepancies was performed at the Nordic Reference Laboratory for Genomic Blood Group Typing at the Department of Clinical Immunology and Transfusion Medicine in Lund, Sweden.

DNA was isolated from EDTA blood with either QIAsymphony DSP DNA Mini Kit (Qiagen) or a simple salting-out method for small volumes.

Routine in-house *RHD* genotyping was initially performed with allele-specific-PCR (ASP-PCR), and in unsolved cases, followed by sequencing of *RHD* exons 1-10 (ABI BigDye v3.1, Life technologies, Carlsbad, CA, USA). Sequence analysis was made with CodonCode Aligner (CodonCode,

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Centerville, MA, USA) using NG_007494.1 (*RHD*) as reference. Quantitative PCR analysis using *RHD* exon 7 was performed to confirm *RHD* zygosity in some samples[16].

Statistical analysis

A MedCalc Software Ltd. Diagnostic (Ostend, Belgium) test evaluation calculator (version 20.106) was used for statistical analysis of results, when comparing fetal *RHD* genotyping and serological RhD typing of newborns.

RESULTS

Over a period of 31 months fetal *RHD* screening has been performed on a total of 6,948 blood samples from RhD negative pregnant women early in pregnancy. In five cases, later shown to carry a maternal *RHD* gene variant, *RHD* screening was performed on two blood samples collected from the same woman in different pregnancies. Therefore, our calculations will be based on 6,943 samples. The samples, collected at the maternal primary care centres of the whole Västra Götaland County were analysed at the Department of Transfusion Medicine, Sahlgrenska University Hospital. All samples were analysed in triplicate in parallel with *GAPDH* as an endogenous control, and the results were reported as either positive, negative, or inconclusive.

A total of 4,081/6,943 samples (58.8%) showed *RHD* positive fetal genotype, 2,636/6,943 (38%) samples showed *RHD* negative genotype and inconclusive results were observed in 226/6,943 (3.2%). The outcome of *RHD* genotyping, and the type and frequency of different factors causing inconclusive results are shown in **Figure 1**. The assay showed 1/3 *RHD* positive signal in 86/6,943 (1.2%) cases, of which 50/86 samples were reanalysed. In 30/50 fetal *RHD* genotype result was positive (18/30 on a new sample collected later in pregnancy, and 12/30 after retesting of the same sample); in 20/50 fetal *RHD* genotype result was negative (14/20 after reanalysis of the same sample and 6/20 on a new sample). In 36/86 cases a new sample was requested but not collected possibly due to early termination of pregnancy.

A total of 43/6,943 (0.6%) samples had inconclusive result due the presence of a maternal *RHD* gene, as suggested by *RHD* Ct values lower than *GAPDH* Ct values. Strong positive reactions with all the reagents by IAT in gel cards were observed in 5 samples from pregnant women of European ethnicity. The blood group result was therefore changed to RhD positive.

All the other samples, 38/43 (88.4%), giving either negative, very weak positive, or variable serological results using different reagents, were investigated with ASP-PCR and, in 22/38 (57.9%) cases, also with sequencing of *RHD* exons 1-10. The most common variant detected (12/38, 31.6%) was *RHDψ* (*RHD*08N.01*).

All data related to the serological and molecular investigation performed in these cases are summarized in **Table I**. The frequency of these findings in relation to

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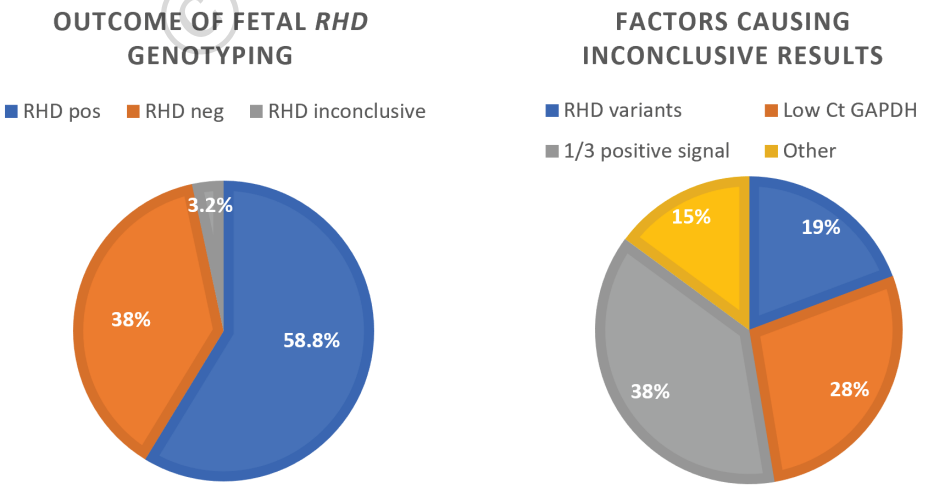


Figure 1 - Outcome of fetal *RHD* genotyping and factors causing inconclusive results

Table I - *Serological and molecular investigation of samples with suspected maternal RHD gene variant*

Number of cases	RhD IAT[a]	ID-partial RhD[b]	*RHCE**	*RHD* Allele	Nucleotide change(s)	D antigen expression
12	Neg	Neg	*ce/ce*[g] *or Ce/ce*	*RHD*08N.01 (RHDψ)*	37-bp insertion in exon 4 and c.609G>A, c.654G>C, c.667T>G, c.674C>T, c.807T>G	Neg
2	Neg	Not performed	*Ce/ce*	*RHD*01EL.01*	c.1227G>A	DEL
		Neg	*Ce/ce*			
1	Neg	Not performed	*Ce/ce*	*RHD*01EL.08*	c.486+1G>A	DEL
1	Weak pos[c] with all reagents	Weak pos[c] with 3/6 reagents	*cE/ce*	*RHD*01EL.43*	c.46T>C	DEL
4	Neg	Weak pos[c] with 2/6 reagents	*Ce/ce*	*RHD*11 (or RHD*weak partial 11)*[f]	c.885G>T	DEL alt weak/ partial
		Neg	*Ce/ce*			
			Ce/Ce			
	Weak pos[c] with 1/2 reagents	Weak pos[c] with 2/6 reagents	*Ce/ce*			
1	Neg	Neg	ce/ce	Not identified	Possible insertion in exon 2 Not further investigated	Neg
1	Neg	Neg	Ce/Ce	Novel	c.421G>A, c.422delT	Neg
1	Neg	Neg	*cE/ce*	*RHD*01N.86* (novel)	c.806_807insA	Neg
1	Neg	Neg	*Ce/ce*	*RHD*01N.73*	c.443C>G	Neg
1	Neg	Neg	*Ce/ce*	*RHD*01N.41*	c.361delTTGTCGGTGCT	Neg
1	Neg	Neg	ce/ce[g]	*RHD*01N.72*	Deletion of exon 3, changes in exons 4-6	Neg
1	Neg Ads/elu neg	Neg	*Ce/ce*	Possible *RHD*01N.67*	Possible deletion of exon 1 Not further investigated	Neg
1	Strong pos[d] with 4/4 reagents	Strong pos[d] with 4/6 reagents	*Ce/ce*	*RHD*05.07 (RHD*V.7)*	c.667T>G, c.697G>C, c.712G>A, c.733G>C, c.744C>T, c.787G>A	Partial
1[e]	Weak pos[c] with reagent 1, Strong pos[d] with reagent 2,3,4	Pos with 3/6 reagents, variable strength	*Ce/ce*	*RHD*05.05*	c.697G>A	Partial
1	Neg	Neg	*ce/ce*[g]	Not identified	Changes in exon 3 och 4 Not further investigated	Probably partial
1	Weak pos[c] 3/4 reagents	Neg	*cE/ce*[g]	Not identified[h] Hemizygote	602C>G, 667T>G, 819G>A, c.486+2T>A Not further investigated	Weak alt partial
1	Neg	Neg	*Ce/ce*	Not identified All *RHD*-exons detected Hemizygote	Not identified	Probably neg or DEL
2	Weak pos[c] with 3/4 reagents	Weak pos[c] with 2/6 reagents	*ce/ce*	Not identified	c.340C>T, c.1136C>T Not further investigated	Weak alt partial
	Weak pos with all reagents		ce/ce			
1	Pos with all reagents, variable strength	Strong pos[d] with 4/6 reagents	*cE/ce*	*RHD*01W.02*	1154G>C	Weak
1	Neg	Weak pos[c] with 2/6 reagents	*Ce/ce*	*RHD*01W.03*	c.8C>G.	Weak
1	Strong pos[d] all reagents	Strong pos[d] with 4/6 reagents	*Ce/ce*	*RHD*01W.92*	c.1145T>C	Weak
1	Weak pos[c] with 1/2 reagents	Weak pos[c] with 2/6 reagents	*Ce/Ce*	Not identified All *RHD* exons detected Homozygote	Not identified	Very weak

Pos: positive, neg: negative, alt: alternative. [a]2 to 4 reagents used. [b]6 reagents used. [c]1+ or less. [d]Strong pos: 2+/4+. [e]2+ with direct agglutination with Bio-Rad DVI+ reagent, negative with DVI- reagent. [f]This variant can be associated with a cDe haplotype giving rise to a weak D shown by indirect agglutination; it can also be associated with a CDe haplotype generating a phenotype with much lower antigen density which is borderline weak D/DEL. [g]At least one allele being *RHCE*ce.01(c.48G>C)*. [h]The detected SNPs have previously been found in *RHD*09.03.01* and *RHD*01EL.09*, respectively.

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ethnical background of the local obstetric population is shown in **Table II**.

The deliveries took place at different local hospitals. A surveillance system for quality assurance was planned; the outcome of fetal *RHD* genotyping was compared to serological results from RhD typing of newborns, performed by direct agglutination. In Gothenburg, a comparison between fetal *RHD* genotyping and serological results was performed in 2,277 newborns (1,418/2,277 with RhD positive blood group and 859/2,277 with RhD negative blood group) showing full concordance.

A total of seven discrepancies were observed in Western Sweden; all these cases were reported to Transfusion Medicine, Sahlgrenska University Hospital, and further investigated and clarified. The investigation performed and the outcome are summarized in **Table III**.

Overall, considering the whole Västra Götaland County, the assay has shown high sensitivity 99.95% (CI 99.82 to 99.99%), specificity 100% (CI 99.86 to 100%) and accuracy 99.97% (CI 99.89 to 100%).

Table II - *RHD gene variants found in Western Sweden correlated to ethnicity*

Ethnicity	Frequency
Asia	13% (No.=5)
Middle East	21% (No.=8)
Africa	32% (No.=12)
Europe	26% (No.=10)
Unknown	8% (No.=3)

The frequency was calculated on a total of 38 cases.

DISCUSSION

Non-invasive single-exon fetal *RHD* genotyping was implemented in Västra Götaland County, Western Sweden, November 2020[14]. The frequency and characterization of *RHD* variants in RhD negative pregnant women is of practical clinical importance to assess the appropriateness of antenatal administration of anti-D prophylaxis. Therefore, in the present study, we show the results of a 31-month follow up performed to evaluate the efficiency of the assay in the local obstetric population on a larger scale.

Table III - *Investigation of discrepant results between fetal RHD genotype and serological RhD blood group of newborns*

Case No.	Fetal *RHD* genotype	Newborn RhD blood group	Serological investigation	Molecular investigation
1[a]	Pos	Neg	DAT pos (2+) D-IAT weak pos[c] after elution performed due to DAT pos	All *RHD*-gene-exons detected with ASP-PCR Negative for the most common alleles causing weak D antigen expression Sequencing not performed due to insufficient blood volume Flow cytometry: weak D antigen expression
2	Neg	Pos with DVI+ reagent, neg with DVI- reagent	D-IAT pos (DAT-) ID-Partial RhD Typing Set consistent with DFR variant	All *RHD*-gene-exons detected with ASP-PCR except exon 4 Genomic result concordant with *DFR* variant Sequencing not performed
3	Pos	Neg	D-IAT weak pos[c] with 1/4 reagents (DAT-) ID-Partial RhD Typing Set weak pos[c] with 1/6 reagents	All *RHD*-gene-exons detected with ASP-PCR Sequencing: *RHD*11 (or RHD*weak partial 11)*[d]
4	Pos	Neg	D-IAT neg with all reagents ID-Partial RhD Typing Set weak pos[c] with 1/6 reagents	All *RHD*-gene-exons detected with ASP-PCR Sequencing: *RHD*01EL.33* causing DEL phenotype
5[b]	Neg	Pos with DVI+ reagent, neg with DVI- reagent	D-IAT 3+ (DAT-) ID-Partial RhD Typing Set consistent with DVI variant	Not performed
6	Pos	Neg	D-IAT weak pos[c] with 2 reagents (DAT-) ID-Partial RhD Typing Set consistent with DFR variant	Not performed
7	Pos	Neg	D-IAT neg with all reagents ID-Partial RhD Typing Set neg	All *RHD*-gene-exons detected with ASP-PCR *RHDψ* (*RHD*08N.01*) detected with ASP-PCR

Pos: positive, neg: negative. [a]Case 1: maternal serology showed RhD immunization with anti-D titer increasing from 8 to 128 during pregnancy. [b]Case 5: maternal serological investigation after the delivery showed the presence of DVI variant also in the mother. [c]1+ or less. [d]This variant can be associated with a cDe haplotype giving rise to a weak D shown by indirect agglutination; it can also be associated with a CDe haplotype generating a phenotype with much lower antigen density which is borderline weak D/DEL.

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We investigated all cases of suspected maternal *RHD* gene and the correlation to ethnicity, and we explored the discrepancies between molecular and serological results in newborn samples.

The frequency of *RHD* gene variants detected in the local obstetric population was 0.6%, which is in concordance with other studies performed in a Caucasian population with mixed ethnicities[17-19].

In 5/43 women of European descent, previously typed as RhD negative by direct agglutination, a weak D antigen was detected by serological RhD typing and the blood group was changed to RhD positive, according to our standard routine. In 38/43 the serological investigation with indirect antiglobulin test was negative or weakly positive with some reagents. Therefore, further investigation using ASP-PCR and Sanger sequencing was performed, elucidating the molecular basis of most of these variants. Interestingly, two novel alleles were identified, of which one has recently been published[20]. In 2/38, we could not identify any genetic variation resulting in weak or partial antigen expression. In addition, there were five cases in which the presence of a *RHD* gene variant was suspected, but it could not be confirmed because further studies resolving the whole gene were not performed.

The most common variants detected in our cohort were the non-functional *RHDΨ* allele (*RHD*08N.01*) and alleles generating DEL phenotypes. It was previously shown that the *RHDΨ* allele has a frequency of approximately 66% in people of African ancestry with RhD negative phenotype[10]; this observation could be confirmed in samples from women with African background in our cohort. Importantly, the RhD negative phenotype determined by the presence of this variant can be associated with RhD alloimmunization. Regarding the DEL phenotype, four variants were identified among eight cases: *RHD*01EL.01* (No.=2), *RHD*01EL.08* (No.=1), *RHD*01EL.43* (No.=1), *RHD*11* (*RHD*11 weak partial 11*) (No.=4). The variant *RHD*01EL.01* (named Asian DEL type), mainly found in people of Asian descent, is mistyped as RhD negative by serological typing[7-9]. Interestingly, the two cases found in our study were confirmed to be associated with women of Asian descent. The *RHD*11* (*RHD*weak partial 11*) may generate either DEL phenotype when associated with CDe haplotype or a weakly expressed D antigen reacting with indirect agglutination when associated with a cDe haplotype[21,22]. In 1/4 case, this variant was found in a woman from Middle East while the other ones were found in European women (one from Northern Europe and two from Southern Europe).

In most of the women in our cohort, 25/38 (66%), a non-European descent was ascertained. In 3/38 (8%) cases the ethnicity was unknown, but in two of them a non-European descent could be presumed. Overall, our data show that the frequency of *RHD* variants in the obstetric population of Western Sweden is at present very low and mostly related to women with non-European ancestry. However, possible changes in the population might occur in the future, which can lead to the necessity of introducing new diagnostic tools.

Serological blood group typing was performed in all neonates born to RhD negative women. Considering Västra Götaland County, a total of seven cases with discrepant results were observed between fetal *RHD* genotyping and serology. Two false negative results were observed, both due to *RHD* variants with changes in exon 4, hence not detectable by the used *RHD* screening kit. One of them was serologically consistent with a DFR phenotype, which was confirmed by molecular investigation. The second one was serologically consistent with a DVI phenotype, and interestingly, also detected in the mother and therefore, not entailing a risk for maternal RhD immunization. However, both variants have low incidence. In the remaining five cases with positive fetal *RHD* genotype and negative RhD blood group in newborns, *RHD* gene variants causing no or very low D antigen expression in newborns were detected, confirming *RHD* screening results. Thus, the present study has confirmed our previous data on the efficiency and reliability of our system for fetal *RHD* genotyping and has shown an even higher sensitivity of the test when considering a larger scale of samples. Several studies evaluating the diagnostic accuracy of fetal *RHD* screening in the second trimester have been published, highlighting that the accuracy of the test is high enough, particularly in a population of mainly European descent, to guide both antenatal and postnatal administration of anti-D prophylaxis[13]. Notably, our study shows high accuracy of fetal *RHD* genotyping even when performing the test in early gestation.

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CONCLUSIONS

In summary, the single-exon approach for fetal *RHD* screening early in pregnancy is an appropriate choice in the population of Western Sweden with a very low frequency of inconclusive results caused by the presence of maternal *RHD* gene variants. Antenatal anti-D prophylaxis is recommended as a standard routine in these cases. Due to the high sensitivity, specificity, and accuracy of the test, serological blood typing of neonates born to RhD negative women has not been performed routinely at our laboratory since June 2023.

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AUTHORSHIP CONTRIBUTIONS

CP and PI contributed to the project development, data collection and preparation of the manuscript. ÅH performed the molecular investigation of discrepant results and reviewed the manuscript.

The Authors declare no conflict of interest.

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