Sex chromosome aneuploidy screening in a general population

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Abstract

Cell-free fetal DNA testing is being used in parallel or in contingency screening as part of the first trimester screen. The test has high sensitivity and specificity for the trisomies 21, 18 and 13. The test also offers the option of assessing sex chromosome aneuploidies (SCA) which are recognised to be the next most common group of aneuploidies in the live birth population. Companies that offer the sex chromosome assessment report an accuracy rate of above 99% and a significant number of high-risk results have been detected in a multi-site Australian ultrasound practice. A high proportion of these women underwent prenatal testing to further assess the sex chromosomes. This study reports the results of these invasive investigations and results show that many of the high-risk SCA results appear to be false positives. This study reports the clinical experience of cell-free fetal DNA (cfDNA) testing with regard to sex chromosome aneuploidies in singleton pregnancies for a multi-site Sydney specialist O&G Ultrasound practice.

Keywords: cell-free fetal DNA test, NIPT, screening, sex chromosome.

Aim

To review the clinical experience of cell-free fetal DNA (cfDNA) testing with regard to sex chromosome aneuploidies in singleton pregnancies for a multi-site Sydney specialist O&G Ultrasound practice.

Background

Screening for trisomy 21 (T21) by the analysis of cell-free fetal DNA in the maternal circulation has been shown to be superior to that of traditional screening methods such a nuchal translucency testing with a higher detection rate and a lower false-positive rate.[1] In addition to autosomal variants, cell-free fetal DNA testing can be offered for sex chromosome aneuploidy.[2]

In a recent meta-analysis, the detection rate of monosomy X varied between 67% and 100% and the false-positive rate varied between 0% and 0.52%. For sex chromosome aneuploidies for other than monosomy X, the pooled detection rate was 93% with a false-positive rate of 0.14%.[3,4]

Our practice covers six Sydney sites located in areas of high socioeconomic status, older maternal age and high IVF rates.[5,6] The majority of patients have private obstetric care for their pregnancies. The remainder of pregnancies are cared for by GP shared antenatal care, team- or hospital-based midwifery models. Consequently, given the circumstances of care, there is a high rate of cell-free fetal DNA testing in our population.

Our practice currently offers three cell-free fetal DNA tests. All examine the trisomies 21, 18 and 13. Harmony (Ariosa technologies, San Jose, CA, USA) offers the option of both gender testing and an 'opt in' sex chromosome aneuploidy panel. This is different from Panorama (Natera, San Carlos, CA, USA) and the Genesyte (Genea, Sydney, Australia, operating the Illumina platform). Both Panorama and Genesyte perform assessment of sex chromosome aneuploidy on each sample without prior approval or as requested by patients and report when a high-risk result is obtained.

In addition, Natera also offer a microdeletion screen that includes 22q11.2 deletion syndrome, present in 1:2000 of the population with high phenotypic variability. Discussion of the accuracy or clinical utility of these microdeletion panels is beyond the scope of this study.

Harmony has constituted the largest group of tests in our practise. Given the 'opt-in' method Harmony employs when it comes to SCA reporting women are provided with pre-test counselling regarding sex chromosome aneuploidy. To be able to provide accurate information during the pre-test counselling,

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we reviewed 2 years of Harmony data and outcomes for patients. Our findings are presented in this brief report.

Method

Data from each cfDNA request and the outcome of each test is recorded at our practice. Data from October 2013 to October 2015 were reviewed. Where a high-risk result for a sex chromosome aneuploidy was received we reviewed our medical records for each patient and documented the number of women who had prenatal testing and the karyotypes following prenatal testing. These data are presented in this report.

Results

During the outlined time period 3280 Ariosa Diagnostics 'Harmony' cell-free fetal DNA tests were requested and reported. From this total sample 67 (2%) were twin pregnancies with no gender information reported by Ariosa. Of the remaining 3213 singleton pregnancies, 398 (12%) women did not want X and Y chromosome analysis; 314 (10%) women requested gender information only; and 2501 (78%) requested full X and Y chromosome analysis (see Figure 1).

A result for sex chromosome assessment was not obtained in seven cases (0.28%). The majority of the sample received a sex chromosome assessment reporting no abnormality detected (N = 2471; 98.8%; 47.5% females; 52.5% males). The remaining 23 women (0.92%) received a high risk-result (see Table 1).

Eight couples decided not to undertake invasive testing following counselling. Invasive testing was performed in the other 15 cases, amniocentesis in 14 cases and chorionic villus sampling in 1 case. A sex chromosome anomaly was confirmed in only 2 of 15 cases (13%).

Discussion

Both pre-test and post-test counselling for sex chromosomes is important. Should the result be a low-risk result, careful ultrasound examination of the fetus is still recommended at this

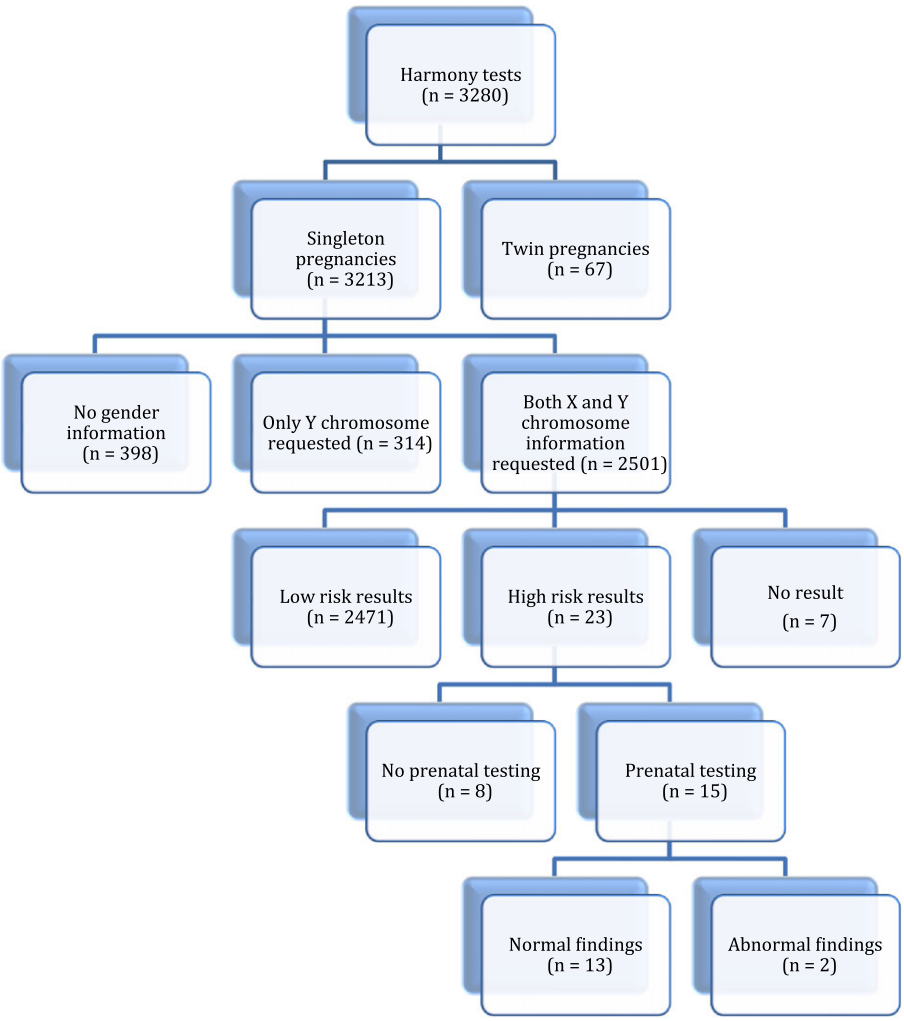

Figure 1: Outline of the Samples Test Results and Outcome of Prenatal Testing.

Table 1: Description of high-risk results and outcome following prenatal testing.

Harmony XY Analysis Result	Prenatal Diagnostic Test	Final Result
XO	Amniocentesis	46,XX
XO	Amniocentesis	46,XX
XO	Amniocentesis	46,XY
XO	Amniocentesis	46,XX
XO	Amniocentesis	Majority cell line 45,X
XO	Amniocentesis	46,XX
XO	Amniocentesis	46,XX
XO	Amniocentesis	46,XX
XO	Amniocentesis	46,XX
XXX	No	N/A
XXX	No	N/A
XXX	No	N/A
XXX	Amniocentesis	46,XX
XXY	No	N/A
XXY	Amniocentesis	46,XY
XXY	Amniocentesis	46,XY
XXY	Amniocentesis	46,XY
XXY	No	N/A
XXY	No	N/A
XYY	No	N/A
XYY	CVS	46,XY
XYY	Amniocentesis	XYY
XYY	No	N/A

practice. The presence of fetal anomalies may reveal another chromosomal or genetic anomaly which may not be tested by the cell-free fetal DNA test, or represent a false negative. Use of the cell-free fetal DNA test in the absence of ultrasonographic assessment would potentially miss significant anomalies which would warrant further investigation.

Counselling following a high-risk result for sex chromosome aneuploidy on cfDNA is additionally important for several reasons. Cases of sex chromosome aneuploidy are often non-fatal, without physical or intellectual disability. In eight of our cases, couples elected not to proceed to confirmatory invasive testing following genetic counselling regarding the results. This suggests that although they wished to be informed about a potential sex chromosome issue, they would not act on the information even if the result was confirmed.

The test assumes a normal maternal karyotype. Having a high-risk result for a sex chromosome aneuploidy relies on the assumption that under-representation and over-representation of cell-free DNA is fetal in origin. There are several reports suggesting this is not always the case. McNamara and colleagues[7] reported a series of three cases in which maternal sex chromosome aneuploidy resulted in false-positive results. There have also been reports of discordance of fetal sex on cfDNA and ultrasound and karyotyping and possible causes including co-twin demise, maternal transplant from a male donor and ambiguous genitalia have been presented.[8] Pre-test counselling should therefore include a discussion regarding limitations of cell-free fetal DNA testing.

Sex chromosome aneuploidies suggested in cfDNA should be treated with caution and invasive diagnostic testing should be performed particularly if termination of pregnancy is to be considered. In this series 87% of those cases which underwent invasive testing were false positives. It appears that the detection of sex chromosome aneuploidies on cfDNA is not as accurate at that for common aneuploidies with many of these results being false positives.

Women who wish to test for SCA on cfDNA need adequate pre-test counselling about the high false-positive rate and the potential for generating additional anxiety and possible path to diagnostic testing. Even in experienced hands amniocentesis is associated with a risk of miscarriage. Hence, the desire to have karyotypic information may result in unintended fetal loss.

This analysis has only discussed results from a single-cell-free fetal DNA test, being the Harmony test (Ariosa). The accuracy of sex chromosome aneuploidy assessment may differ across different platforms. At this practice clinicians operate under the principle of patients making informed decisions about including sex chromosome aneuploidy assessment to their cfDNA tests. This involves discussion about the seemingly high false-positive rate and also the variable phenotypes of the conditions.

Screening for common disorders such as Trisomy 21 is well established and meets the Wilson and Jungner criteria for a screening test (1968).[9] The first item in Wilson and Jungner checklist is 'The condition sought should be an important health problem'. It is much less clear if it could be claimed that all of the sex chromosome aneuploidies could be classified as an important health problem, particularly in the context of our results where 86% of women who has an invasive procedure during pregnancy returned a normal fetal karyotype. It remains to be seen whether these tests meet the modified WHO criteria for utility in the general population and we welcome open discourse between professional bodies to make this clearer over time. Our aim is to avoid situations where the ability to screen for rare genetic conditions as part of routine screening outpaces professional and patient comprehension.[10]

Conclusion

Although cell-free fetal DNA testing continues to be highly sensitive and specific for the most common aneuploidies of T21, 18 and 13, the screen-positive rate for the sex chromosome aneuploidies is approximately 1%. High-risk results on cfDNA regarding sex chromosome aneuploidy should be treated with caution as many appear to be false positives. Pre-test counselling to review the limitations of screening for sex chromosome aneuploidy is important for women to make and provide informed consent.

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