

The introduction of QF-PCR in prenatal diagnosis of fetal aneuploidies: time for reconsideration

Umberto Nicolini^{1,3}, Faustina Lalatta¹, Federica Natacci¹, Cristina Curcio¹
and The-Hung Bui²

¹Department of Obstetrics and Gynaecology and Medical Genetics, Ospedale V Buzzi, University of Milano, Milano, Italy and ²Karolinska Institutet, Department of Molecular Medicine, Clinical Genetics Unit, Karolinska University Hospital Solna, SE-17176 Stockholm, Sweden

³To whom correspondence should be addressed. E-mail: umberto.nicolini@unimi.it

Quantitative fluorescent polymerase chain reaction (QF-PCR) has recently entered the field of prenatal diagnosis to overcome the need to culture fetal cells, hence to allow rapid diagnosis of some selected chromosomal anomalies. We reviewed the studies on the accuracy of QF-PCR in detecting chromosomal anomalies at prenatal diagnosis. Overall, 22 504 samples have been analysed. The detection rate of aneuploidies of the selected chromosomes (13, 18 and 21, and X and Y) was 98.6% (95% confidence interval 97.8–99.3). QF-PCR might play a major role and be considered a valid alternative to the full karyotype. Being less expensive, and almost entirely automated, more women could undergo invasive prenatal diagnosis without significant increase in health expenditure. By using QF-PCR as a stand-alone test, the chances of non diagnosing the commonest, and the only chromosome anomalies which do increase in frequency with maternal age, are approximately one in 150 abnormal karyotypes, or one in 10–30 000 samples, based on the age distribution. These error rates might be deemed acceptable, although most structural chromosomal anomalies will be missed. At present, women are rarely informed about the full spectrum of the conditions which might be diagnosed via amniocentesis or chorionic villous sampling. Some of these anomalies might be acceptable, in view of their limited or uncertain clinical relevance, and decision analysis might, in the majority of cases, confine the full karyotype to selected women who have specific indications.

Key words: cytogenetics/Down's syndrome/genetic counselling/prenatal diagnosis/QF-PCR

Introduction

Valenti *et al.*, (1969) reported the first prenatal diagnosis of Down's syndrome 3 years after the achievement of amniotic cell growth by Steele and Berg (1966). For several years, the use of amniocentesis was confined to few referral centres, mainly due to the perception that the whole process of invasive prenatal diagnosis was a highly specialized task both for clinical and laboratory personnel.

More than a quarter of a century later, significant technical developments have occurred. The introduction and extensive use of real time sonography have resulted in an easier and safer approach to invasive procedures in obstetrics. The development of chorionic villous sampling (CVS) in the early 1980's has allowed anticipation of diagnosis in the first trimester (Brambati and Simoni, 1983; Spencer and Cox, 1987, Spencer and Cox, 1988; Abramsky and Rodeck, 1991; Lippman *et al.*, 1992). The application of strategies for improving cell culture and chromosome banding has expanded the number of laboratories which may perform successfully fetal chromosome analysis (Porreco

et al., 1980; Brackertz *et al.*, 1983; Tabor *et al.*, 1984; Lawce, 1986; Cheung *et al.*, 1987; Speit *et al.*, 1990). Despite these developments, the commonest indication for amniocentesis or CVS is still the same as when prenatal diagnosis was introduced, i.e. an increased risk of having a child with trisomy 21. Down's syndrome is the most frequent autosomal aneuploidy, occurring in about one in 600 newborn infants (Hook and Chambers, 1977). Without prenatal diagnosis and selective termination, trisomy 21 would account for approximately one-quarter of all cases of mental disability in children (Nielsen *et al.*, 1982).

In most developed countries the option of whether to have prenatal diagnosis is discussed as part of routine antenatal care. The introduction of first and second trimester biochemical and ultrasonographic markers of Down's syndrome and, to a lesser extent, of other chromosomal anomalies, allows a better risk estimation compared to that based on maternal age alone. However, even with the best screening strategy, a false positive rate of 5% is expected (Haddow *et al.*, 1994; Cuckle, 2000; Petticrew *et al.*, 2000). Indeed, one in 20 pregnant women in the UK

chooses to undergo amniocentesis or CVS (Hultén *et al.*, 2003) and ~117 000 fetal karyotype analyses have been performed in Italy in the year 2002, accounting for one in five pregnancies, with an increase of 30% compared to 2 years previously (Dallapiccola, 2003). Thus, the main bulk of invasive prenatal diagnosis concerns women at low risk (much less than the one in four entailed by autosomal recessive conditions), which is perceived as being significant enough to warrant the risk of miscarriage of a healthy fetus associated with invasive sampling (Kuppermann *et al.*, 2000). In these cases, fetal cells (chorionic villi, amniotic fluid cells or blood) are cultured and a full karyotype analysis is performed. This requires considerable technical expertise. Despite laboratory technique improvements and partial automation, labour costs are still significant and there is a limit to the number of analyses which an experienced technician may handle safely. In 1995, this number was estimated to be a maximum of 300 per annum by a consensus conference of the Italian Association of Medical Cytogenetics (Associazione Italiana di Citogenetica Medica (AICM), 1995).

Fetal karyotype: the gold standard of prenatal diagnosis

The standard karyotype analysis implies the study of the number and structure of the 23 chromosome pairs. The most common chromosome anomalies, those which increase in frequency with maternal age and are related to non-disjunctional errors, result in an extra copy or loss of one chromosome, either in all cells or as a mosaic state, and are easily detected (Ferguson-Smith and Yates, 1984). Error rates of 0.1–0.6% have been reported in the 1970's and early 1980's (Garver *et al.*, 1976; Hsu, 1992), with the vast majority being incorrect sex assignment resulting from either maternal cell contamination or laboratory error, but occasional missed trisomies or monosomies have also been described (Berry *et al.*, 1992; Griffiths *et al.*, 1996). Test failure, as opposed to error, arising from a failure to culture cells from the amniocytes is relatively rare at $\leq 0.5\%$ (Milunsky, 1998). Thus, the overall error and failure rate of fetal karyotyping is low, with about 4 to 14 cases per 1000 expected to experience culture failure and up to a further six cases per 1000 to be misdiagnosed (Association of Clinical Cytogeneticists, 1990).

Although high resolution banding could allow diagnosis of anomalies as small as 2 Mb ($\times 10^6$ bases), structural anomalies are routinely detected only when they involve ≥ 4 Mb. These anomalies are relatively common, since they account for ~1% of all fetal karyotypes analysed following traditional indications (maternal age, biochemical and/or ultrasonographic screening, maternal anxiety). However, they are incidental findings, because their frequency does not increase with maternal age nor current screening programmes are aimed at their detection. In other words, serendipity is the basis of diagnosis of as many as 30% of abnormal fetal karyotypes detected at prenatal diagnosis. Furthermore, balanced translocations and inversions, which are the commonest structural anomalies, are clinically irrelevant for the current pregnancies, but require genetic counselling and additional investigations which often include karyotype of the couple and high resolution fetal ultrasonography in selected cases (Jacobs, 1974; Warburton, 1982, Warburton, 1984)

To summarize the current state of affairs, a full karyotype is the gold standard of prenatal diagnosis. Yet, errors may occur

and structural chromosome anomalies may not be identified if they involve very small fragments of the chromosome. On the other hand, many of those diagnosed are clinically irrelevant. Moreover, most women who choose or are advised to undergo invasive prenatal diagnosis and fetal karyotype analysis do so with the expectation that they are at risk of aneuploidies, and in most instances of Down's syndrome, but in a third of cases the diagnosis involves anomalies of which they are not aware and for which they are not at increased risk (Warburton, 1991). However, there is a general consensus among cytogeneticists and physicians that the extra knowledge provided by full karyotype is beneficial. Some structural anomalies are indeed clinically relevant, and since banding has been introduced to facilitate chromosome recognition, no extra processing is needed generally to detect these anomalies. Although coincidental, it is generally thought that it would be gross misconduct not to pursue the opportunity to make such diagnoses.

The time needed to culture fetal cells and complete the analysis ranges from 10 to 21 days (Salk *et al.*, 1983), which is generally considered to be a psychological burden and results in late terminations following a pathological diagnosis. The shift that has occurred in some, albeit not all, countries towards first trimester screening and diagnosis of chromosome anomalies is largely due to the attempt to overcome the time factor, but the interval from sampling to complete the analysis of the full karyotype remains exceedingly long (Simoni *et al.*, 1983; Niazi *et al.*, 1981).

New techniques aimed at diagnosing fetal chromosome anomalies

In the early 1990s, fluorescence *in situ* hybridization (FISH) and, more recently, QF-PCR (quantitative fluorescent polymerase chain reaction) entered the field of prenatal diagnosis to overcome the need to culture fetal cells, hence to allow rapid diagnosis of some selected chromosomal anomalies (Von Eggeling *et al.*, 1993; Divane *et al.*, 1994; Pertl *et al.*, 1994).

FISH uses specific DNA probes labelled by incorporating chemically modified nucleotides that fluoresce directly or can be detected by binding a fluorescently tagged reporter molecule (Kuo *et al.*, 1991; Whiteman and Klinger, 1991; Divane *et al.*, 1994; Roberts *et al.*, 1999; Leung *et al.*, 2001; Tepperberg *et al.*, 2001). When the nuclei of fetal cells are analysed by fluorescent microscope, two spots, one for each of the analysed chromosomes, are visualized in normal subjects, while trisomies are revealed by the presence of an extra spot and monosomies by the absence of one spot.

QF-PCR is based on the amplification of chromosome-specific DNA sequences (STR, short tandem repeats) polymorphic in length between subjects. By means of fluorescent primers, the amplified segments can be visualized and quantified as peak areas on automated DNA scanners. Normal heterozygous subjects are expected to show two peak areas (peaks ratio 1:1) for each chromosome analysed, while trisomies are visualized either as an extra peak (triallelic subjects) or as a 2:1 ratio peak between the two areas (Adinolfi *et al.*, 1997).

FISH and QF-PCR provide rapid (within 24–48 h) diagnosis of whether there is aneuploidy of the analysed chromosomes. Although both techniques could be applied to identify all

chromosomes, only the 13, 18 and 21 pairs, as well as the sex chromosomes, are routinely hybridized. The techniques can also disclose triploidies. The obvious candidates to apply these new technologies are high risk patients (those with fetal malformations/soft markers) or late bookers. The result was, and still is, sufficient to take action if a chromosomal anomaly is thus identified, but is considered only a preliminary step while awaiting the result of full karyotype. In fact, the percentage of

chromosomal anomalies correctly identified by FISH in a high risk population could be as low as 65% (Evans *et al.*, 1994).

QF-PCR has some advantages over FISH (Cirigliano *et al.*, 2001; Hultén *et al.*, 2003). QF-PCR is feasible on fewer cells, and since the analysis can easily be automated, many samples can be processed at the same time, the whole process taking ~30 min. FISH can use commercial probes, which do not need any validation, but QF-PCR can detect maternal cell contamination,

Table I. Results of studies of prenatal diagnosis of chromosomal aneuploidies using quantitative fluorescent PCR

Reference	No. of analyses	No. of AF	No. of CVS	No. of PUBS	No. of TOP	Chromosome/ no. of markers	No. of detected trisomies/ no. of actual trisomies
Verma <i>et al.</i> , (1998)	2139	2139				21/2-3	32/33
Pertl <i>et al.</i> , (1999a)	247		247			21/4 13/2 18/3	15/15 1/1 4/5
Pertl <i>et al.</i> , (1999b)	52	52				21/4 13/3 18/3 XY/3	2/2 0/0 3/3 0/3
Schmidt <i>et al.</i> , (2000)	662	662				21/3 13/3 18/3 XY/3	5/5 0/0 4/5 5/5
Levett <i>et al.</i> , (2001)	5000	5000				21/6 13/6 18/6 X/5, Y/2	57/57 8/8 17/17 16/20
Vogliano <i>et al.</i> , (2002)	1653	1302	61	10	280	21 13 18 XY	110/110 15/15 40/40 25/25
Bili <i>et al.</i> , (2002)	1084	1020	64			21/5 13/5 18/5 XY/3	11/13 3/3 4/5 1/1
Mann <i>et al.</i> , (2003) (quoted in Hultén <i>et al.</i> , 2003)	5090					21/2-4 13/2-4 18/2-4 21	189/189 32/32 75/75 58/58
Cirigliano <i>et al.</i> , (2003) (quoted in Hultén <i>et al.</i> , 2003)	3478					13 18 21 13 18 XY	7/7 28/28 24/24 6/6 26/26 1/1
Leung <i>et al.</i> , (2003)	235	235				21/4 13/3 18/3 XY/5	26/26 2/2 8/8 6/6
Curcio <i>et al.</i> , (2003)	1277	996	281			21/4 13/3 18/3 XY/5	6/6 2/2 8/8 6/6
Andonova <i>et al.</i> , (2004)	472	426	34		12	21/4 18/2	8/8 4/4
Quaife <i>et al.</i> , (2004)	1115					21/4 13/4 18/4 XY/4	11/11 6/6 6/6 13/13
Total	22 504	11 832	687	10	292		914/927 (98.6%)

AF = Amniotic Fluid; CVS = chorionic villous sampling; PUBS = Percutaneous umbilical blood sampling; TOP = termination of pregnancy.

which cannot be disclosed by FISH in cases of female fetuses. Based on these considerations, QF-PCR is increasingly being considered and proposed as a complementary investigation or even as an alternative to conventional cytogenetic analysis in prenatal diagnosis (Grimshaw *et al.*, 2003; Ogilvie, 2003; Leung *et al.*, 2004).

Accuracy of QF-PCR

Table I displays the results of the studies reported so far and indexed on Medline (searched using a number of keywords) on the accuracy of QF-PCR in detecting chromosomal anomalies at prenatal diagnosis. Two of the 13 series were not published, but quoted as personal communications in Hultén *et al.*, (2003) and the series from our laboratory (Curcio *et al.*, 2004) was only presented at the 2004 European Human Genetics Conference. In all instances, QF-PCR was used in combination with conventional cytogenetic analysis which was considered the gold standard. QF-PCR has been applied on different fetal tissues (chorionic villi, amniotic fluid, fetal blood and fetal tissues obtained after termination). A minimum of two markers, but, more recently, multiplex PCR (as many as six markers) have been used for each of the analysed chromosomes, thus allowing further reduction in time analysis and costs compared to the initial sequential individual PCR.

Overall, 22 504 samples have been reviewed. The detection rate of aneuploidies of the selected chromosomes (13, 18 and 21, and X and Y) was 98.6% (range 86.3–100%; 95% confidence interval 97.8–99.3). Sex chromosome aneuploidies were those with the highest false negative rates (7/74), whereas there were only 3/551, 3/222 and 0/80 missed diagnoses of trisomy 21, 18 and 13 respectively. In fact, apart from one false negative result reported by Pertl *et al.*, (1999a), the other five missed diagnoses were due to uninformative tests. In addition, five out of six missed trisomies occurred prior to 2001.

Four out of nine of the unbalanced structural anomalies involving the tested chromosomes were detected: QF-PCR could not identify four ring chromosomes (two involving chromosome 21 and two chromosome 18) and one unbalanced translocation involving chromosome 21. Only 45% of the mosaicisms were correctly diagnosed, those with a percentage of abnormal cells >30%. Surprisingly, most studies did not report the rate of failures to obtain a result with QF-PCR. Furthermore, only a few studies (Pertl *et al.*, 1999b; Schmidt *et al.*, 2000; Levett *et al.*, 2001; Voglino *et al.*, 2002; Leung *et al.*, 2003; Andonova *et al.*, 2004; Quaife *et al.*, 2004) reported how many clinically significant anomalies involving untested chromosomes would have been missed using QF-PCR alone: there were 26 cases from a total number of 386 non-mosaic autosomal chromosomal anomalies out of 9189 samples. This gives an estimate of one missed diagnosis every 15 abnormal karyotypes and one in 353 analysed samples. If one takes into account also the errors in diagnosing trisomies 13, 18 and 21 (6/853), the estimated number of missed diagnoses would be about one in 13 analyses (one out of 142 plus one out of 15). If, on the other hand, QF-PCR gives a normal or uninformative result, the chances of an autosomal non-mosaic abnormality could be estimated to be one in 310 (six out of 21 657 plus 26 out of 8829).

Admittedly, these rates reflect three possible biases. Firstly, these studies included the initial experiences, hence some errors might be related to the learning curve which is obvious for any newly applied technique and the smaller number of markers compared to more recent series. On the other hand, the data provided were originated in referral laboratories whose accuracy could well be superior to that achieved once the technique spreads out to less specialized centres. Finally, the selection criteria varied from study to study. It may be postulated that, in series including mainly women at risk for age, the rate of numerical or structural anomalies of chromosomes other than those tested would be lower (thus increasing the accuracy of QF-PCR) than in case studies of a large proportion of women undergoing prenatal diagnosis following ultrasound detection of fetal anatomical defects. However, in the former studies, the rate of mosaicisms, especially at CVS, could be higher (thus lowering the accuracy of QF-PCR). Based on these considerations, one may argue that the number of reported cases is still too small to provide an accurate estimation of what would be the rate of clinically significant missed diagnoses per samples performed and/or per anomalies at birth.

The role of QF-PCR in prenatal diagnosis

The authors who reported their experiences displayed in Table I held different views on what might be the role of QF-PCR. Although all considered QF-PCR as being an economic and rapid technique, the evaluation of the achieved accuracy was somewhat different.

Some suggested that QF-PCR has to be used as an adjunctive test to conventional cytogenetics (Pertl *et al.*, 1996; Levett *et al.*, 2001; Bili *et al.*, 2002), or could become in the future the method of choice if prenatal diagnosis of chromosomal anomalies on fetal cells isolated from maternal blood would be a viable option (Verma *et al.*, 1998). The advantage of providing a quick provisional response, while awaiting the full karyotype, may relieve maternal anxiety. One study (Leung *et al.*, 2002), however, has shown that the anxiety related to the time needed for diagnosis persists until the prenatal test is completed.

A distinction was proposed by Mann *et al.*, (2001), who advocated a personalized approach to prenatal diagnosis: those women with abnormal ultrasound findings or with parental chromosomal rearrangements would require a full karyotype analysis, whereas those with advanced maternal age or positive maternal serum screening and women who require prenatal diagnosis because of anxiety could be offered QF-PCR alone. In her opinion, the false negatives of sex chromosome anomalies which would occur with QF-PCR might not be a significant pitfall of the technique. Indeed, in recent years, improved knowledge of the clinical consequences of most sex chromosome anomalies has led to a steady decrease in the rate of terminations of pregnancy, due to a greater acceptance, despite the parental anxiety which the diagnosis generates (Brun *et al.*, 2004).

Recently, Ogilvie (2003) and Leung *et al.*, (2004) proposed replacing conventional prenatal cytogenetic analysis after positive screening for Down's syndrome with QF-PCR. They suggested that the advantages of this technique clearly outweigh the disadvantages. The use of QF-PCR leads to a quicker response, lower costs, and less labour than conventional

cytogenetics and, being a technique which is very accurate in diagnosing or ruling out Down's syndrome, provides the main response to the expectations of the patient and the clinician. One of the objections against QF-PCR as a stand-alone test is the number of conditions that would be missed using the five chromosome polymorphisms. Based on Leung data, however, it is evident that most of those abnormalities (Robertsonian translocations, mosaicisms, chromosome markers) would either be clinically irrelevant or would require intensive genetic counselling with unpredictable outcomes and even unwarranted pregnancy terminations (Ogilvie, 2003; Leung *et al.*, 2004).

It has been claimed that clinically relevant chromosomal aneuploidies, with the exception of Down's syndrome, would most likely be associated with abnormal ultrasound findings. This assumption is the basis of some proposed schemes of utilization of QF-PCR. Women with a normal ultrasound scan can be considered at low risk, hence some authors raise the point that QF-PCR might be the only test offered. Women with fetal structural abnormalities would also benefit from a quick response through QF-PCR which would allow diagnosis of the most common aneuploidies, but there is the general consensus that they should be tested by conventional cytogenetics as well. Therefore, although all women would take advantage by QF-PCR, some women (if not all) should also proceed to have a full karyotype. Besides the fact that such a scheme requires an accurate ultrasound examination, with the implicit costs and limitations in accuracy outside referral centres, it is unknown what might be the residual risk of aneuploidies other than Down's syndrome which would be missed by QF-PCR alone, in the case of a normal fetal anatomy, especially in early gestation, prior to CVS or amniocentesis. Conversely, following detection of fetal anatomical defects on ultrasound, it is not defined to what extent the risk of aneuploidies unlikely to be diagnosed by QF-PCR is increased.

Increased nuchal translucency, for example, is the commonest anatomical marker which can be identified at the first trimester scan. This is without any doubt a robust method of selecting women at increased risk of chromosomal anomalies, a variety of genetic syndromes and some structural malformations which will become apparent later in gestation (Jenderny *et al.*, 2001). However, these two latter conditions cannot be diagnosed by QF-PCR or conventional cytogenetics. Although the authors did not address specifically this issue, the UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal translucency showed that <12% of all chromosomal aneuploidies would be missed using QF-PCR as a diagnostic test (Snijders *et al.*, 1998). Out of a total of 96 127 women, 651 fetuses or newborns had an abnormal karyotype: 326 were trisomy 21 (50%), 325 were other aneuploidies, of which 18.2% were trisomy 18, 7.2% trisomy 13, 8.2% monosomy X, 5% triploidy and 11.3% were unbalanced translocations, deletions, partial trisomies and sex chromosome anomalies other than Turner's syndrome. QF-PCR, therefore, would be a suitable method of analysis for pregnancies with an increased nuchal translucency and conventional cytogenetics would add little in diagnostic accuracy.

Similarly, Comas Gabriel *et al.*, (2002) reported 48 prenatal diagnoses of congenital cardiac anomalies in a series of 330 high-risk pregnancies screened at 12–17 weeks, 31 of which

also had extracardiac anomalies: 27 fetuses had an abnormal karyotype (56.3%) and 23 out of 27 abnormal karyotypes would have been detected by QF-PCR being either a trisomy 21, 18, 13 or monosomy X. There was only one structural rearrangement, one 22q deletion and two rare trisomies (7 and 15). On the other hand, Paladini *et al.*, (2002) described 355 fetuses with cardiac malformations and known karyotype diagnosed at a mean gestational age of 26.2 weeks. The chromosomal abnormalities which were not trisomy 21, 18, 13 and Turner's syndromes were one in seven (14 out of 104).

Howe *et al.*, (1996) reported the results of karyotype analysis in 38 fetuses diagnosed as having congenital diaphragmatic hernia. Twelve fetuses (31%) had an abnormal karyotype: two had trisomy 18, one had trisomy 14 and the remainder had structural abnormalities (deletions or translocations or marker chromosomes). Therefore, QF-PCR would have failed to detect the majority of aneuploidies in a series of fetuses with diaphragmatic hernia.

These examples underline the need to apply different investigative strategies based on the expected rate of non-'classical' chromosomal anomalies with specific ultrasonic fetal malformations or markers. In cases of increased nuchal translucency, or early cardiac malformations, it could be proposed that in view of the low rate of non-detectable abnormalities with QF-PCR, there is no need to perform a full karyotype, whereas this would not be the case with diaphragmatic hernia or some cardiac defects diagnosed later in gestation.

New clinical attitudes to prenatal diagnosis

The main purpose of invasive prenatal diagnosis is to offer prospective parents, who choose to undergo such a procedure, the assurance of having unaffected children when the risk of having a child with a specific genetic disorder (chromosomal or monogenic) is deemed unacceptably high. However, it a selective procedure. In other words, it does not allow diagnosis of all congenital defects, but only of a very limited number of these. The issue is how large this number should be within the framework of current techniques. Public health policies in prenatal diagnosis may collide with the need to maintain individual autonomy of decision-making (Wenstrom, 2003).

Clinical practice is dependent on what has been made available by improvements in technology and by its delivery to large segments of the population. Invasive prenatal diagnosis is nowadays easily accessible, at least in the Western world. Those women who undergo amniocentesis or CVS do so mainly because they perceive or are told that the risk of Down's syndrome is increased above a threshold that is either predefined by the health system or is greater than the one they can accept. In both instances, the risk estimate is based purely on some algorithm which assesses the risk of autosomal aneuploidies and of nothing else. Since Down's syndrome at birth is by far the commonest of these and the one which most people are aware of, the whole process is tailored to identify, and, eventually, offer termination of fetuses with trisomy 21.

Various screening programmes have been implemented in the last decade. All of them, from asking maternal age through biochemical testing of maternal blood to integrated tests which include ultrasonographic and biochemical markers, are evaluated

on the ability to detect the largest number of trisomy 21 fetuses with the lowest percentage of women who are selected to undergo invasive procedures (false positive rates). Yet, once amniocentesis or CVS have been performed, a great deal of information is provided. Fetal sex and heterochromosome anomalies are disclosed, but the impact of these on the health of the child has never been properly assessed, and it might be argued that the benefit of knowing whether the future child has, for example, XYY or XXX is questionable (Abramsky and Chapple, 1997; Christian *et al.*, 2000; Cirigliano *et al.*, 2002; Donague *et al.*, 2003; Brun *et al.*, 2004). Structural anomalies may be relevant, but a large number of these are a cause of parental anxiety and they hardly produce a measurable benefit. In general, these results come unexpectedly, and provide information which is difficult to assess properly and convey. However, the public may be led to believe that invasive tests give additional information of an unknown extent (which may well be greater than what is currently possible), and the opinion is reinforced that all women, irrespective of the risk for which they are selected, have the right to know and missing that information would be a great loss.

This 'holistic' view of prenatal diagnosis is, in our opinion, outdated. It reflects the legacy of how prenatal diagnosis was established: a sophisticated tool for a few high risk patients who were investigated by a complex technique to evaluate the full karyotype, well beyond the initial indication for which the test was done.

In recent years two different phenomena have occurred which may well change this attitude. The number of women who undergo invasive prenatal diagnosis has increased throughout the Western world, which is a burden to the health systems in a period of widespread restriction and careful evaluation of how scarce resources are allocated. On the other hand, a general consensus has emerged that the individual choices of the patient/client should be the main drive of accessing health care, rather than established criteria based on some objective risk estimation by the medical community or health providers. In addition, the public has developed exceedingly high, even unrealistic, expectations about the ability of prenatal diagnosis in identifying congenital defects (Marteau, 2002).

In a recent cost-utility analysis, Harris *et al.*, (2004) have demonstrated that guidelines which recommend prenatal diagnosis based on any risk threshold should be changed, and prenatal testing should be offered to all pregnant women, regardless of maternal age or risk. The desire for reassurance of not having a chromosomally abnormal fetus is the main variable in establishing the cost-effectiveness of prenatal diagnosis, if the decrease in quality of life resulting from not having such information is factored. Although the care providers should have more communication skills than at present, the final decision on whether to undergo prenatal diagnosis or not should be based on both risk estimation and assessment of women's preferences (Marteau and Dormandy, 2001; Petrou and Mugford, 2004).

This would constitute a complete change of direction compared with the current trend towards objective risk assessment based on a combination of age, biochemical and ultrasonographic variables. Such an approach has the advantage of selecting women who are at a higher risk of aneuploidies, and mainly trisomy 21, above a preselected threshold, thus allowing better

allocation of public resources than one based on individual perception of risk. However, the measures by which these combinations of tests are evaluated (false positive and false negative rates) are relevant for health providers, but may be meaningless to individual women for whom positive and negative predictive values are eventually more important. In other words, it may be of no significance for an individual's choice that the detection rate of Down's syndrome using a specific combination of non-invasive tests approaches 90%. The choice is more likely to be driven by the risk of having (positive predictive value) or the chances of not having (negative predictive value) an abnormal fetus, given that specific risk estimation, and the very personal perception of how relevant this estimation is, and how serious is the possible unwanted outcome (whether the loss of healthy fetus or the birth of an affected child).

Within this changed panorama in the field of invasive prenatal diagnosis, QF-PCR might play a major role and be considered a valid alternative to the full karyotype. Being less expensive, and almost entirely automated, more women could undergo invasive prenatal diagnosis without significant increase in health expenditure. By using QF-PCR as a stand-alone test, the chances of non-diagnosing the commonest and the only chromosome anomalies which do increase in frequency with maternal age, are approximately one in 150 abnormal karyotypes, or one in 10–30 000 samples, based on the age distribution. These error rates might be deemed acceptable, although most structural chromosomal anomalies and some sex chromosomes would be missed. At present, however, women are rarely informed about the full spectrum of the conditions which might be diagnosed via amniocentesis or CVS, besides the most common anomalies. Some of these anomalies might be acceptable, in view of their limited or uncertain clinical relevance, and decision analysis might, in the majority of cases, confine the full karyotype to selected women who have specific indications.

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