



## THE CLINICAL APPLICATION OF RAPID ANEUPLOIDY TESTING IN PRENATAL DIAGNOSIS

Dr. Wing-Cheong Leung

Division of Maternal Fetal Medicine, Department of Obstetrics & Gynaecology, Kwong Wah Hospital, Kowloon West Cluster, Hong Kong

### Introduction

The most frequent fetal chromosomal abnormalities involve the autosomes 21, 18, 13 and sex chromosomes X and Y. Aneuploidy or alterations in number of copies of these chromosomes, including Trisomy 21 (Down syndrome), Trisomy 18 (Edwards syndrome), Trisomy 13 (Patau syndrome), 45,X (Turner syndrome), 47,XXY (Klinefelter syndrome) and Triploidy (presence of three copies of each chromosome), account for more than 80% of clinically significant chromosomal abnormalities diagnosed in the prenatal period.

The traditional standard method for prenatal diagnosis of these common aneuploidies involves analysis of banded metaphase chromosomes from cultured amniotic fluid cells (amniocentesis) or chorionic villi (chorionic villous sampling). It is known as karyotyping. All the 23 pairs of chromosomes are examined. Besides aneuploidy, a wide range of chromosomal abnormalities can be identified, including chromosomal rearrangements such as translocations and inversions that may be balanced or unbalanced. Traditional karyotyping is labour intensive and results are not usually available for two weeks or more. Advances in molecular diagnostics, using either fluorescence in situ hybridization (FISH) with chromosome specific DNA probes or quantitative fluorescence-polymerase chain reaction (QF-PCR) with chromosome specific small tandem repeat markers, can be applied to diagnose these common aneuploidies within one to two days. The sensitivity and specificity of FISH and QF-PCR, collectively described as rapid aneuploidy testing (RAT), have been demonstrated in a number of large-scale studies. They compare favorably with traditional karyotyping for the diagnosis of the common aneuploidies. Unlike karyotyping, these technologies will only allow the identification of the chromosomal abnormalities that are specifically being sought.

The challenge now is how to apply RAT clinically in the most cost-effective manner. There has been a hot debate in this area of prenatal diagnosis in recent years. If RAT is being used to give a preliminary rapid result for the common aneuploidies as an adjunct to karyotyping, it

would clearly increase the cost of prenatal diagnosis. On one hand, if the indication for prenatal diagnosis is an increased risk of Down syndrome, such as positive screening test or advanced maternal age, karyotyping could be effectively replaced by RAT. On the other hand, certain chromosomal abnormalities, although of a small number and might not be clinically significant, would be missed.<sup>1</sup>

### Fluorescence in situ hybridization (FISH)<sup>2-6</sup>

FISH involves hybridization of selected chromosome specific DNA sequences that have been labeled with fluorescent dyes to chromosome preparations. The fluorescently labeled sequences stick to the corresponding DNA of the chromosomes and can be visualized under the microscope. Normal samples are expected to show two dots per cell nucleus, whereas those that are trisomic will show three dots. Between 50 and 100 cells are usually analysed to allow for low-level background and signal overlay that can occur during FISH procedures.

### Quantitative fluorescence-polymerase chain reaction (QF-PCR)<sup>7-12</sup>

QF-PCR involves the amplification of chromosome-specific repeated DNA sequences known as small tandem repeats (STRs). STRs are stable and polymorphic, varying in length between subjects, depending on the number of times the tri-, tetra- or penta-nucleotides are repeated. The sample DNA from amniotic fluid or chorionic villi is amplified by PCR using fluorescent primers so that products can be visualized and quantified as peak areas of the respective repeat lengths using an automated DNA sequencer with the gene-scan software. DNA amplified from normal subjects who are heterozygous (having alleles of different STR lengths) will show two peaks with the same area. DNA amplified from subjects who are trisomic will show either an extra peak (being triallelic) with the same area, or only two peaks (being diallelic), one of them twice as large as the other. The

number and variety of STR markers multiplexed together differ between assays and determine assay efficiency.

### QF-PCR versus FISH<sup>13</sup>

1. The risk for misdiagnosis of the common aneuploidies by either FISH or QF-PCR is relatively small.
2. FISH is more labour intensive than QF-PCR.
3. Maternal cell contamination may constitute more of a problem with FISH than with QF-PCR.
4. Fetal mosaicism remains a challenge by either method.

### Maternal cell contamination

Maternal cell contamination of fetal material may arise during any of the invasive prenatal sampling procedures. With FISH, mixture of maternal and fetal XY cells are readily detectable but maternal and fetal XX cells are indistinguishable. Using QF-PCR, maternal cell contamination is readily detected by the characteristic pattern with extra alleles or skewed ratios between peaks for all target chromosomes.<sup>14</sup>

### Fetal mosaicism

It refers to the occurrence of more than one cell line containing different chromosomal composition. Using FISH, examination of a large number of interphase nuclei can facilitate the diagnosis of mosaicism. Low-grade mosaicism is likely to be missed. QF-PCR is also capable of identifying autosomal mosaicism, when the trisomy is present in more than 15%.<sup>15</sup>

### RAT versus karyotyping<sup>6,12,16-27</sup>

When the results of RAT and karyotyping are compared, they can be divided into four groups: concordant normal, concordant abnormal, false positive and false negative.

#### 1. *Concordant normal*

Concordant normal RAT with normal karyotyping results are the most common scenario (96.6%). In other words, karyotyping does not give additional information to RAT in the great majority of women having prenatal tests such as amniocentesis or chorionic villous sampling. Most of these prenatal tests are performed because of positive Down screening test result or advanced maternal age. The major advantage of RAT in this great majority group is that the rapid normal result within one to two days can relieve the anxiety of the women and their partners much earlier than when they have to wait for the karyotyping result which can take up to three weeks. However, a randomized controlled trial has suggested that this advantage of RAT may be lost if the women still have to wait for the karyotyping result.<sup>28</sup> One possible explanation is that the woman, although being told that the RAT result is normal, is still having a significant degree of anxiety as she has to wait for the 'confirmation' by the karyotyping result. This anxiety

can be alleviated if the RAT report is considered to be final.

#### 2. *Concordant abnormal*

Concordant abnormal RAT with the same abnormal karyotyping results accounted for 2.4% of the results. In this group of women, we can again see that karyotyping does not give additional information to RAT. At present, many centers<sup>29</sup>, including ours, are already offering the option of termination of pregnancy based on abnormal RAT results without waiting for the karyotyping confirmation. In this regard, RAT can again be considered as a stand-alone test.

#### 3. *False positive*

This refers to the scenario when the RAT result is falsely abnormal in the presence of a normal karyotype which can potentially result in termination of a normal pregnancy. It has occurred in one<sup>17</sup> out of the 233,496 cases in the twelve recent studies<sup>6,12,17-26</sup> that have been reviewed. This case was a 45,X/46,XX mosaic predicted by FISH but 46,XX found on karyotyping. The author gave the explanation that it was the result of extreme variation in size of the alpha satellite centromeric region of the X chromosome. This was a rare occurrence and manifested as very low signal strengths on fluorescent microscopy. When very low signal strengths are encountered, FISH analysis is repeated with alternative probes or reliance should be placed on karyotyping. Nevertheless, the absence of false-positive result is a basic pre-requisite if RAT is to be used as a stand-alone test.

#### 4. *False negative*

This refers to the scenario when the RAT result is normal but the karyotyping result is abnormal. It accounted for 1.0% of the results. These 'abnormal' karyotypes could be divided into two groups: clinically not significant (0.6%) and clinically significant (0.4%). The clinically not significant group refers to those balanced translocations or other chromosomal rearrangements of known familial origin. The clinically significant group includes rare aneuploidies (other than chromosomes 21, 18, 13, X and Y), unbalanced translocations or other chromosomal rearrangements, balanced de novo translocations and marker chromosomes. Strictly speaking, they should not be considered as false negative RAT results because the FISH or QF-PCR that had been used could only detect the common aneuploidies (chromosomes 21, 18, 13, X and Y). However, this is the group of chromosomal abnormalities that would be missed if RAT is to replace karyotyping.

Some, but not all, of the clinically significant chromosomal abnormalities that cannot be detected by RAT would have evidence of major structural abnormalities or soft markers of aneuploidy on ultrasound examination.<sup>18,23</sup> A policy offering RAT to all patients, but restricting karyotyping to cases with ultrasound anomalies, would reduce the number of karyotyping by 70%, but maintain a 95% detection rate for all clinically important chromosomal abnormalities.<sup>26</sup> Chitty et al has also shown that, after first trimester screening for trisomy 21, a policy of RAT for all samples

and karyotyping only if the fetal nuchal translucency thickness is increased would identify 99% of the clinically significant chromosomal abnormalities.<sup>30</sup>

Nevertheless, there would be some clinically significant false negative RAT chromosomal abnormalities that do not have ultrasound abnormalities. It is important to note that the clinical significance of these chromosomal abnormalities, in particular, balanced de novo translocations and marker chromosomes, is very different from that of Trisomy 21, 18 or 13.<sup>31</sup> The risk of an adverse clinical outcome (including impaired intellectual development, learning difficulties and physical abnormalities) for this cohort of chromosomal abnormalities varies from 5% to 15%.<sup>31</sup> Identification of these balanced de novo translocations and marker chromosomes in the absence of ultrasound abnormalities often poses difficult counseling issues, may not be in the best interest of the parents or the fetus, and presents a difficult choice regarding the continuation of the pregnancy.

In addition to the above clinically significant chromosomal abnormalities, false negative RAT results also include those balanced translocations or other chromosomal rearrangements of known familial origin. Although they are not clinically significant, they have the potential to result in unbalanced products in future pregnancies.

### Cost-effectiveness

One of the advantages of using RAT as a stand-alone test is cost-saving. Instead of adding the cost of RAT on top of that of karyotyping, the cost of the latter can be saved by the RAT alone approach. In the age of ever-escalating cost in the provision of health care, especially in a government-funded public medical care system, the savings can be redirected to enhance existing or fund new programmes, thus maximizing the effect of limited resources. Grimshaw et al<sup>25</sup> has conducted a cost-effectiveness analysis on five testing policies:

1. RAT and karyotyping for all women
2. RAT as a replacement for karyotyping
3. RAT for all plus karyotyping for high-risk women
4. Karyotyping for all plus RAT for high-risk women
5. Parental choice plus karyotyping for high-risk women

Policies 2, 3 and 5 are found to be more cost-effective than karyotyping based on the cost per case (chromosomal abnormality) detected.

### Ethical issue

If RAT is to replace karyotyping for indications such as positive Down screening or advanced maternal age when no ultrasound abnormality is detected, one has to accept the risk that for every 1000 amniocenteses performed, up to four potentially clinical significant chromosomal abnormalities may be missed. Some people may argue from an ethical point of view that since amniocentesis is an invasive procedure that carries a small risk of miscarriage, we should maximize the information that can be obtained

by performing karyotyping to examine all the 23 pairs of chromosomes. However, even the performance of karyotyping doesn't mean that the information is maximized e.g. microdeletions and common mutations are not tested. We must also realize that Down screening is a programme designed to detect primarily Down syndrome and therefore in principle, follow up test with RAT alone would have realistically fulfilled the expectations of the couples and obstetricians (targeted testing). A recent conjoint analysis study showed that women would prefer simple information on just knowing whether the fetus has Down syndrome as long as the result is received 6 days sooner than karyotyping.<sup>32</sup>

### Clinical application of RAT in Hong Kong and mainland China

RAT for prenatal diagnosis is widely available in Hong Kong, both in public and private sectors. The major advantages of RAT include fast reporting within 24 to 48 hours and earlier relief of anxiety. Like in other developed cities, RAT is likely to remain as an adjunct to karyotyping in Hong Kong, although the option of termination of pregnancy can usually be offered based on abnormal RAT results without waiting for the karyotyping confirmation.

The best opportunity when RAT can be applied as a cost-effective stand alone test is when a national screening program for Down's syndrome is to be launched in a country, like mainland China, with its large population and thus high-throughput of samples and where traditional karyotyping is not very deep seated or widely available.

### Links

Prenatal Diagnosis and Counselling Service:

- Individual HA hospitals  
Hospital Authority: [www.ha.org.hk](http://www.ha.org.hk)
- The University of Hong Kong  
[www.hku.hk/obsgyn/clinicalServ/prenatalDiag\\_Conse.htm](http://www.hku.hk/obsgyn/clinicalServ/prenatalDiag_Conse.htm)
- The Chinese University of Hong Kong  
<http://www.fetalmedicine.hk/en/mainmenu.asp>

### References

1. Caine A, Maltby AE, Parkin CA, Waters JJ, Crolla JA, for the UK Association of Clinical Cytogeneticists (ACC). Prenatal detection of Down's syndrome by rapid aneuploidy testing for chromosomes 13, 18, and 21 by FISH or PCR without a full karyotype: a cytogenetic risk assessment. *Lancet* 2005;366:123-8.
2. Ward BE, Gersen SL, Carelli MP, et al. Rapid prenatal diagnosis of chromosomal aneuploidies by fluorescence in situ hybridization: clinical experience with 4,500 specimens. *Am J Hum Genet* 1993;52:854-65.
3. Eiben B, Trawicki W, Hammans W, Goebel R, Pruggmayer M, Epplen JT. Rapid prenatal diagnosis of aneuploidies in uncultured amniocytes by fluorescence in situ hybridization. Evaluation of >3,000 cases. *Fetal Diagn Ther* 1999;14:193-7.
4. Estabrooks LL, Sapeta M, Lytle C, et al. Prenatal interphase FISH using the AneuVysion probe set in over 10,000

- samples. *Am J Hum Genet* 1999;65:A162.
5. Pergament E, Chen PX, Thangavelu M, Fiddler M. The clinical application of interphase FISH in prenatal diagnosis. *Prenat Diagn* 2000;20:215-20.
  6. Witters I, Devriendt K, Legius E, et al. Rapid prenatal diagnosis of trisomy 21 in 5049 consecutive uncultured amniotic fluid samples by fluorescence in situ hybridization (FISH). *Prenat Diagn* 2002;22:29-33.
  7. Pertl B, Yau SC, Sherlock J, Davies AF, Mathew CG, Adinolfi M. Rapid molecular method for prenatal detection of Down's syndrome. *Lancet* 1994;343:1197-8.
  8. Verma L, Macdonald F, Leedham P, McConachie M, Dhanjal S, Hulten M. Rapid and simple prenatal DNA diagnosis of Down's syndrome. *Lancet* 1998;352:9-12.
  9. Levett LJ, Liddle S, Meredith R. A large-scale evaluation of amnio-PCR for the rapid prenatal diagnosis of fetal trisomy. *Ultrasound Obstet Gynecol* 2001;17:115-8.
  10. Cirigliano V, Lewin P, Szpiro-Tapias S, Fuster C, Adinolfi M. Assessment of new markers for the rapid detection of aneuploidies by quantitative fluorescent PCR (QF-PCR). *Ann Hum Genet* 2001;65:421-7.
  11. Mann K, Fox SP, Abbs SJ, et al. Development and implementation of a new rapid aneuploidy diagnostic service within the UK National Health Service and implications for the future of prenatal diagnosis. *Lancet* 2001;358:1057-61.
  12. Cirigliano V, Voglino G, Canadas MP, et al. Rapid prenatal diagnosis of common chromosome aneuploidies by QF-PCR. Assessment on 18,000 consecutive clinical samples. *Mol Hum Reprod* 2004;10:839-46.
  13. Hulten MA, Dhanjal S, Pertl B. Rapid and simple prenatal diagnosis of common chromosome disorders: advantages and disadvantages of the molecular methods FISH and QF-PCR. *Reproduction* 2003;126:279-97.
  14. Stojilkovic-Mikic T, Mann K, Docherty Z, Mackie Ogilvie C. Maternal cell contamination of prenatal samples assessed by QF-PCR genotyping. *Prenat Diagn* 2005;25:79-83.
  15. Donaghue C, Mann K, Docherty Z, Ogilvie CM. Detection of mosaicism for primary trisomies in prenatal samples by QF-PCR and karyotype analysis. *Prenat Diagn* 2005;25:65-72.
  16. Leung WC, Lau ET, Lao TT, Tang MHY. Rapid aneuploidy screening (FISH or QF-PCR): the changing scene in prenatal diagnosis? *Expert Rev Mol Diagn* 2004;4:333-7.
  17. Thilaganathan B, Sairam S, Ballard T, Peterson C, Meredith R. Effectiveness of prenatal chromosomal analysis using multicolour fluorescent in situ hybridization. *BJOG* 2000;107:262-6.
  18. Thein AT, Abdel-Fattah SA, Kyle PM, Soothill PW. An assessment of the use of interphase FISH with chromosome specific probes as an alternative to cytogenetics in prenatal diagnosis. *Prenat Diagn* 2000;20:275-80.
  19. Ryall RG, Callen D, Cocciolone R, et al. Karyotypes found in the population declared at increased risk of Down syndrome following maternal serum screening. *Prenat Diagn* 2001;21:553-7.
  20. Leung WC, Lau ET, Lao TT, Tang MHY. Can amniopolymerase chain reaction alone replace conventional cytogenetic study for women with positive biochemical screening for fetal Down syndrome? *Obstet Gynecol* 2003;101:856-61.
  21. Evans MI, Henry GP, Miller WA, et al. International, collaborative assessment of 146,000 prenatal karyotypes: expected limitations if only chromosome-specific probes and fluorescent in-situ hybridization are used. *Hum Reprod* 1999;14:1213-6.
  22. Lewin P, Kleinfinger P, Bazin A, Mossafa H, Szpiro-Tapia S. Defining the efficiency of fluorescence in situ hybridization on uncultured amniocytes on a retrospective cohort of 27,407 prenatal diagnoses. *Prenat Diagn* 2000;20:1-6.
  23. Leung WC, Chitayat D, Seaward G, et al. Role of amniotic fluid interphase fluorescence in situ hybridization (FISH) analysis in patient management. *Prenat Diagn* 2001;21:327-32.
  24. Homer J, Bhatt S, Huang B, Thangavelu M. Residual risk for cytogenetic abnormalities after prenatal diagnosis by interphase fluorescence in situ hybridization (FISH). *Prenat Diagn* 2003;23:566-71.
  25. Grimshaw GM, Szczepura A, Hulten M, et al. Evaluation of molecular tests for prenatal diagnosis of chromosome abnormalities. *Health Technol Assess* 2003;7:1-146.
  26. Leung WC, Waters JJ, Chitty L. Prenatal diagnosis by rapid aneuploidy detection and karyotyping: a prospective study of the role of ultrasound in 1589 second-trimester amniocenteses. *Prenat Diagn* 2004;24:790-5.
  27. Leung WC, Lao TT. Rapid aneuploidy testing, traditional karyotyping, or both? *Lancet* 2005;366:97-8.
  28. Leung WC, Lam YH, Wong Y, Lau ET, Tang MHY. The effect of fast reporting by amnio-PCR on anxiety levels in women with positive biochemical screening for Down syndrome - a randomized controlled trial. *Prenat Diagn* 2002;22:256-9.
  29. Adinolfi M, Sherlock J. Prenatal detection of chromosome disorders by QF-PCR. *Lancet* 2001;358:1030-1.
  30. Chitty LS, Kagan KO, Molina FS, Waters JJ, Nicolaides KH. Fetal nuchal translucency scan and early prenatal diagnosis of chromosomal abnormalities by rapid aneuploidy screening: observational study. *BMJ* 2006;332:452-5.
  31. Warburton D. De novo balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints. *Am J Hum Genet* 1991;49:995-1013.
  32. Ryan M, Diack J, Watson V, Smith N. Rapid prenatal diagnostic testing for Down syndrome only or longer wait for full karyotype: the views of pregnant women. *Prenat Diagn* 2005;25:1206-11.