Non-invasive fetal *RHD* genotyping in D- (RH:-1) patients with *RHD* genomic sequences : retrospective study over 11 years

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Conflict of interest

☑ We have no conflict of interest

Non-invasive Fetal *RHD* genotyping

- indications in France (CNGOF (French obstetricians college) 2017 recommendations) -

Anti-D allo-immunized pregnant woman

Risk of fetal anemia in case of *RHD* positive fetus

Results of the test

<u>*RHD*+ FETUS</u>

Feto-maternal incompatibility
→ monitoring : regular anti-D quantification +/- weekly fetal ultrasound and MCA-PSV measurements

<u>*RHD*- FETUS</u>
(confirmed on 2 separate samples)

No feto-maternal incompatibility
→ No specific monitoring

UNDETERMINED (discordant exons amplification) **/ UNINTERPRETABLE** (early (=mother) exons amplication) ***RHD* FETUS**

Potential feto-maternal incompatibility
→ same monitoring as for RHD+ fetus

Non-alloimmunized D- pregnant women

Risk of anti-D allo-immunization in case of *RHD* positive fetus

Results of the test

<u>*RHD*+ FETUS</u>

RhIg Injection
-systematic prophylaxis (28GW)
-targeted prophylaxis
-post partum prophylaxis

<u>*RHD*- FETUS</u>
(confirmed on 2 separate samples)

No RhIg injection
- no antenatal prophylaxis (systematic / targeted)
- Newborn D phenotype to perform at birth to guide post-partum prophylaxis

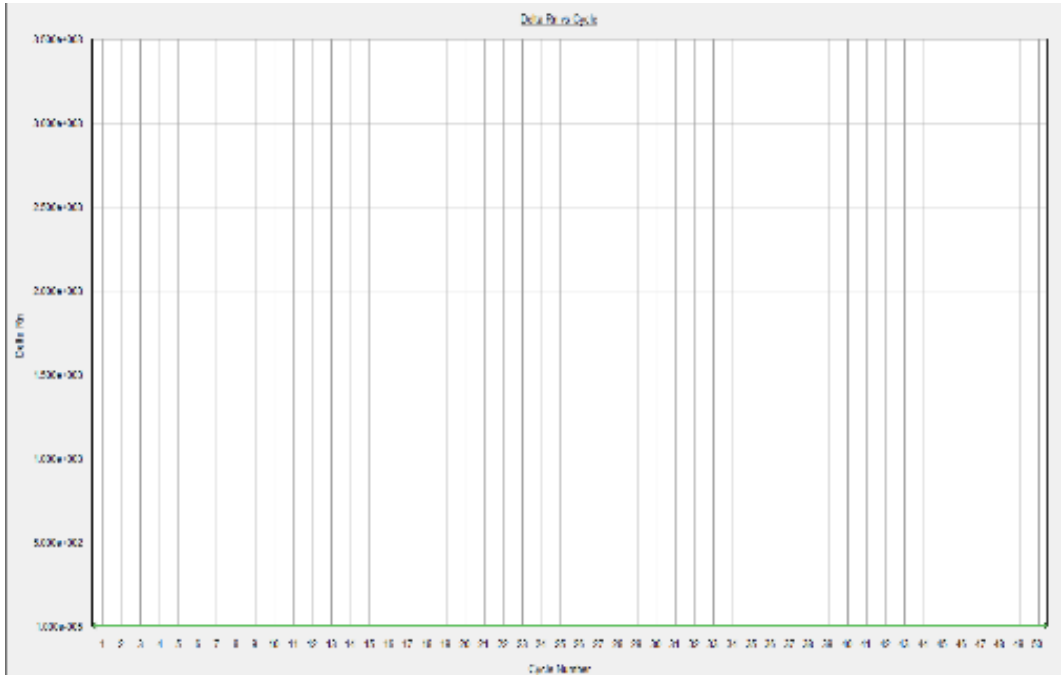
UNDETERMINED (discordant exons amplification) **/ UNINTERPRETABLE** (early (=mother) exons amplication) ***RHD* FETUS**

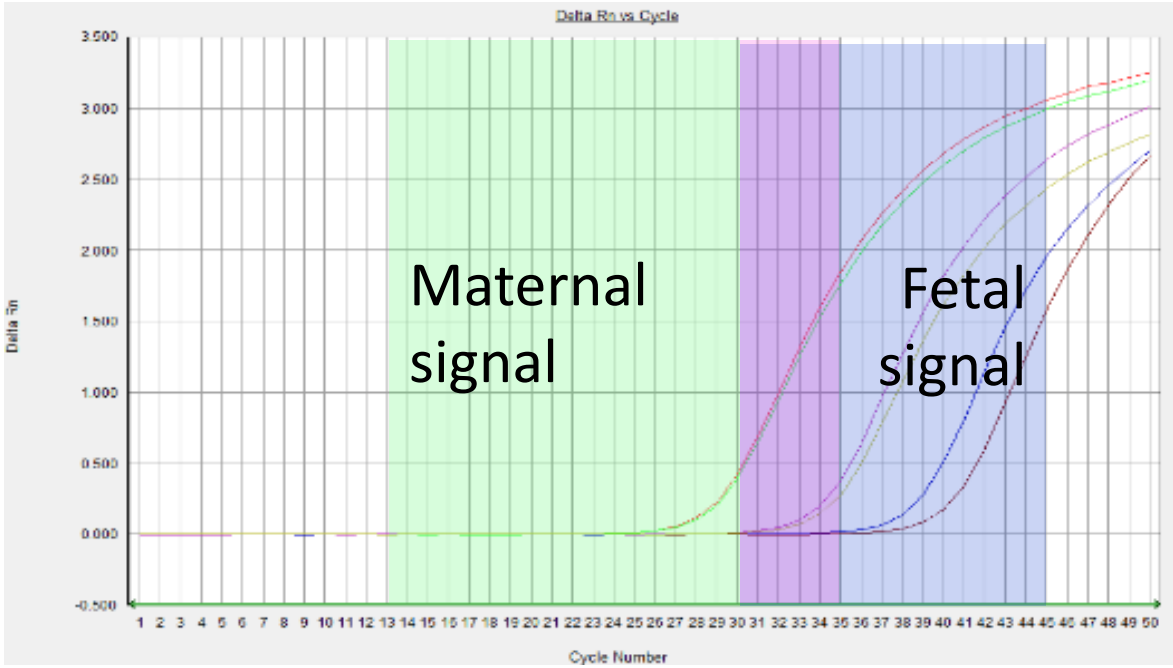
RhIg injection
-systematic and targeted prophylaxis
- Newborn D phenotype to perform at birth to guide post-partum prophylaxis

Non-invasive Fetal *RHD* genotyping at the CNRHP

Principle : Identification of *RHD* sequences present in the fetus and absent in the mother by Real Time PCR

(exons 5,7 and 10 – Free DNA Fetal Kit® RhD – Institut Biotechnologie Jacques Boy)





No signal = no *RHD* sequence
Mother with deleted *RHD* alleles
→*RHD* negative fetus (default diagnosis)

Late signal after 35 cycles (Ct) for all exons tested = Mother with deleted *RHD* alleles →*RHD* positive fetus

Early signal before 30 cycles (Ct) for at least one exon = maternal *RHD* allele amplification (interference)

Signal between 30 and 35 cycles (Ct) for at least one exon = maternal or fetal signal

What is the frequency of non-invasive fetal *RHD* genotyping results with early signal (Ct < 35) for at least one exon in the CNRHP?

Distribution of interpretable PCR signals (negative or Ct ≥ 35) versus uninterpretable (Ct <35) for at least one exon among exons 5,7 or 10). N= 31 722 pregnancies.

92,4 %

PCR signal: negative or Ct ≥ 35

Mother with deleted *RHD alleles:*
the fetal *RHD* genotype could be interpreted

antenatal prophylaxis /
pregnancy monitoring if allo-immunization

7,6 %

PCR signal: Ct < 35

Maternal DNA interference OR
presence of a large amount of fetal DNA
(advanced gestational age, twin pregnancy, etc.)

→ Maternal D phenotype and targeted research for *RHD* exons 4, 5, 6, 7 and 10 and *DPsi* allele mutations in maternal genomic DNA

What are the causes of non-invasive fetal *RHD* genotyping results with early signal (Ct <35) in the CNRHP ?

Distribution of maternal *RHD* variants and conclusion for the fetus considering all tested exons when one or several PCR Ct signals are < 35

Deleted maternal *RHD* alleles

D-CE(4-7)-D
exon 10 Ct < 35

DΨ
exons 10 and 7 Cts <35

Silent maternal *RHD* allele(s)

Silent, variant or positive maternal *RHD* allele(s)
exons 5, 7 and 10 Ct<35

Large amount of fetal DNA *RHD* positive fetus

Determination of the fetal *RHD* status with the extra help of exons 4 and 6 amplifications on maternal plasma (in-house method)

Uninterpretable results for the fetus

Antenatal prophylaxis / pregnancy monitoring if allo-immunization

Risk of false positive results if no Ct threshold is defined to identify maternal variants

Non-invasive Fetal *RHD* genotyping : conclusion

In 11 years, **7.6% of non-invasive fetal RHD genotyping were found with early signal amplifications (Ct <35)**.

Among these cases, in our population coming from Paris area (multiethnic population including Afro-caribbean and sub-Saharan African populations):

- near than **77% corresponds to mothers with *Dpsi* or *D-CE(4-7)-D* silent *RHD* alleles.** In these cases, the **fetal *RHD* genotype could be determined thanks to extra exon amplifications (exons 4 and 6).**

This strategy, **preventing false positive results,** allows to give an **accurate result for the *RHD* genotype of the fetus in most of first « uninterpretable » categorized cases** .

It allows to avoid unnecessary RhIg injection for non allo-immunized D- women and stressful fetal ultrasound monitoring for allo-immunized pregnant women.

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Thank you for your attention

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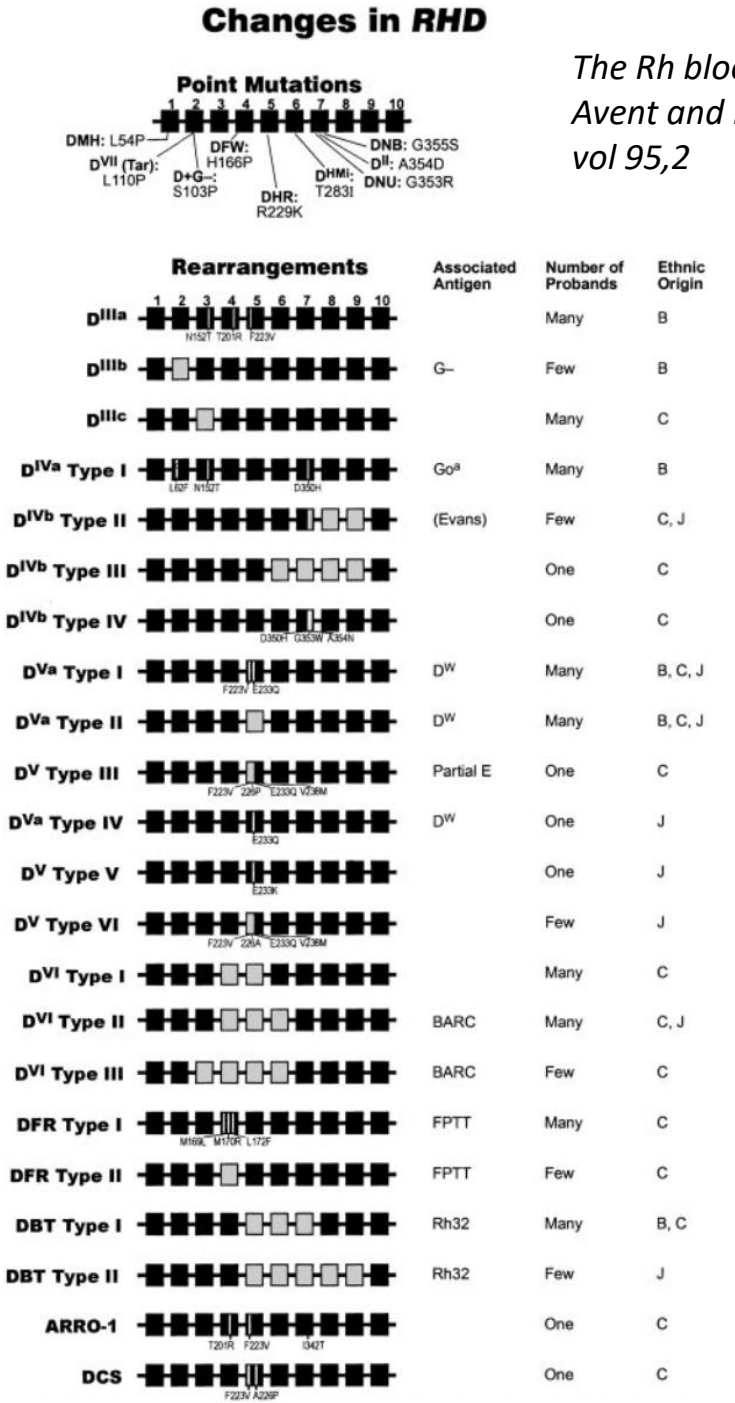
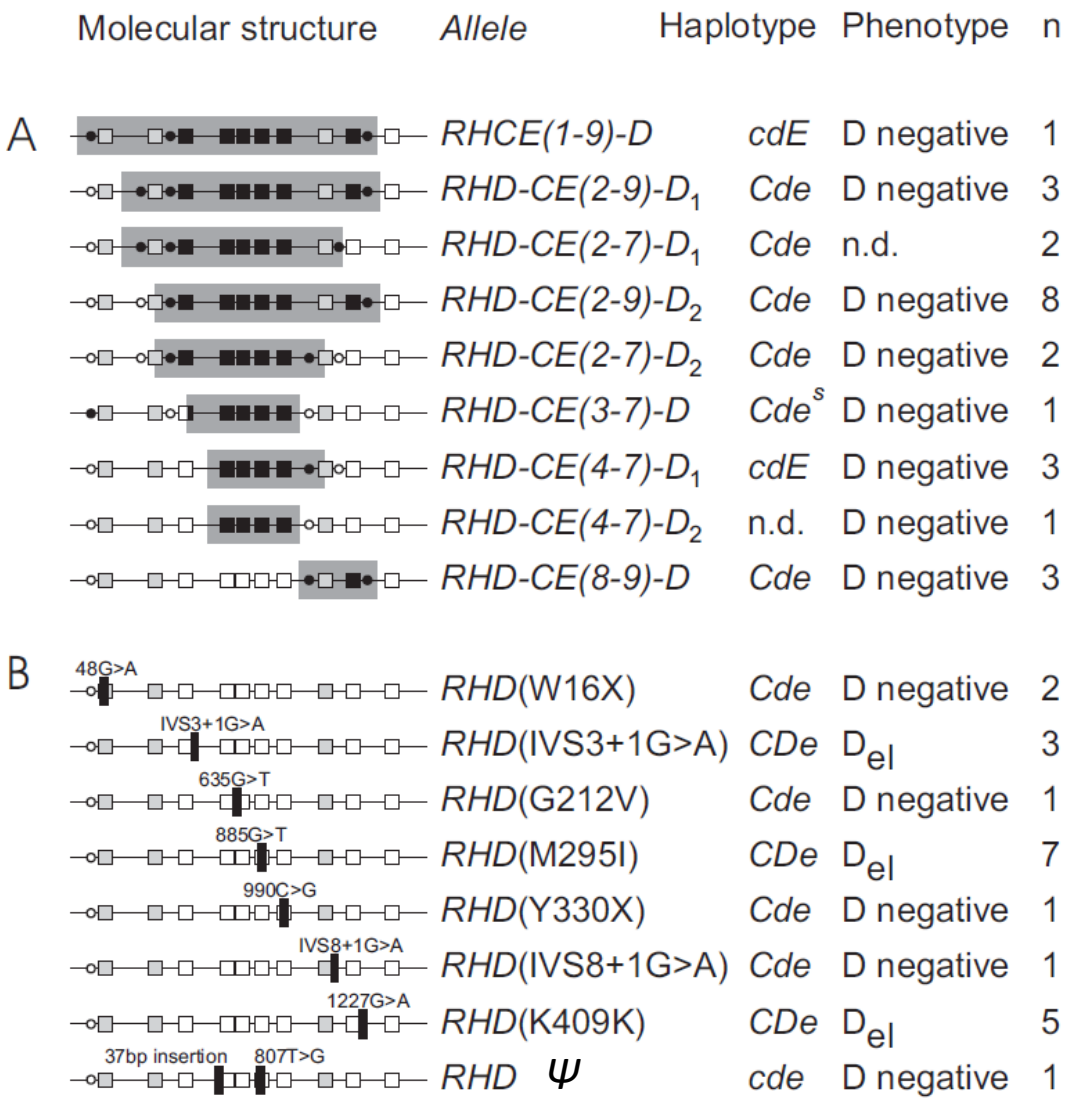


The Rh blood group system: a review; ND Avent and ME Reid, Blood Janurary 2000, vol 95,2



RHD positive haplotypes in D negative Europeans; Wagner F, Frohmajer A and Felgel WA, BMC Genteics 2001

Mother genotype on maternal cells of D- women									Plasma amplication and fetal RHD genotype interpretation					
Exon10	Exon7	Exon6	Exon6ψ	Exon5	Intron 4	Exon4	Exon4ψ	Interpretation	Exon10	Exon7	Exon6	Exon5	Exon4	Interpretation
-	-	NR	NR	NR	-	-	-	***RHD* deleted mother**	+	+	NR	+	NR	***RHD* + fetus**
									+	+	-	-	-	Uninterpretable *RHD* fetus (potential DPsi, ask father's sample)
+	-	NR	NR	NR	-	-	-	**Rearranged RH haplotype** (Conversion). Only exons 4 to 7 will be informative for the fetus on the plasma	**+ (Ct<35)**	**+**	**NR**	**+**	**NR**	***RHD* + fetus**
									+ (Ct<35)	**-**	**-**	**-**	**-**	***RHD* - fetus**
									+ (Ct<35)	+	-	-	-	Uninter. *RHD* fetus
									+ (Ct<35)	-	+/-	+/-	-	Uninter. *RHD* fetus
									+ (Ct<35)	-	+/-	+	+/-	Uninter. *RHD* fetus
									+ (Ct<35)	-	+/-	+/-	+	Uninter. *RHD* fetus
+	+	NR	+	NR	+	-	+	***RHD* ψ mother**	**+ (Ct<35)**	**+ (Ct<35)**	**+**	**+**	**+**	***RHD* + fetus**
									+ (Ct<35)	**+ (Ct<35)**	**-**	**-**	**-**	***RHD* - fetus**
									+ (Ct<35)	+ (Ct<35)	+	-	+/-	Uninter. *RHD* fetus
									+ (Ct<35)	+ (Ct<35)	-	+	+/-	Uninter. *RHD* fetus
									+ (Ct<35)	+ (Ct<35)	-	+/-	+	Uninter. *RHD* fetus
									+ (Ct<35)	+ (Ct<35)	+	+/-	-	Uninter. *RHD* fetus
+	+	NR	-	NR	+	+	-	Non characterized maternal silent *RHD* alleles	+ (Ct<35)	+ (Ct<35)	NR	+ (Ct<35)	NR	Uninter. *RHD* fetus
+	-	+	-	+	+	+	-	Partial or Silent RHD alleles with only 1 or 0 informative exon	+ (Ct<35)	+	NR	+ (Ct<35)	NR	Uninter. *RHD* fetus
									+ (Ct<35)	-	NR	+ (Ct<35)	NR	Uninter. *RHD* fetus

NR: non realized