Noninvasive prenatal diagnosis for early detection of fetal sex in X-linked disorders

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INTRODUCTION

Early prenatal determination of fetal sex is required for pregnant women who are carriers of X-linked disorders (such as haemophilia), for the management of congenital adrenal hyperplasia or conditions associated with ambiguous development of the external genitalia. This is traditionally performed by invasive procedures, using either chorionic villus sampling or amniocentesis, which carry a small but significant risk of miscarriage (0.5-1%) and cannot be performed until 11 weeks of gestation. The discovery of cell-free fetal DNA (cffDNA) in maternal plasma has opened up new possibilities for non-invasive procedures in prenatal diagnosis (NIPD), with no risk of fetal loss. The non-invasive procedures consist in multiplex qRT-PCR, targeting Y chromosome-specific sequences, on DNA samples isolated from maternal plasma. Since a female fetus is predicted by a null result (absence of Y chromosome-specific sequences amplification), which could also reflect the absence of cffDNA or a failure of the test, the analysis of a fetal-specific DNA marker, as internal control, is important to confirm the presence of cffDNA.

OBJECTIVES

The aims of the study were:

i) Optimization of a rapid and easily applicable procedure for fetal sex determination and validation on a large cohort of pregnant women between 8 and 11 weeks of gestation.

ii) Set-up of fetal-specific (epigenetic) marker analysis – i.e. sequences that are differentially methylated in maternal blood cells and placenta - to attest the presence of cffDNA and therefore to avoid false negative results.

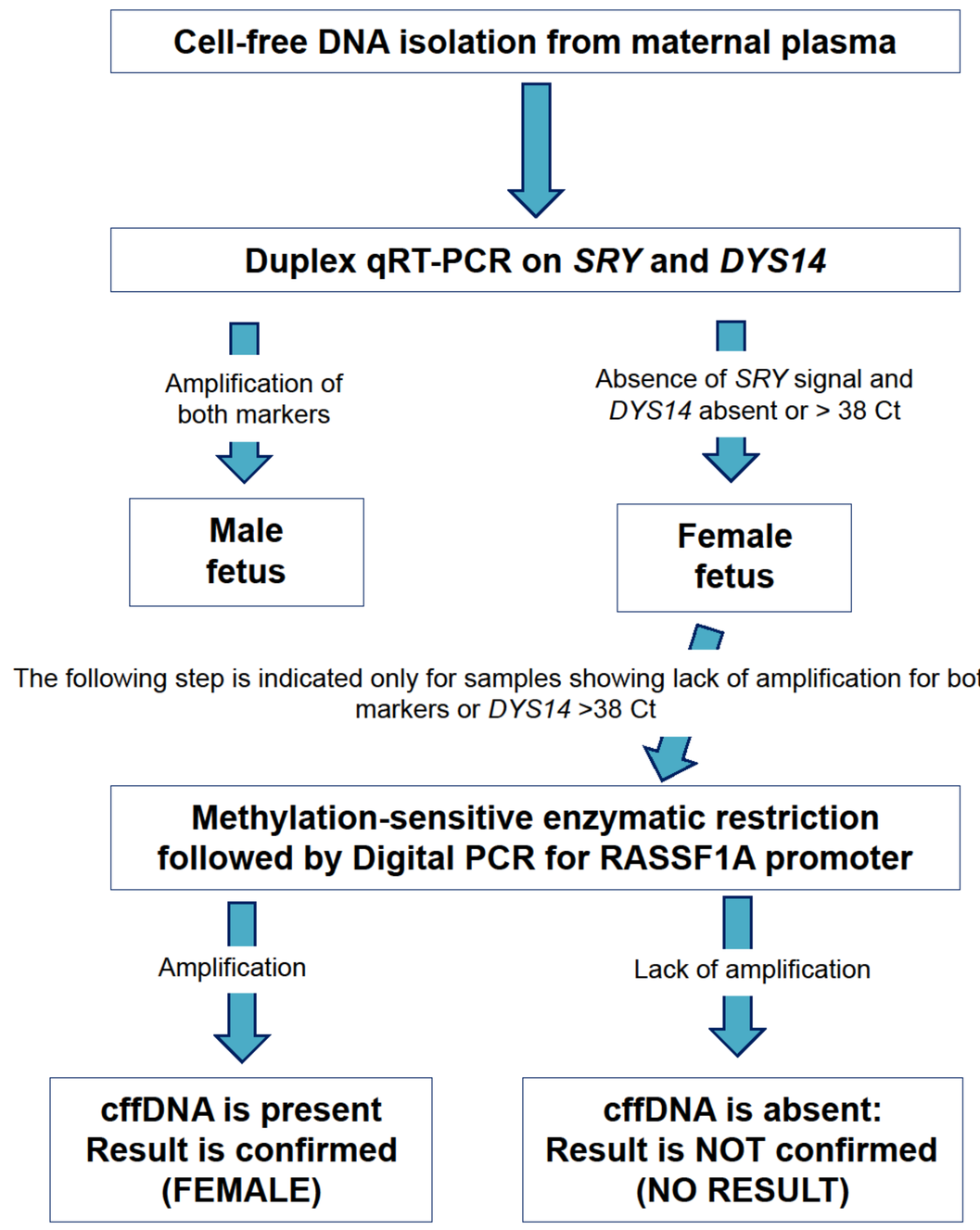
METHODS

A total of 1533 pregnant women undergoing the first trimester screening (8-11 weeks of gestation) have been enrolled and cell-free DNA has been isolated from plasma.

A multiplex PCR was designed to allow for the simultaneous amplification of two Y chromosome specific sequences (*SRY* and the multicopy *DYS14*) for fetal sex determination.

Fetal gender was confirmed by QF-PCR/karyotype results after invasive testing (if available) and/or by neonatal follow-up data.

STUDY DESIGN



RESULTS

A first set of 526 samples have been analysed for fetal gender by qRT-PCR. We obtained conclusive results in 519 of 526 samples (98,7%). To date, postnatal sex identification or fetal karyotype results were available for 312 samples, showing 100% concordance with qRT-PCR results. Five samples are from haemophilia-carriers. qRT-PCR analysis correctly identified the presence of a male fetus in 2 out of 5 pregnancies and a female fetus in the other three. Fetal sex was confirmed by ultrasonography at 16 weeks of gestation.

CONCLUSIONS

Preliminary data show that noninvasive fetal sex determination is highly reliable and it could be considered as an early “pre-test” in pregnancies at risk for X-linked disorders. The introduction of this test will allow to avoid unnecessary invasive testing in case of female fetus that would be, at worst, carrier of the disease. The analysis carried out in haemophilia-carrier women allowed to avoid unnecessary invasive testing in the 3 pregnancies with a female fetus. The set-up of fetal specific marker is completed while absolute quantification of cell free DNA by Digital PCR is ongoing.

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Carrier Issues

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