Validation of first trimester non-invasive fetal RhD genotyping for targeted antenatal anti-D prophylaxis

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Background and objectives

Alloimmunisation against Rhesus D (RhD) red cell antigen is the most common cause of severe haemolytic disease of the fetus or newborn (HDFN). In many countries, antenatal anti-D prophylaxis is administered to all RhD-negative pregnant women including those carrying a RhD-negative foetus (30%) while countries like the Netherlands, Denmark and Finland have a national implementation of non-invasive fetal RhD genotyping with targeted anti-D prophylaxis. We evaluated the accuracy of fetal RhD genotyping on cell-free fetal DNA (cffDNA) in maternal plasma which was available for aneuploidy testing at 12 weeks of gestation.

Patients and Methods

Cell-free fetal DNA from 39 RhD-negative pregnant women was manually extracted using the QIAamp Circulating Nucleic Acid Kit. RhD genotyping was performed by RQ-PCR specific for RhD exon 5 and 7 according to Grootkerk-Tax et al., 2006¹. The results were compared to serological cord blood typing after birth. For accuracy testing, genomic DNA from known RhD variants (8 partial D and 11 weak D samples) was tested. Dilution experiments of RhD+/RhD- healthy donor cfDNA in RhD-/RhD- healthy donor cfDNA were performed to determine analytical sensitivity.

Results

Fetal RhD genotyping vs serological cord blood typing

Fetal RhD typing resulted in 13 RhD-negative (no amplification detected) and 26 RhD-positive fetuses with mean Ct's of 36,56 for RhD exon 5 and 35,55 for exon 7. The cffDNA concentrations ranged between 0,148 and 0,794 ng/µl (mean 0,343 ng/µl). Hitherto, 14 results can be compared to cord blood serological RhD typing which give a 100% concordance.

Real-time PCR Ct values of 39 cffDNA samples compared to serological RhD typing after birth

Genomic DNA of **8 partial and 11 weak RhD variants** were correctly typed as RhD positive since either exon 5 or exon 7 were amplified corresponding to the genomic structure of the RhD variant.

	Type	Number of samples tested	Exon-5	Exon-7
Weak D	Type 1	3	+++	+++
	Type 2	3	+++	+++
	Type 3	3	+++	+++
	Type 4.0/4.1/4.3 (A)	1	+++	+++
	Type 15	1	+++	+++
Partial D	DVI type 1	1	-	+++
	DVI type 2	1	-	+++
	DAR	1	+++	-
	DAU 1,2,3	3	+++	+++
	DAU 4	1	+	+++
	DHMI	1	+++	+++

Real-time PCR results of 8 partial and 11 weak RhD variants :+++ strong positive; + weak positive, - negative

Dilution experiments revealed an **analytical sensitivity of 2% RhD+/RhD- DNA**. This corresponds to the fraction of cell free fetal DNA in maternal plasma during pregnancy (3-13%) .

RhD+/RhD- %	Ct (exon 5)	Ct (exon 7)
100%	30,941	30,55
10%	35,122	34,777
4%	36,567	36,027
2%	38,593	37,916
0%	UND	UND
NTC	UND	UND

Real time PCR Ct values of RhD+/RhD- healthy donor cfDNA diluted in RhD-/RhD- healthy donor cfDNA

Non-invasive fetal RhD genotyping is a feasible test that can easily be implemented along with prenatal aneuploidy testing. There was a 100% concordance with serologic cord blood typing and partial/weak D typing. Implementing this test could avoid unnecessary administration of RhIG in 30% of RhD negative pregnancies.

¹ Grootkerk-Tax MG, Soussan AA, De Haas M, Maaskant Van Wijk PA, Van der Schoot CE. Evaluation of prenatal RHD typing strategies on cell-free fetal DNA from maternal plasma.. Transfusion 2006 Dec;46(12):2142-8.