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Fetal *RHD* Genotyping in Maternal Plasma at 11–13 Weeks of Gestation

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Key Words
Fetal *RHD* genotyping · Cell-free fetal DNA · First trimester · Pyramid of prenatal care

Abstract
Objective: To examine the feasibility of fetal *RHD* genotyping at 11–13 weeks' gestation from analysis of circulating cell-free fetal DNA (ccffDNA) in the plasma of RhD negative pregnant women using a high-throughput robotic technique. ***Methods:*** Stored plasma (0.5 ml) from 591 RhD negative women was used for extraction of ccffDNA by a robotic technique. Real-time quantitative polymerase chain reaction (PCR) with probes for exons 5 and 7 of the *RHD* gene was then used to determine the fetal *RHD* genotype, which was compared to the neonatal RhD phenotype. ***Results:*** In total there were 502 (85.7%) cases with a conclusive result and 84 (14.3%) with an inconclusive result. The prenatal test predicted that the fetus was RhD positive in 332 cases and in all of these the prediction was correct, giving a positive predictive value of 100% (95% CI 96.8–100). The test predicted that the fetus was RhD negative in 170 cases and in 164 of these the prediction was correct, giving a negative predictive value for RhD positive fetuses of 96.5% (95% CI 93.7–99.2). ***Conclusion:*** The findings demonstrate the feasibility and accuracy of non-invasive fetal *RHD* genotyping at 11–13 weeks with a high-throughput technique.

Introduction

In RhD negative pregnant women the fetal *RHD* genotype can be determined from analysis of circulating cell-free fetal DNA (ccffDNA) in maternal plasma [1–4]. The potential benefits of this non-invasive prenatal test are in the management of pregnancies in RhD negative women with *RHD* hemizygous partners. In such pregnancies with no RhD hemolytic antibodies, knowledge that the fetus is *RHD* negative avoids the need for antenatal and postnatal immunoprophylaxis with anti-D either routinely or following an episode of vaginal bleeding or an invasive procedure, such as chorionic villous sampling (CVS). This would be beneficial because of the cost of anti-D immunoglobulin and also the risks associated with the administration of blood products. In the case of isoimmunized patients, knowledge that the fetus is *RHD* negative avoids the need for intensive antenatal monitoring to predict and treat fetal anaemia. In isoimmunized patients requesting invasive prenatal diagnosis of fetal aneuploidies or inherited genetic disorders, such as sickle cell disease, knowledge of the fetal *RHD* status may influence the decision on the choice between CVS and amniocentesis. Although in general CVS is preferable because it provides results in the first rather than in the second trimester, the associated risk of feto-maternal haemorrhage and therefore worsening of the severity of isoim-

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munization is greater and therefore, in the presence of an *RHD* positive fetus, the preferred technique may be amniocentesis [5, 6].

The usefulness of non-invasive determination of the fetal *RHD* status ultimately depends on firstly, the accuracy of the test, and secondly, the ability to screen large numbers of patients. Several studies, mainly from the second and third trimesters of pregnancy, reported contradictory results on the accuracy of the test which ranged from 32 to 100% [7–9]. In most studies the method used for fetal *RHD* genotyping is labor-intensive and expensive and therefore not suitable for mass screening. The introduction of robotic techniques for isolation of fetal DNA have made it possible to offer a high-throughput test which could be a cost-effective alternative to routine administration of anti-D to all RhD negative pregnant women [8, 10, 11].

The aim of our study was to examine the feasibility of fetal *RHD* genotyping at 11–13 weeks' gestation in a large screening population and to evaluate the diagnostic accuracy of the test using a high-throughput technique utilizing robotic isolation of DNA from maternal plasma.

Methods

This study was part of a large prospective observational study for early diagnosis of pregnancy complications in women attending for their routine hospital visit in pregnancy at King's College Hospital, London, UK. In this visit, which is held at 11^{+0}–13^{+6} weeks of gestation, we perform screening for chromosomal defects by a combination of fetal nuchal translucency thickness and maternal serum pregnancy-associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG) [12, 13]. All women attending for this visit were invited to participate in a research study for pregnancy complications which was approved by King's College Hospital Ethics Committee. The study included blood collection and storage of maternal plasma at –80°C for future analysis.

In this study, 591 rhesus D negative women were selected from the database and their stored plasma (0.5 ml) was used to extract ccffDNA for determination of fetal *RHD* status. We included singleton pregnancies, who subsequently had live births and had neonatal rhesus (D) status determined serologically at delivery. All the samples, which had not been previously thawed or refrozen, were transferred at –80°C to the International Blood Group Reference Laboratory (NHS Blood and Transplant, Bristol, UK) for subsequent analysis. The laboratory personnel analyzing the samples and interpreting the results were not aware of the neonatal RhD phenotype.

Sample Preparation

Maternal venous blood collected in ethylene diamine tetraacetic acid (EDTA) BD Vacutainer® tubes (Becton Dickinson UK Ltd., Oxford, UK) was processed within 15 min of collection and centrifuged at 3,000 rpm for 10 min to separate plasma from packed cells and buffy coat and subsequently at 13,000 rpm for 10 min to further separate cell debris. The maternal plasma obtained after double centrifugation was then divided into 0.5-ml aliquots and stored at –80°C until subsequent analysis. The buffy coat separated at the first centrifugation was stored along with the maternal plasma samples at –80°C.

Robotic Isolation of ccffDNA and Real-Time Polymerase Chain Reaction to Detect the Presence of RHD

Bar-coded tubes containing 0.5 ml maternal plasma were transferred to the MDx BioRobot (Qiagen, Hilden, Germany) for robotic isolation of DNA. The extracted DNA was eluted into nuclease-free water in a 96-well plate and then subsequently used for real-time quantitative polymerase chain reaction (PCR) to detect the presence of *RHD* sequences in maternal plasma. The QIAgility liquid handling robot (Qiagen) was used to dispense a mix of Universal PCR MasterMix (Applied Biosystems, Foster City, Calif., USA) and primers and probes for exons 5 and 7 of the *RHD* gene (as a multiplex) or the CCR5 gene into a 384-well PCR plate. DNA (5 μl) from each maternal sample was dispensed into three wells containing reaction mix for the *RHD* gene and into a single well containing reaction mix for CCR5 [11]. Control wells for the *RHD* assay containing RhD positive DNA, RhD negative DNA, *RHDΨ* positive DNA, and no DNA were also dispensed. Real-time quantitative PCRs were performed on the ABI Prism 7900HT (Applied Biosystems) to detect the presence of fetal *RHD* gene sequences and to quantify total (maternal and fetal) DNA in the plasma. The procedure took about 5 h with a capacity of DNA from 88 samples per run.

Calling Rules for Determination of Fetal RHD Status

Confirmation of successful DNA extraction and an estimation of the amount of total DNA in the sample was provided by the single amplification of chemokine receptor 5 (CCR5) gene demonstrated by a cycle threshold (Ct) value of <35.4. Human CCR5 gene maps to the short arm of chromosome 3 at position 21 and amplifies both maternal and fetal DNA [9, 14]. Samples with no demonstrable amplification of CCR5 were considered as having insufficient DNA for subsequent analysis.

Samples with demonstrable amplification of CCR5 were analyzed for the presence of *RHD* exons 5 and 7. The exon 5 assay amplified *RHD* only, whereas the exon 7 assay amplified both *RHD* and the highly homologous but inactive gene *RHDΨ*, common in Black Africans. A cycle threshold value of <42 was interpreted as a positive signal. An algorithm was created to predict fetal RhD phenotype depending on the number of positive signals obtained for both *RHD* exons 5 and 7. The sample was classified as *RHD* positive if at least two positive replicates for both exons 5 and 7 were seen and as *RHD* negative if there was a maximum of one positive replicate for either exon 5 or exon 7. The presence of a maternal *RHD* variant gene was suspected if the difference between the average Ct value of *RHD* exon 7 and the Ct value of CCR5 was <1. The presence of a fetal *RHD* variant gene was predicted if the difference between the average Ct value of *RHD* exon 7 and the CCR5 Ct value was >1, and there were at least two positive replicates for *RHD* exon 7 but no positive replicates for exon 5. All other permutations of replicates were classified as an equivocal result.

In the cases of suspected maternal D variant, DNA from the buffy coat of the stored maternal sample was analyzed by allele-

specific PCR and agarose gel electrophoresis to detect exon 7 and intron 4 of *RHD*, and regions of exon 2 and intron 2 of the *RHCE* gene responsible for c and C antigen expression, respectively. Women were classified as D variant (*RHDΨ*) if a larger PCR amplicon for *RHD* intron 4 was identified (due to the presence of a 37 base pair insertion in *RHDΨ*) [22]. Women were classified as D variant (other) if found to have either or both *RHD* intron 4 and exon 7. In these cases a prediction of fetal D status was not made. Women were classified as D negative by the lack of amplification of *RHD* intron 4 and exon 7, but positive identification of at least one *RHCE* PCR product. In such cases the fetus was subsequently predicted to be D positive.

Statistical Analysis

The sensitivity, specificity and diagnostic accuracy of the test and their 95% confidence intervals (CI) were determined. The statistical software package XLSTAT-Pro 2010 (Addinsoft, USA) was used for the analysis.

Results

In the study population of 591 pregnancies the median gestational age at collection of the maternal blood sample was 12.4 (range 11–14) weeks and the racial origin of the women was Caucasian in 457 (77.3%), African in 114 (19.3%), mixed in 13 (2.2%) and South Asian in 7 (1.2%). The neonatal RhD phenotype was positive in 413 (70%) cases and negative in 178 (30%) cases.

Prediction of Fetal RHD Status

In 5 (0.9%) of the 591 cases there was no amplification of CCR5 and these were not analyzed further (fig. 1). In 451 (77.0%) of the 586 samples with sufficient DNA extraction the result was conclusive, in 40 (6.8%) it was equivocal and in 95 (16.2%) maternal *RHD* variant was suspected. The 95 cases with suspected maternal *RHD* variant were reclassified, after assessment of the maternal buffy coat, into conclusive in 51 (53.7%) and equivocal in 44 (46.3%). Therefore, in total there were 502 (85.7%) cases with a conclusive result and 84 (14.3%) with an inconclusive result. In 72 (85.7%) of the 84 cases with an inconclusive result the neonatal RhD phenotype was positive.

There were 42 cases with a suspected fetal or confirmed maternal *RHD* variant gene and in 38 of these cases the maternal racial origin was African.

Accuracy of Prediction of Fetal RHD Status

In the 502 samples with sufficient DNA extraction and a conclusive result the neonatal RhD phenotype was positive in 338 cases and negative in 164 cases. The prenatal sample was classified as *RHD* positive in 332 cases and in all cases the neonatal RhD phenotype was positive. The prenatal sample was classified as *RHD* negative in 170 cases, including 164 in which the neonatal RhD phenotype was negative and 6 in which it was positive.

The non-invasive test correctly predicted the neonatal RhD phenotype in 496 of 502 cases and therefore the accuracy of the test was 98.8% (95% CI 97.9–99.8). In 332 of the 338 pregnancies with RhD positive fetuses the test predicted that the fetus was positive and in 6 that it was negative and therefore the sensitivity of the prenatal test for the prediction of *RHD* positivity was 98.2% (95% CI 96.1–99.3). In all 164 pregnancies with RhD negative fetuses the prenatal test predicted that the fetus was negative and therefore the specificity of the test for the prediction of *RHD* positivity was 100% (95% CI 97.2–100).

In total, the prenatal test predicted that the fetus was *RHD* positive in 332 cases and in all of these the prediction was correct, giving a positive predictive value of 100% (95% CI 96.8–100). The prenatal test predicted that the fetus was *RHD* negative in 170 cases and in 164 of these the prediction was correct, giving a negative predictive value for *RHD* positive fetuses of 96.5% (95% CI 93.7–99.2).

Discussion

The findings of this study demonstrate the feasibility of fetal *RHD* genotyping at 11–13 weeks' gestation with a high-throughput technique utilizing robotic isolation of DNA from maternal plasma. In about 15% of the cases the result was equivocal. In those with a conclusive result indicating *RHD* positivity the neonatal RhD phenotype was always positive. In those with a *RHD* negative result the neonatal RhD phenotype was negative in 96.5% and positive in 3.5% of cases.

The clinical implications of our findings that a *RHD* positive result indicates that the fetus is RhD positive are, firstly, if the mother is isoimmunized the pregnancy should be monitored closely by Doppler assessment of the fetal middle cerebral artery for evidence of a hyperdynamic circulation and fetal anaemia in need for intrauterine fetal blood transfusions, secondly, if the mother is isoimmunized and she requests invasive prenatal diagnosis it would be preferable to perform amniocentesis rather than CVS, and thirdly, if the mother is not isoimmunized she should receive anti-D immunoprophylaxis both antenatally and in the neonatal period. Most previous first-trimester studies on non-invasive diagnosis of the fetal *RHD* genotype have also reported that the false positive rate is 0% [2, 15–21].

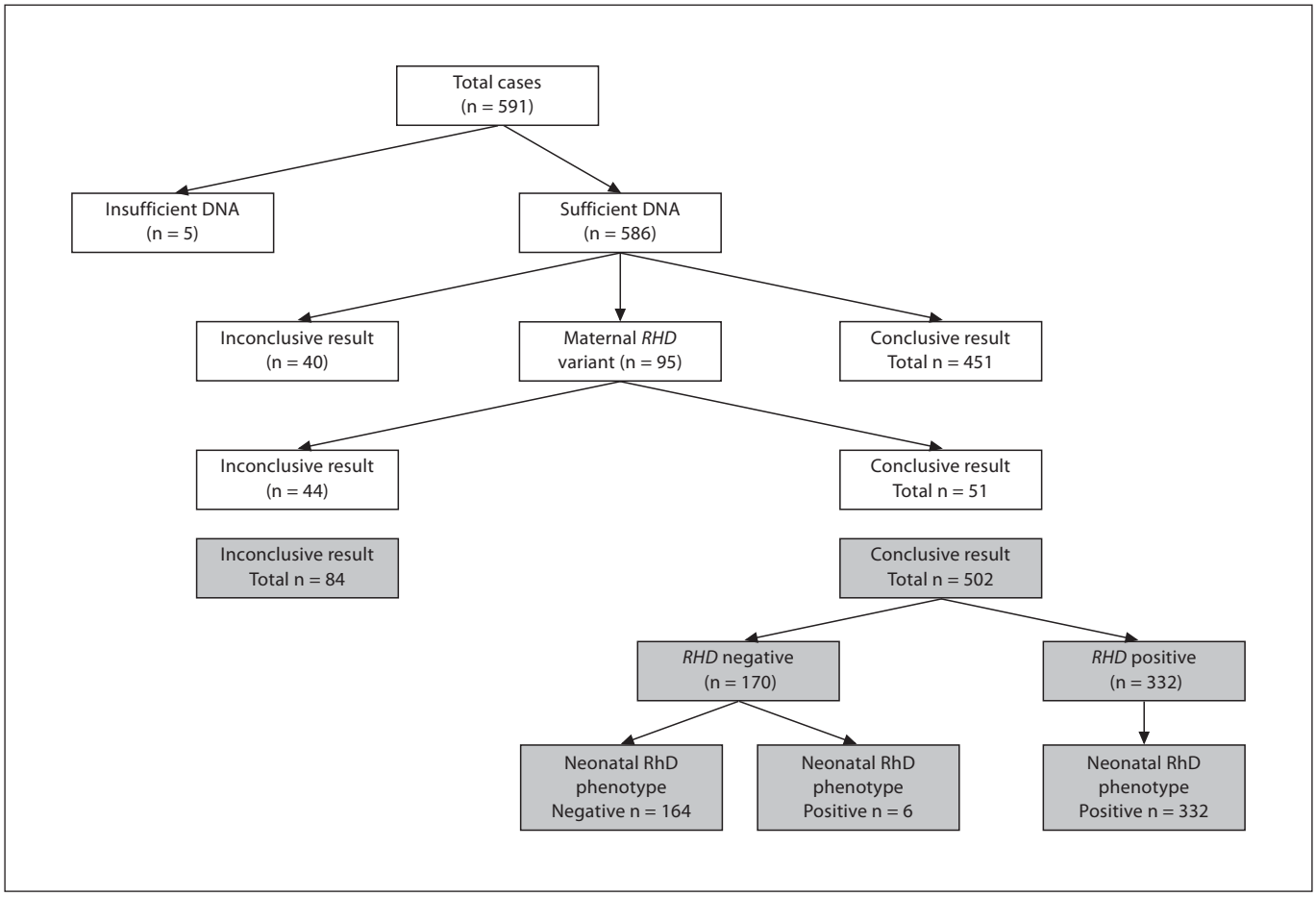

Fig. 1. Flow chart summarizing the results of non-invasive fetal *RHD* genotyping.

An inconclusive result was reported in about 15% of our cases and this group included fetuses or mothers with *RHD* variant genes and those with an equivocal result. Our incidence of inconclusive result is substantially higher than in previous reports [2, 15–21]. There are three possible explanations for this difference: firstly, many of the previous studies have excluded such cases [16], secondly, a high proportion of the women in our study were of African racial origin and in these women there is a very high prevalence of a *RHD* variant pseudogene [9, 22], and thirdly, in cases of a fetal or maternal *RHD* variant gene further analysis is necessary to ascertain the fetal genotype accurately, but in our study this was not possible due to limited availability of stored maternal plasma. In about 85% of our cases with an inconclusive result the neonatal RhD phenotype was positive and therefore the clinical management of such cases should be the same as in cases with a *RHD* positive result.

A *RHD* negative result should be interpreted with caution because in a small proportion of such cases the fetus is actually RhD positive. The false negative rate in our study was 3.5% and in previous first-trimester studies it varied between 0 and 50% [2, 15–21]. Such false negative results are likely to be the consequence of failure to detect ccffDNA in maternal plasma [23, 24]. The presence of ccffDNA in a sample is ascertained by utilizing internal controls, such as SRY or insertion deletion polymorphisms [9, 25, 26]. However, SRY can be used only in pregnancies with male fetuses and use of insertion deletion polymorphisms is time-consuming, labour-intensive and has a low sensitivity [9, 23]. Consequently, it is currently not possible to distinguish a true negative from a false negative result.

The false negative rate of 3.5% at 11–13 weeks is substantially higher than the reported rate of <0.2% using the same high-throughput robotic DNA isolation tech-

nique at 26–32 weeks [11]. The most likely explanation for this gestation-related difference is that cffDNA increases with gestational age [27]. There are three possible implications from such results in terms of pregnancy management. Firstly, non-invasive fetal *RHD* genotyping should not be carried out at 12 weeks because irrespective of the results of such a test the management of all pregnancies would be based on the assumption that the fetus is RhD positive. Non-invasive testing should instead be carried out at 26 weeks when the false negative rate is very low. Secondly, testing should be performed at 11–13 weeks and the false negative rate of 3.5% should be ignored. The advantage of this approach is that women with a result indicating that the fetus is *RHD* negative will have the option of having CVS rather than amniocentesis for fetal karyotyping or genetic testing and these women are not given anti-D either after episodes of vaginal bleeding or miscarriage or prophylactically in the antenatal and neonatal periods. The third option is to perform testing at 11–13 weeks and if the result is negative, the pregnancies are managed as such at between 12 and 26 weeks but the test is then repeated at 26 weeks to reassess the need for prophylactic anti-D. The disadvantage of this approach relates to the additional cost from a second test and the advantages are, firstly, the vast majority of women with a true negative result are not deprived of the option of having CVS rather than amniocentesis and, secondly, the few (3.5%) with a false negative result are not deprived of receiving prophylactic anti-D.

In a Caucasian population about 15% of pregnant women are RhD negative and in 40% of these the fetus would be *RHD* negative [28]. This is because in 15% of such cases the Caucasian partner is also *RHD* negative, in 50% he is hemizygous *RHD* positive and in 35% homozygous *RHD* positive. One controversial issue in the assessment of pregnancies from RhD negative women is whether the management should include determination of zygosity in the father. This would require specialized quantitative PCR methods to determine *RHD* gene copy number, which would be as expensive as the fetal testing. Predicting *RHD* zygosity from serology is inaccurate in Caucasians and of no value at all in people of African racial origin. Additionally, since such testing may not always be possible because of unavailability of the partner or is against the wishes of the woman, we may have to offer non-invasive testing to all RhD negative women rather than only to the 50% of cases whose partners are hemizygous.

The findings of our study concerning non-invasive fetal *RHD* genotyping at 11–13 weeks, that a *RHD* positive result is always true and that a negative result is false in 3.5% of cases, as well as the results of a previous study, that with testing at 26–32 weeks the false negative rate is only 0.2% [11], can form the basis for prospective studies to determine the optimum gestation for fetal *RHD* genotyping from maternal blood. If these results are confirmed there would be three management options: firstly, testing all RhD negative women only at 12 weeks, secondly, testing only at 26 weeks, and thirdly, testing for all at 12 weeks and for those with a negative result repeating the test at 26 weeks.

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References

1 Lo YM, Corbetta N, Chamberlain PF, Rai V, Sargent IL, Redman CW, Wainscoat JS: Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485–487.

2 Lo YM, Hjelm NM, Fidler C, Sargent IL, Murphy MF, Chamberlain PF, Poon PM, Redman CW, Wainscoat JS: Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. N Engl J Med 1998; 339:1734–1738.

3 Faas BH, Beuling EA, Christiaens GC, von dem Borne AE, van der Schoot CE: Detection of fetal RhD-specific sequences in maternal plasma. Lancet 1998;352:1196.

4 Finning KM, Martin PG, Soothill PW, Avent ND: Prediction of fetal D status from maternal plasma: introduction of a new noninvasive fetal RHD genotyping service. Transfusion 2002 42:1079–1085.

5 Tabor A, Bang J, Nørgaard-Pedersen B: Feto-maternal haemorrhage associated with genetic amniocentesis: results of a randomized trial. Br J Obstet Gynaecol 1987;94:528–534.

6 Brambati B, Guercilena S, Bonacchi I, Oldrini A, Lanzani A, Piceni L: Feto-maternal transfusion after chorionic villus sampling: clinical implications. Hum Reprod 1986;1: 37–40.

7 Geifman-Holtzman O, Grotegut CA, Gaughan JP: Diagnostic accuracy of noninvasive fetal Rh genotyping from maternal blood – a meta-analysis. Am J Obstet Gynecol 2006; 195:1163–1173.

8 Legler TJ, Muller SP, Haverkamp A, Grill S, Hahn S: Prenatal RhD testing: A review of studies published from 2006 to 2008. Transfus Med Hemother 2009;36:189–198.

9 Daniels G, Finning K, Martin P, Massey E: Noninvasive prenatal diagnosis of fetal blood group phenotypes: current practice and future prospects. Prenat Diagn 2009;29: 101–107.

10 Van der Schoot CE, Soussan AA, Koelewijn J, Bonsel G, Paget-Christiaens LG, de Haas M: Non-invasive antenatal RHD typing. Transfus Clin Biol 2006;13:53–57.
11 Finning K, Martin P, Summers J, Massey E, Poole G, Daniels G: Effect of high-throughput RHD typing of fetal DNA in maternal plasma on use of anti-RhD immunoglobulin in RhD negative pregnant women: prospective feasibility study. BMJ 2008;336:816–818.
12 Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH: UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Lancet 1998;352:343–346.
13 Kagan KO, Wright D, Baker A, Sahota D, Nicolaides KH: Screening for trisomy 21 by maternal age, fetal nuchal translucency thickness, free β-human chorionic gonadotropin, and pregnancy-associated plasma protein-A. Ultrasound Obstet Gynecol 2008;31:618–624.
14 Raport CJ, Gosling J, Schweickart VL, Gray PW, Charo IF: Molecular cloning and functional characterization of a novel human CC chemokine receptor (CCR5) for RANTES, MIP-1β, and MIP-1α. J Biol Chem 1996;271:17161–17166.
15 Zhang J, Fidler C, Murphy MF, Chamberlain PF, Sargent IL, Redman CW, Hjelm NM, Wainscoat JS, Lo YM: Determination of fetal RhD status by maternal plasma DNA analysis. Ann NY Acad Sci 2000;906:153–155.
16 Costa JM, Giovangrandi Y, Ernault P, Lohmann L, Nataf V, El Halali N, Gautier E: Fetal RHD genotyping in maternal serum during the first trimester of pregnancy. Br J Haematol 2002;119:255–260.
17 Randen I, Hauge R, Kjeldsen-Kragh J, Fagerhol MK: Prenatal genotyping of RHD and SRY using maternal blood. Vox Sang 2003;85:300–306.
18 Brojer E, Zupanska B, Guz K, Orzińska A, Kaliñska A: Noninvasive determination of fetal RHD status by examination of cell-free DNA in maternal plasma. Transfusion 2005:45:1473–1480.
19 Al-Yatama MK, Mustafa AS, Al-Kandari FM, Khaja N, Zohra K, Monem RA, Abraham S: Polymerase-chain-reaction-based detection of fetal rhesus D and Y-chromosome-specific DNA in the whole blood of pregnant women during different trimesters of pregnancy. Med Princ Pract 2007;16:327–332.
20 Machado IN, Castilho L, Pellegrino J Jr, Barini R: Fetal RHD genotyping from maternal plasma in a population with a highly diverse ethnic background. Rev Assoc Med Bras 2006;52:232–235.
21 Minon JM, Gerard C, Senterre JM, Schaaps JP, Foidart JM: Routine fetal RHD genotyping with maternal plasma: a four-year experience in Belgium. Transfusion 2008;48:373–381.
22 Singleton BK, Green CA, Avent ND, Martin PG, Smart E, Daka A, Narter-Olaga EG, Hawthorne LM, Daniels G: The presence of an RHD pseudogene containing a 37 base pair duplication and a nonsense mutation in Africans with the Rh D-negative blood group phenotype. Blood 2000;95:12–18.
23 Freeman K, Szczepura A, Osipenko L: Non-invasive fetal RHD genotyping tests: a systematic review of the quality of reporting of diagnostic accuracy in published studies. Eur J Obstet Gynecol Reprod Biol 2009;142:91–98.
24 Wright CF, Burton H: The use of cell-free fetal nucleic acids in maternal blood for non-invasive prenatal diagnosis. Hum Reprod Update 2009;15:139–151.
25 Page-Christiaens GC, Bossers B, van der Schoot CE, DE Haas M: Use of bi-allelic insertion/deletion polymorphisms as a positive control for fetal genotyping in maternal blood: first clinical experience. Ann NY Acad Sci. 2006;1075:123–129.
26 Chan KC, Ding C, Gerovassili A, Yeung SW, Chiu RW, Leung TN, Lau TK, Chim SS, Chung GT, Nicolaides KH, Lo YM: Hypermethylated RASSF1A in maternal plasma: a universal fetal DNA marker that improves the reliability of noninvasive prenatal diagnosis. Clin Chem 2006;52:2211–2218.
27 Lo YM, Tein MS, Lau TK, Haines CJ, Leung TN, Poon PM, Wainscoat JS, Johnson PJ, Chang AM, Hjelm NM: Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. Am J Hum Genet 1998;62:768–775.
28 Daniels G: Human Blood Groups, ed 2. Oxford, Blackwell Science, 2002.