Frequency and characterization of *RHD* variant alleles in serologically D-negative Surinamese pregnant women

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Introduction

The D antigen is highly polymorphic and over 250 *RHD* variants have been described. Next to the normal D+ and D- expression, three types of variant D expression exist: weak D, Del and partial D. Individuals with partial D variants express D antigens that lack one or several of the 30 D-epitopes, which can have an effect on D antigen expression, hampering detection by routine serology. Characterization of *RHD* alleles, especially in ethnic groups with limited access to preventive measures is of practical clinical importance.

In Suriname, the D phenotype of transfusion recipients, pregnant women and newborns is only serologically determined, and anti-D prophylaxis for women at risk for D immunization is not routine. The population of Suriname is a mixture of different ethnic groups, of which Hindustani, Maroons, Creoles, and Javanese represent the four most common. Our previous studies revealed 4.3% serological D negativity and anti-D in 12% of multigravida D- women in Suriname.

The aim of this study was to determine the frequency and characterization of *RHD* variant alleles in Surinamese serologically D- pregnant women from different ethnic groups.

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1 A cross-sectional study, in D- pregnant women, who visited one of the four hospitals (Academic Hospital Paramaribo, Diakonessen Hospital, 's Lands Hospital and Sint Vincentius Hospital) in Paramaribo Suriname for routine pregnancy care, was performed (the RheSuN study). The Commission for Human Research of Suriname's Ministry of Health approved the study (VG-022-14).

In Suriname, pregnant women are routinely tested once for the D blood group using a conventional serologic direct agglutination test with a single monoclonal antibody. From D- women residual blood was shipped to Sanquin, the Netherlands.

After genomic DNA extraction, qPCR targeting *RHD* exons 5 and 7 and *RH*-Multiplex Ligation-dependent Probe Amplification (*RH*-MLPA) were used for *RHD* variant allele testing. Samples with either exon 5 or 7 present or with discrepancies between molecular and serological D were further analyzed with *RH*-MLPA as single samples. *RH*-MLPA revealing normal D genes, were *RHD* sequenced (exons 1-10). The remainder (i.e. both exons absent) were first tested with *RH*-MLPA in batches of 5-6 DNA samples, and when variants were suspected as single samples.

2 Five different variant *RHD* alleles were detected in 36 of the 87 Surinamese pregnant serologically D- women with DNA available for analysis.

RHD allele (ISBT notation)	Allele name	Number of alleles
*RHD*01N.01*	*RHD* deletion	121
*RHD*01*	Normal *RHD*	9
*RHD*03N.01*	*RHD*DIIIa-CEVS (4-7)-D*	22
*RHD*08N.01*	*RHD*Pseudo-gene* or *RHD*Ψ*	18
*RHD*09.06*	*RHD*DAR6*	2
*RHD*10.00*	*RHD*DAU0*[1]	1
*RHD*01EL.01*	*DEL*	1

[1] This woman had the *RHD*10.00/RHD*03N.01* genotype confirmed by *RHD* sequencing.

3 *RHD* variants were most frequent in Maroon women, while two variant alleles most often present in women of mixed ethnicity.

Ethnicity	Number of women	Women with *RHD* variants	*RHD* variant alleles*†	*RHD* alleles (ISBT notation)
Maroons	46	25 (54)	29 (32)	15 *RHD*03N.01*; 12 *RHD*08N.01*; 2 *RHD*09.06*
Mixed	13	5 (38)	8 (31)	4 *RHD*03N.01*; 3 *RHD*08N.01*; 1 *RHD*01EL.01*
Creoles	17	4 (24)	6 (18)	3 *RHD*08N.01*; 2 *RHD*03N.01*; 1 *RHD*10.00*
Hindustani	6	0 (0)	0 (0)	
Unknown	6	1 (17)	1 (8)	1 *RHD*03N.01*
Total	87	35 (40)	44 (25)	

Data presented as n (%) unless stated otherwise; *The denominator for calculating the percentage of *RHD* variant alleles per ethnicity was two times the number of individuals; †Two variant alleles were present in nine women: two were homozygous *RHD*08N.01*, two homozygous *RHD*03N.01* and five women carried, next to *RHD*03N.01* alleles, *RHD*08N.01* (n=2), *RHD*01EL.01*, *RHD*10.00* and *RHD*09.06* alleles (one each).

- *RHD*-MLPA showed that these nine women had a *D* gene.
- Complete *RHD* sequencing (so far performed in seven women) revealed no *RHD* mutations explaining serological D negativity.

4 Our previous study showed D antibodies in eleven of the 87 pregnant women.[1] A homozygous *RHD* deletion was present in five of these women, and five women had a heterozygous *RHD* deletion next to *RHD*08N.01* (n=4) or *RHD*03N.01* (n=1) alleles. One women was homozygous *RHD*08N.01*.

[1]Zonneveld R, Kanhai HHH, Lamers M, Brand A, Zijlmans WCWR, Schonewille H. D antibodies in pregnant women in multiethnic Suriname: the observational RheSuN study. Transfusion. 2017;57:2490-2495

Conclusions

- In 40% of serologically D- Surinamese pregnant women *RHD* variant alleles were present.
- The frequency of variant *RHD* alleles was 25%, with the D-null alleles, *RHD*03N.01* and *RHD*08N.01,* representing 91% of variant alleles.
- *RHD* variants were most frequent in Maroon women.
- In seven women serological D negativity was discrepant with genotyping.
- D antibodies were only present in women with genotypes commonly known to be at risk for anti-D.

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