

## Rapid aneuploidy testing versus traditional karyotyping: is it better to know more?

Chromosomal anomalies remain the commonest cause of mental retardation and congenital malformation. Traditionally, the prenatal diagnosis of chromosomal aberration relies on cytogenetic analysis of the cells obtained from amniocentesis or chorionic villus sampling (CVS). First, the amniotic fluid or chorionic villi samples need to be cultured for 14 days or more before adequate foetal cells can be harvested. Then the cells are spread on slides and stained. Finally, the copy number and the structural arrangement of each chromosome are examined. This procedure, called karyotyping, has for many years been the gold standard for prenatal diagnosis of chromosomal anomalies in developed countries and in Hong Kong.

The main drawback of the traditional karyotyping is the delay in obtaining the results. It has been shown that the women's anxiety level remained high following the invasive prenatal diagnosis test until they know the result is normal.<sup>1</sup> Obviously, both the women and the obstetricians would like to have an earlier report, if possible. With the advent of molecular genetics techniques, accurate diagnosis of the commonest numerical chromosomal abnormalities (aneuploidies) has become feasible within 1 or 2 days.<sup>2</sup> Techniques commonly employed include fluorescence in-situ hybridisation or quantitative fluorescence-polymerase chain reaction. The aneuploidies in question include: trisomies 13, 18, or 21, and the sex chromosomal abnormalities. This rapid aneuploidy testing (RAT), however, has its own limitations. In contrast to the traditional karyotyping, it only allows identification of the numerical chromosomal abnormalities that are specifically targeted for and will miss structural anomalies such as translocations, inversions, and marker chromosomes.

Currently, most prenatal diagnosis units offer either traditional karyotyping only or RAT in addition to karyotyping. If cost is not an issue, the latter appears ideal. However, in the publicly funded system, money spent in one area means deprivation in another. Prioritisation is essential. In 1997, the National Health Service (NHS) Health Technology Assessment (HTA) Programme in the United Kingdom evaluated the cost of the RAT as well as traditional karyotyping and came to a conclusion that RAT was less expensive than karyotyping.<sup>2</sup> Hence, there has been a suggestion that RAT could replace traditional karyotyping, given that the majority of clinically significant chromosomal abnormalities can be picked up by this technique.<sup>3</sup> However, no matter how infrequent, certain abnormal karyotypes missed by RAT could be of clinical

significance and carry potential medical, emotional, and financial consequences.<sup>4,5</sup>

In this issue of the Journal, Leung et al,<sup>6</sup> on behalf of the Working Group on Prenatal Diagnosis and Counselling of the Hospital Authority (HA), report a retrospective study on the karyotyping of 19 517 samples. The amniocentesis was performed in HA hospitals in Hong Kong between 1997 and 2002 for advanced maternal age, which was the commonest indication for the procedure during the study period. As expected, the majority of samples yielded normal results; only 1.7% were abnormal. Nearly half (47.4%) of the abnormal karyotypes would have been missed by RAT, of which only 18.9% were regarded to be of clinical significance. Viewed from another perspective, only 0.8% of all 19 517 procedures had abnormal karyotypes which would be missed by RAT and only 0.3% were of potential clinical significance. These figures are comparable to reported findings from other similar studies.<sup>4,7</sup> In a meta-analysis of 12 studies involving amniocentesis and CVS, the risk of having chromosomal anomalies missed by RAT was estimated to be 0.9% and this figure dropped to 0.4% if only those with clinically significant anomalies were considered.<sup>7</sup>

Faced with abnormal karyotypes with potential clinical significance, all obstetricians find prenatal counselling extremely difficult, unless the outcome is certain. Many with uncertain outcomes, ranging from completely normal to mental, physical or developmental disability, might not be apparent at birth. Take de-novo balanced translocations as an example. The overall prognosis is good but there remains a 6% chance of serious congenital anomalies.<sup>8</sup> Continuing the pregnancy would mean coping with the uncertainty until after the birth of the child, while a termination of pregnancy (TOP) for an over 90% chance of having a normal child is equally difficult for many women. It is uncertain whether knowing more about these aspects is beneficial to the women, the foetus, or the family.

Table 2 of Leung et al<sup>6</sup> should give us some idea about the impact of such information. Of the 63 pregnancies with abnormal karyotypes of potential clinical significance, one abnormality was major (5p- syndrome) and four others had major structural anomalies detected on ultrasound; all five mothers had TOP. It is not known whether the structural anomalies would have been picked up on a routine ultrasound, if the abnormal karyotypes were not known to the

sonographers, as the level of vigilance during scanning and the extent of antenatal ultrasound use might have differed. Another six pregnancies without ultrasound features of structural malformation were terminated as the parents could not accept the uncertainty of the clinical outcomes. If only RAT was offered, rightly or wrongly, these six pregnancies would not have been terminated.

If knowing more is not necessarily advantageous, who should decide which test to order? In the NHS HTA study,<sup>2</sup> the attitudes of patients, medical professionals, and the general public were examined on this aspect, using a questionnaire-based approach. Most obstetricians (57%), midwives (71.4%), and pregnant women (67%) preferred RAT to karyotyping, whereas the majority of the non-pregnant general public (60%) expressed a preference for karyotyping.<sup>2</sup> Clearly, there is disagreement even among each category of respondent. Offering women the autonomy to choose RAT, karyotyping, or both is unlikely to be clinically practical, because the counselling could be labour-intensive and many women would remain confused. Too many choices, like too much information, could make life difficult!

While the controversy surrounding RAT and karyotyping is likely to continue, it is worth mentioning newer technology, such as array-based comparative genomic hybridisation (aCGH). In contrast to RAT, aCGH is a comprehensive, high-resolution, genome-wide screening strategy for obtaining DNA copy number information in a single measurement. Compared with traditional karyotyping, it is rapid, less labour-intensive, and readily amendable to automation.<sup>9</sup> It is not ready for routine use yet due to the costs, but it is likely to become increasingly important and has the potential to replace traditional karyotyping in the future. Of course, the advent of molecular genetics also foresees a future involving non-invasive prenatal diagnosis.<sup>10,11</sup>

**TN Leung, MD, FRCOG**

E-mail: dannyleung@hksh.com

Maternal Fetal Medicine

Obstetrics and Gynaecology Centre

Hong Kong Sanatorium and Hospital

Happy Valley

Hong Kong

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