Title: Maternal liver transplant: another cause of discordant fetal sex determination using cell free DNA.

Running Head: Organ transplant interferes with NIPT outcome

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ABSTRACT

NIPT can very accurately determine fetal sex during pregnancy. We present an exceptional case where NIPT contradicts the ultrasound based sex determination. The pregnant woman was recipient of a liver transplant from a male donor. Graft-derived cell-free DNA released into the maternal circulation clouded the NIPT based sex determination. Hence, NIPT is not advisable when the pregnant mother underwent an organ transplant.

Non-invasive prenatal testing (NIPT) for chromosomal aneuploidies offers higher sensitivities and specificities for common aneuploidies and sex determination at an earlier gestational age compared to traditional biochemical and sonographic screening. As a consequence, NIPT is now widely adopted as a first line prenatal screening test for common fetal aneuploidies and is often the primary source of fetal sex determination. NIPT is based on examining the cell-free fetal DNA which derives from the placenta and circulates freely along the high background of maternal DNA (1). Since the fetal circulating cell-free DNA (ccfDNA) fraction is small compared to the maternal fraction, any deviations in the maternal genomic constitution can confound test results. It has been shown that maternal mosaicism and copy number variations, as small as 500kb, can be a cause of false positives results. In addition, maternal presymptomatic or even undiagnosed cancers have been shown to interfere with NIPT (2). More recently, it has been suggested that biochemical features, such as vitamin B12 deficiency, can influence the DNA sequencing profiles (3). Here, we present an exceptional case where fetal sex determination by NIPT versus ultrasound screening is discrepant in a pregnant woman who has had a liver transplantation.

The pregnant woman was 26 years old when she underwent a combined first trimester screening. The risk for chromosomal aneuploidy was >1/283 instigating a NIPT. Maternal blood was collected at 13^{+6} weeks of gestation and testing was performed by massively parallel sequencing of ccfDNA isolated from plasma as described (4). This case was part of a study approved by the institutional ethics review board (S60268). The fetal fraction estimated from the Y-chromosomal read counts was 32.7%, in contrast to the 2.9% fetal fraction predicted by SeqFF (5). SeqFF utilizes subtle mapped read length differences between regions across the genome and is thus less sensitive to the overrepresentation of ccfDNA originating from Y or any other individual chromosome. Despite this descrepancy, a male fetus with a normal profile for chromosome 21, 18 and 13 was reported. Five weeks following the report, the referral gynaecologist contacted the laboratory and pointed out that the ultrasound scans identified a female fetus during the first and second trimester whereas the NIPT report stated the fetus to be male. To exclude a misdiagnosis, a second blood sample was obtained at 18 weeks of pregnancy. The Y chromosome based fetal fraction determination showed a male fetus with an unexpected higher fetal fraction of 40.2%, again in disagreement with SeqFF outcome (5.35%). In Figure 1, SeqFF derived fetal fraction values are plotted against the Y-chromosome based fetal fraction values.. As has been shown (5) the plot shows a positive correlation between SeqFF and fetal fraction based on Chromosome Y. Clearly the two samples, marked by red arrows, separate from the clustering of male cases around the fitted line, suggesting a biological reason for the sex discrepancy. A potential sample mix-up was excluded since STR profiles of maternal DNA and the NIPT library were shown to be identical. During the discussion of these atypical findings between the clinical geneticists and the

couple it was revealed that the woman was recipient of a liver transplant from a male donor 10 months earlier. The indication for the liver transplant was polyadenomatosis. In addition, at 20 weeks gestation, a progressive disturbance of liver function tests was observed, and at 30 weeks gestation, a liver biopsy revealed mild graft rejection. Graft rejection and the accompanying liver cell apoptosis and necrosis from the transplanted tissue can explain the high levels of male-derived ccfDNA in the recipient's circulation.

Previous studies based on stem cell and liver transplant recipients have shown that plasma ccfDNA, although predominantly hematopoietic in origin, can also derive from transplanted organs and contributes significantly to the ccfDNA. In the blood of heart transplant recipients significant increased levels of ccfDNA of the donor's genome were observed, indicative of acute cellular rejection (6). Similarly, Gadi *et al.* report that donor DNA concentrations based on HLA-specific quantitative PCR assays, were significantly higher in serum of patients with pancreas-kidney rejection compared to those who had not (7). Findings of comparable performance of plasma cell-free donor derived DNA (cfdDNA) testing to the results obtained from invasive biopsy, creates several possibilities for exploring the role of cfdDNA as a noninvasive early rejection marker and to assess graft integrity (8) (9) (10).

A fetal sex discrepancy following organ transplant has been observed once previously (11). Considering that organ transplants can shed relatively large amounts of donor cell-free DNA in the blood stream, the proportion of transplant derived DNA fragments would be high and reduce the overall fraction of placental derived DNA in cfDNA. As a consequence, a fetal trisomy would be masked by the larger fraction of 'normal' diploid chromosomes which would impair the accuracy of fetal trisomy detection.

NIPT as a screening tool for fetal aneuploidies and fetal sex determination may not always be reliable in cases with previous history of organ transplantation, and therefore it should always be reported prior to testing. This case also demonstrates that careful follow-up of discordant test results is essential to provide appropriate counselling. Finally, with novel algorithms taking into account the origin of the cell-free DNA and advanced approaches to measure fetal fraction in combination with improved algorithms for aneuploidy detection, it may well be possible to identify and exclude analysis of graft-derived cell-free DNA and avoid misdiagnoses.

What's already known about this topic ?

NIPT is overall a very accurate predictor of the sex of the fetus during pregnancy. Inaccurate sex determination can usually be attributed to technical mistakes and exceptional cases of sex reversal. In transplant recipients, circulating cell-free DNA found in plasma contains graft-derived DNA belonging to the donor's genome.

What does this study add ?

Discordant results between NIPT and ultrasound screening may occur in organ transplant recipients following sequencing of plasma cell-free DNA. Graft-derived cell-free DNA released into the maternal circulation is another rare cause of atypical genomic profiles identified by NIPT.

1. Vermeesch JR, Voet T, Devriendt K. Prenatal and pre-implantation genetic diagnosis. Nat Rev Genet [Internet]. 2016;17(10):643–56. Available from: http://www.nature.com/doifinder/10.1038/nrg.2016.97

2. Bianchi DW, Chudova D, Sehnert AJ, Bhatt S, Murray K, Prosen TL, et al. Noninvasive Prenatal Testing and Incidental Detection of Occult Maternal Malignancies. Jama [Internet]. 2015;314(2):162–9. Available from: http://jama.jamanetwork.com/article.aspx?articleid=2389341%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/26168314

3. Schuring-Blom H, Lichtenbelt K, van Galen K, Elferink M, Weiss M, Vermeesch JR, et al. Maternal vitamin B12 deficiency and abnormal cell-free DNA results in pregnancy. Prenat Diagn. 2016;36(8):790–3.

4. Bayindir B, Dehaspe L, Brison N, Brady P, Ardui S, Kammoun M, et al. Noninvasive prenatal testing using a novel analysis pipeline to screen for all autosomal fetal aneuploidies improves pregnancy management. Eur J Hum Genet [Internet]. 2015;23(10):1286–93. Available from: http://dx.doi.org/10.1038/ejhg.2014.282

5. Kim SK, Hannum G, Geis J, Tynan J, Hogg G, Zhao C, et al. Determination of fetal DNA fraction from the plasma of pregnant women using sequence read counts. Prenat Diagn. 2015;35(8):810–5.

6. De Vlaminck I, Valantine HA, Snyder TM, Strehl C, Cohen G, Luikart H, et al. Circulating cell-free DNA enables noninvasive diagnosis of heart transplant rejection. Sci Transl Med [Internet]. 2014;6(241):241ra77. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4326260&tool=pmcentrez&rendertype=abstract

7. Gadi VK, Nelson JL, Boespflug ND, Guthrie KA, Kuhr CS. Soluble donor DNA concentrations in recipient serum correlate with pancreas-kidney rejection. Clin Chem. 2006;52(3):379–82.

8. De Vlaminck I, Martin L, Kertesz M, Patel K, Kowarsky M, Strehl C, et al. Noninvasive monitoring of infection and rejection after lung transplantation. Proc Natl Acad Sci [Internet]. 2015;112(43):13336–41. Available from: http://www.pnas.org/lookup/doi/10.1073/pnas.1517494112

9. Schütz E, Fischer A, Beck J, Harden M, Koch M, Wuensch T, et al. Graft-derived cell-free DNA, a noninvasive early rejection and graft damage marker in liver transplantation: A prospective, observational, multicenter cohort study. PLOS Med [Internet]. 2017;14(4):e1002286. Available from: http://dx.plos.org/10.1371/journal.pmed.1002286

10. Snyder TM, Khush KK, Valantine HA, Quake SR. Universal noninvasive detection of solid organ transplant rejection. Proc Natl Acad Sci U S A [Internet]. 2011;108(15):6229–34. Available from: http://www.pnas.org.ezproxy1.lib.asu.edu/content/108/15/6229.short

11. Neufeld-Kaiser WA, Cheng EY, Liu YJ. Positive predictive value of non-invasive prenatal screening for fetal chromosome disorders using cell-free DNA in maternal serum: independent clinical experience of a tertiary referral center. BMC Med [Internet]. 2015;13(1):129. Available from: http://www.biomedcentral.com/1741-7015/13/129

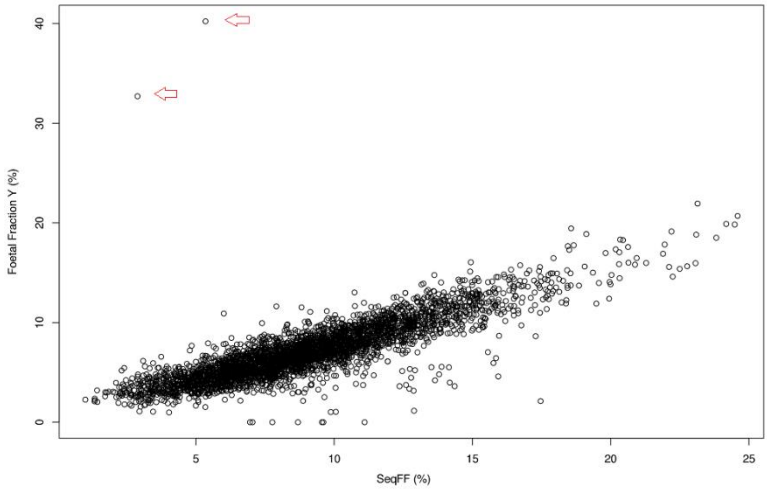

Figure 1

Chromosome Y based estimation of fetal fraction (Y-axis) was plotted against the SeqFF (X-axis). A high correlation can be observed between chromosome Y fetal fraction and the SeqFF for 4000 male pregnancies. The two outliers in fetal DNA fraction (arrows) are two successive samples from the same pregnant woman with liver transplant.