QF-PCR as a stand-alone test for prenatal samples: the first 2 years' experience in the London region

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Objective   To analyse the results of the first 2 years of a QF-PCR stand-alone testing strategy for the prenatal diagnosis of aneuploidy in the London region and to determine the advantages and disadvantages of this policy.

Methods   A review of the results of 9737 prenatal samples received for exclusion of chromosome abnormalities. All samples were subjected to QF-PCR testing for common aneuploidies but only samples fulfilling specific criteria subsequently had a full karyotype analysis.

Results   Of the 9737 samples received, 10.3% had a chromosome abnormality detected by QF-PCR testing. Of the 7284 samples received with no indication for karyotype analysis, 25 (0.3%) received a normal QF-PCR result but subsequently had an abnormal karyotype detected either prenatally as a privately funded test or postnatally. Of these samples, without subsequent abnormal ultrasound findings, five had a chromosome abnormality associated with a poor prognosis, representing 0.069% of samples referred for Down syndrome testing.

Conclusion   While back-up karyotyping is required for some samples, using QF-PCR as a stand-alone prenatal test for pregnancies without ultrasound abnormalities reduces costs, provides rapid delivery of results, and avoids ambiguous and uncertain karyotype results, reducing parental anxiety. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: QF-PCR; karyotyping; prenatal diagnosis; aneuploidy; nuchal translucency

INTRODUCTION

Prenatal screening of blood samples from pregnant women is carried out to determine their risk of fetal aneuploidy, in particular Down syndrome. A number of different screening tests are available, some in the first and some in the second trimester. Integrated testing (first trimester followed by second trimester sampling) offers the best test performance in terms of high trisomy 21 detection rate in association with a low false positive rate (Wald *et al*., 2003). However, in light of the requirement for earlier diagnosis, NICE have recently recommended the combined test [nuchal translucency (NT) measurement plus serum screening for free beta hCG and PAPP-A], carried out in the first trimester, as the best option for National Health Service patients (NICE, 2008). Whichever screening method is used, women with a risk of 1 : 150 or more in the first trimester or 1 : 200 in the second trimester are given the option of an invasive test (chorionic villus or amniotic fluid sampling) in order to exclude chromosomal abnormalities. Since prenatal diagnosis was introduced in the 1960s,

this has until recently been achieved via karyotype analysis of cultured cells.

QF-PCR for the most common autosomal trisomies (13, 18 and 21) (Mansfield *et al*., 1993) was introduced as an NHS diagnostic test in 2000 (Mann *et al*., 2001, 2004), and is now a well-established rapid test for most invasive prenatal samples within NHS Genetics Centres. It is simple, cheap and can provide a 24-h turnaround time from receipt by the laboratory for 95% of samples. Between May 2005 and May 2007, all prenatal samples received by the four London regional cytogenetics laboratories were tested using a two-tiered approach of rapid QF-PCR testing for trisomies 13, 18 and 21, followed by a full karyotype analysis of G-banded chromosomes.

Since the development and availability of molecular methods, such as QF-PCR for assessing chromosome copy number, the suitability of full karyotype analysis for samples referred solely for raised risk of trisomy has been questioned (Ogilvie, 2003; Nicolini *et al*., 2004; Leung *et al*., 2004; Ogilvie *et al*., 2005; Sparkes *et al*., 2008; Cirigliano *et al*., 2009). Published audits of chromosome abnormalities found by karyotyping at prenatal diagnosis have consistently shown a prevalence of between 0.07% and 0.1% for clinically significant abnormalities that would not be detected by QF-PCR in samples from pregnancies without fetal ultrasound

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abnormalities (Thein *et al.*, 2000; Lewin *et al.*, 2000; Ryall *et al.*, 2001; Ogilvie *et al.*, 2005), although a more cautiously interpreted audit concluded that approximately 1 in 100 samples referred with a Down syndrome risk which received QF-PCR only would have an undetected autosomal chromosome abnormality and that 33% of these would have a substantial risk of serious phenotypic consequences, equivalent to a prevalence of 0.33% (Caine *et al.*, 2005). The anxiety, distress and potentially unnecessary pregnancy terminations that can follow equivocal karyotype results, plus the resource implications, especially for laboratories and counselling services, were put forward as an argument for replacing the traditional approach to prenatal diagnosis for women in this specific referral group.

Following these more recent audits, a National Screening Committee (http://www.screening.nhs.uk/) recommendation in 2006 stated that a full karyotype analysis was not required for pregnancies referred for raised risk of trisomy, and without fetal ultrasound abnormalities; the Greater London Genetic Commissioning Group for SE England therefore decided that it would no longer commission karyotyping for these samples. As a result of this decision, from May 2007, all samples in the London Commissioning Region received QF-PCR as a first test, but only in samples with ultrasound abnormalities indicative of a chromosomal abnormality was this followed by a full karyotype. Here we describe the first 2 years' experience of this testing strategy and discuss the results, advantages and disadvantages.

METHODS

Chorionic villus (CV) and amniotic fluid (AF) samples were received by the four London regional cytogenetics laboratories. An aliquot from each sample was then sent to the cytogenetics laboratory at Guy's Hospital, where QF-PCR testing using a single multiplex of polymorphic microsatellite markers for chromosomes 13, 18 and 21 was carried out as described previously (Mann *et al.*, 2001; Ogilvie *et al.*, 2005) and in line with the current CMGS/ACC (2007) Best Practice Guidelines (http://www.cytogenetics.org.uk/prof_standards/professional_standards.htm).

Samples from pregnancies with ultrasound abnormalities indicative of Turner syndrome (cystic hygroma, hydrops, NT > 4 mm, specific cardiac defects) or with a history of sex chromosome aneuploidies or sex-linked disease were additionally tested using a multiplex containing markers for the sex chromosomes (Donaghue *et al.*, 2003; Ogilvie *et al.*, 2005).

Samples were prepared for culturing and full karyotyping according to standard protocols if any of the following criteria were fulfilled: the presence of structural abnormalities detected at ultrasound, the presence of ≥2 soft markers for Down syndrome (see Table 1 for details), NT > 3 mm at <14 weeks' gestation or >6 mm at ≥14 weeks' gestation, family history of chromosome rearrangement.

Table 1—Testing strategy according to referral indication

Referral	QF-PCR	Karyotype
Increased screening risk NT < 3 mm Raised maternal age Maternal anxiety	13, 18, 21	No
Ultrasound abnormalities: Structural abnormalities NT 3–3.9 mm	13, 18, 21	Yes
Ultrasound abnormalities indicative of Turner syndrome: NT > 4 mm Coarctation of the aorta Hydrops Nuchal oedema	13, 18, 21, X, Y	Yes
≥2 soft markers for Down syndrome, e.g Hypoplastic nasal bone Hyperechogenic bowel Echogenic intracardiac focus Renal pyelectasis Shortened femur or humerus Two vessel umbilical cord Choroid plexus cysts Clinodactyly Clenched hands Ductus venosus NT >2.5 <3 mm	13, 18, 21	Yes
Familial autosomal rearrangements	13, 18, 21	Yes
Familial sex chromosome rearrangements	13, 18, 21, X, Y	Yes

Samples which were normal by QF-PCR testing and which were not referred with an indication for karyotyping were reported and no further testing was carried out. Samples which were abnormal or ambiguous by QF-PCR or which had initially been referred for karyotype testing were reported and a full karyotype analysis report followed in approximately 10–14 days. Samples with an abnormal QF-PCR result were karyotyped in order to exclude a chromosome rearrangement.

Cases were not actively followed up; pregnancy outcome and newborn phenotype data for the majority of pregnancies were not available to the laboratories. Newborns with phenotype indicating possible chromosome abnormality were expected to be referred to one of the regional London laboratories, all of which are involved in this study. Such samples were associated with the prenatal sample based on referring information, name and/or address and the results collated.

RESULTS

Summary of the QF-PCR service at Guy's Hospital

In a 2-year period from May 2007 to May 2009, 9737 prenatal samples were received by the cytogenetics

laboratory at Guy's Hospital for rapid QF-PCR aneuploidy testing. This figure comprised 5878 (60%) AF samples and 3859 (40%) CV samples. Ninety-five per cent of samples received a result within one working day of receipt into the laboratory.

In total, 2453 samples (25% of the total samples; 22% of all AF samples, 29% of all CV samples) had a referral indicating karyotype analysis. The largest referral category was for samples with increased risk from the Down syndrome screening programmes (47%). Referral indications of the remaining 28% of samples in the 'other' category include NT > 2.5 < 3 mm, previously known single gene defects, maternal anxiety and raised maternal age in the absence of other indications.

Eighty-eight per cent of all samples received a normal QF-PCR result. Trisomy 21 was the largest abnormal results category comprising 55% of abnormal results (Figure 1). Ten point six per cent (1035) of samples were targeted for sex chromosome testing. Samples identified as monosomy X by QF-PCR comprised 0.7% of the total samples and 7% of those targeted for rapid sex chromosome testing. The 1% of abnormal samples with results in the 'other' category include those with partial chromosome imbalance and a molar pregnancy.

Within this referral period, QF-PCR results were concordant with the karyotype in all cases with the exception of one case of confined placental mosaicism (CPM) for trisomy 13 in which QF-PCR indicated trisomy 13 but the karyotype was normal. Review of this and several previously documented cases (Mann *et al.*, 2007; Waters *et al.*, 2007) has led to the modification of CV sample preparation techniques and careful reporting of such results.

Figure 2 illustrates the flow of samples through the testing system and the results obtained at each point. Of the 2453 samples (25%) with a referral indicating karyotype analysis, 651 (27%) received an abnormal QF-PCR result. Of the remaining 75% of samples referred with no clinical indication for karyotype analysis, 351 (5.2%) received an abnormal QF-PCR result (including one sample which subsequently had a normal karyotype due to CPM). In total therefore, QF-PCR detected
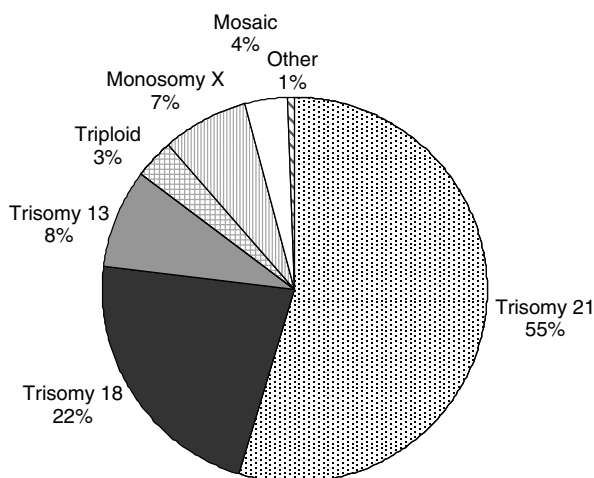

Figure 1—Abnormal QF-PCR results. $n = 1002$

1002 abnormal samples, 10.3% of the total received. Abnormality rates differed according to sample type; 16.6% of all CV samples referred had an abnormal QF-PCR result compared to 6% of AF samples.

Of the samples referred for karyotyping which had normal QF-PCR results, 107 (6%) were found to have other chromosome abnormalities following karyotype analysis. Of the samples not clinically indicated for karyotype analysis, 909 were actually karyotyped due to commissioning differences between referring hospitals, and to the availability within the regional laboratories of privately funded karyotyping to some patients. In this group, which is discussed in more detail below, 17 samples had an abnormal karyotype result. In total, 1126 samples with abnormal prenatal results were found (11.5% of the total samples received). QF-PCR therefore detected 89% of all prenatally detected abnormalities and 99.8% of all abnormalities in samples referred with no clinical indication for karyotyping.

Samples unsuitable for QF-PCR

Amplification failure

QF-PCR was attempted on all samples received, including bloodstained AF samples. DNA from the vast majority of samples was amplified to produce a result that could be interpreted. However, four AF samples that were heavily bloodstained with old blood, giving a brown appearance, failed to amplify. In addition, two CV samples of poor size and/or quality also failed to amplify, giving a total amplification failure rate of 0.06%. All the AF and one of the CV samples proceeded to normal karyotype results. The remaining CV sample failed to grow in culture due to poor sample size/morphology. A repeat CV sample was taken which subsequently received a normal QF-PCR result and was not referred with an indication for karyotyping.

Maternal cell contamination

Figure 3 shows the testing process of bloodstained AF samples. One-hundred and twenty-six AF samples (2.1% of all AF samples) had levels of a second genotype assumed to be maternal cell contamination (MCC) which skewed peak ratios sufficiently to compromise QF-PCR detection of trisomy. These samples received a report stating that the sample was unsuitable for QF-PCR analysis and that karyotype analysis would follow. All but four of these AF samples went on to successful karyotype analysis.

AF samples for which the cell pellet was >90% bloodstained were additionally tested with the QF-PCR sex chromosome multiplex. If a single male genotype was detected, this was reported as normal for chromosomes 13, 18 and 21 (with or without the sex chromosome result depending on referral indication). When a single female genotype was detected for such samples, a preliminary report was issued stating that it was not possible to be certain that the genotype analysed was fetal (Stojilkovic-Mikic *et al.*, 2005) and
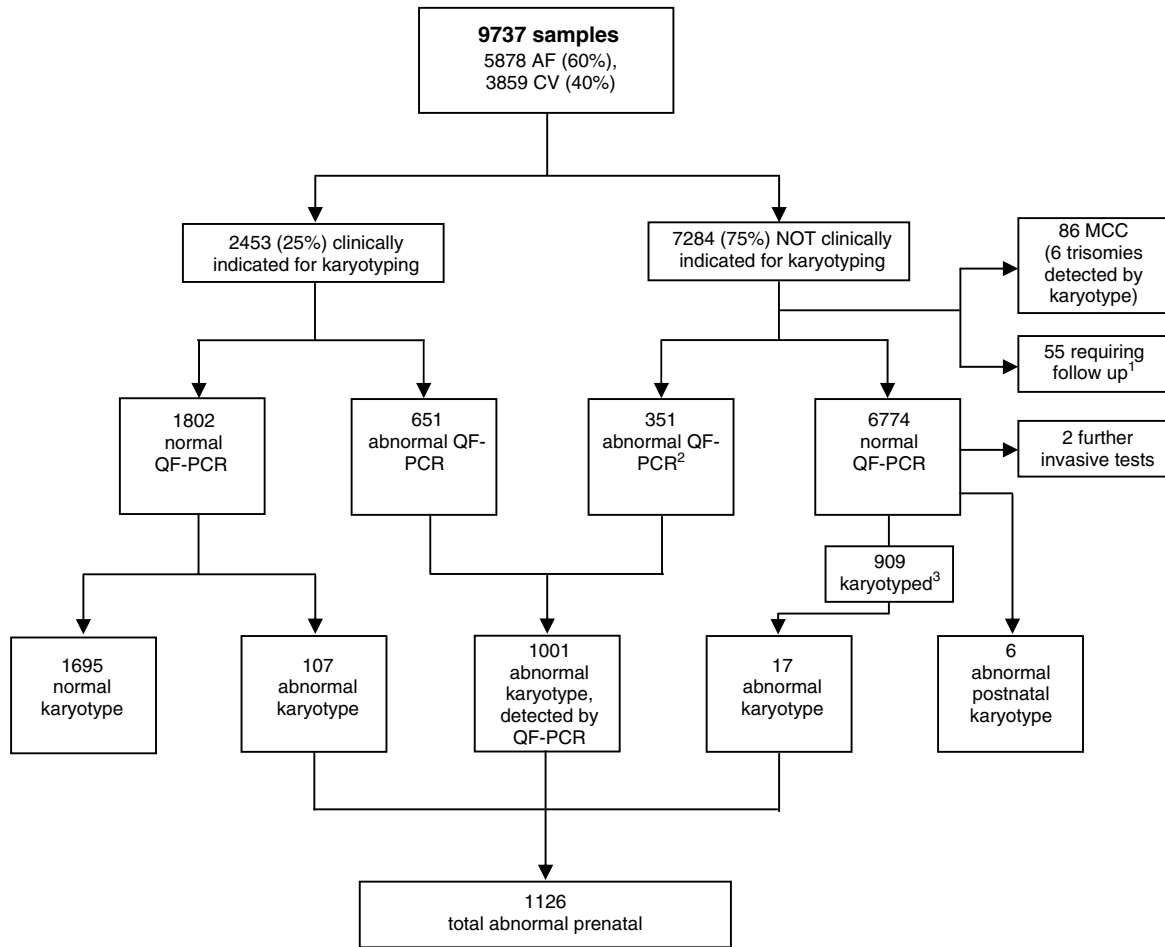
Figure 2—Flow chart depicting testing strategy and outcomes at each stage of sample testing. [1]Fifty five samples required follow up testing of parental bloods in order to exclude MCC from heavily bloodstained AF samples or to investigate inheritance of anomalous QF-PCR results. [2]Including one sample which subsequently had a normal karyotype due to CPM as described above. [3]Nine hundred nine samples received full karyotype analysis without a clinical indication. These samples were from private-funded or from hospitals with service arrangements with individual referring laboratories
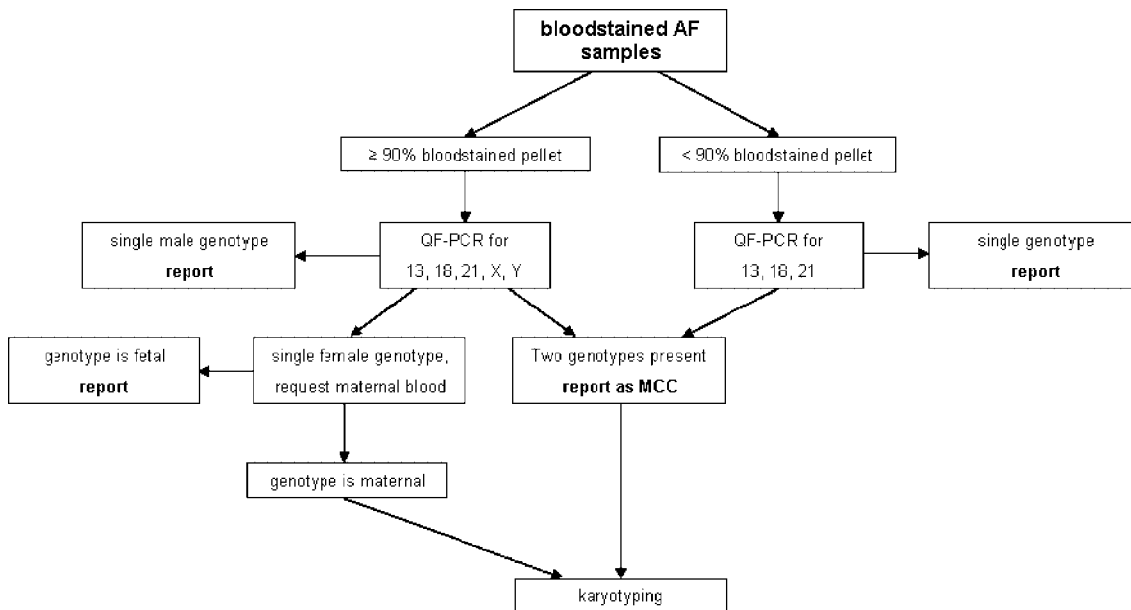

Figure 3—Testing and reporting process for bloodstained AF samples

that therefore a sample of maternal blood was required to exclude MCC. During our evaluation, maternal blood was requested in 65 such cases (1.1% of AF samples). If the genotype was found to be fetal after comparison with the maternal genotype, this was reported as normal for chromosomes 13, 18 and 21 and karyotyping proceeded only if warranted by the original referral indication.

Five CV samples (0.13% of all CV samples) were found to be unsuitable for QF-PCR due to the presence of two genotypes; these samples had been inadequately cleaned of contaminating maternal decidua at sample preparation or were of recognisably poor morphology and/or small size upon receipt. In these cases, an aliquot of the cultured CV cells was subsequently genotyped by QF-PCR prior to reporting the karyotype result, to ensure that only fetal cells had grown and been analysed. One CV sample with MCC failed at karyotype due to poor sample quality. A repeat CV sample gave a normal QF-PCR result.

Samples with ambiguous QF-PCR results

Submicroscopic duplications

Submicroscopic duplications (SMDs) are occasionally detected by QF-PCR, manifesting as a trisomic result for a single marker on a chromosome when all other markers are consistent with a normal complement. The CMGS/ACC Best Practice Guidelines v2.01 state that

> Abnormal markers that are flanked by normal markers may represent CNV. If the marker has previously been reported to represent a CNV inherited from a normal parent then it is acceptable not to report the abnormal marker result.

In cases where a marker was not flanked by normal markers and/or had not previously been seen to be inherited, parental blood samples were requested on the QF-PCR report in order to determine whether the anomaly represented an inherited CNV. In the period of this study, parental samples were requested on eight such occasions. In seven of these cases the SMD proved to be an inherited CNV. The remaining case was a trisomy 13 CV sample with an SMD on a terminal sex chromosome marker which frequently exhibits SMDs. Parental samples were requested but not received. Audit within the cytogenetics laboratory at Guy's Hospital identified 14 markers prone to exhibiting CNV, two of which were subsequently removed from our multiplexes. It is anticipated that a nationally available database detailing such SMDs will be completed in the near future. Such information would reduce the need for follow-up sampling from parents. However, it is important to consider that trisomic markers not flanked by normal markers could indeed represent a clinically significant duplication. These results should lead to more detailed karyotype analysis and/or further follow-up studies.

Uninformative markers

Five samples received a QF-PCR result, where only one marker was informative and normal on a specific chromosome. The ACC/CMGS QF-PCR Best Practice Guidelines state that

> It is acceptable to report cases where only a single marker shows a normal diallelic pattern, all other markers being uninformative, as this is consistent with a normal chromosome complement. The report should state that the result is based on a single marker result and that this result will be confirmed by another technique (either full karyotype or FISH on uncultured cells).

Four of the five cases had initial referrals indicating the need for karyotyping and the remaining case was referred for a family history of Sandhoff disease. Four samples had a normal karyotype result and one failed at karyotype.

Two cases received a QF-PCR result where no markers were informative for a specific chromosome. Both of these cases were referred with ultrasound abnormalities and proceeded to karyotyping, with no abnormality detected in either case.

From the data above regarding heavily bloodstained and SMD samples, in 73 cases (0.74% of total samples received), the strategy of QF-PCR as a stand-alone test has necessitated the need for parental blood sampling which would not have been necessary if all samples had a full karyotype analysis without QF-PCR testing. No additional invasive procedures were required following ambiguous QF-PCR results.

Further invasive procedures

Two pregnancies without an initial clinical indication for karyotyping, which received a normal QF-PCR result for chromosomes 13, 18 and 21, subsequently underwent a further invasive test. The first was a CV sample received at 13 weeks' gestation with a 1 : 11 combined risk. An AF sample was subsequently taken at 20 weeks' gestation after detection of hydrocephaly and ventriculomegaly on ultrasound. Karyotype analysis revealed an apparently balanced Robertsonian translocation between chromosomes 13 and 14 (45,XX,der(13;14)(q10;q10)). Karyotype analysis of parental blood showed that the translocation had arisen *de novo*. Microsatellite analysis of the AF cells and parental blood using chromosome 14 markers excluded uniparental disomy for chromosome 14. The pregnancy continued to term and birth was via caesarean section due to breech presentation. Neonatal brain MRI scans were abnormal. Further follow-up information was not available.

The second sample was an AF received at 16 weeks' gestation with a 1 : 171 combined test risk. A second AF sample was taken at 26 weeks after detection of severe IUGR on ultrasound. Karyotype analysis showed mosaic tetraploidy (mos 92,XXYY[18]/46,XY[42]). The pregnancy was complicated by pre-eclampsia and was terminated. Karyotype analysis of fetal blood taken prior to termination of pregnancy showed a 46,XY karyotype. Verbal reports indicated a potentially abnormal placenta.

Karyotyping of samples with no clinical indication

Of the 7284 samples referred without a clinical indication for karyotyping, 909 (12.5%) received a normal QF-PCR result and subsequently a full karyotype analysis. These samples were either from patients wishing to pay a fee for full karyotype analysis or from referring hospitals which had made specific arrangements with one of the regional laboratories to have all samples karyotyped. Of these samples, 17 had an abnormal karyotype which would not have been detected by the QF-PCR only strategy. These 17 cases have been classified according to the severity of the prognosis associated with the abnormal karyotype (Table 2). In the good prognosis category were four inherited inversions (cases 1–4), an inherited (case 5) and a *de novo* (case 6) Robertsonian translocation, a mosaic and a non-mosaic triple X (cases 7 and 8), and a mosaic balanced translocation with pericentric breakpoints (case 9).

Cases with an uncertain prognosis were a *de novo* balanced translocation (case 10) and two cases of mosaicism for balanced translocations with breakpoints in euchromatic regions (cases 11 and 12). Where inheritance status of these cases is unknown there would remain a 5–6% residual risk of phenotypic abnormality associated with these abnormalities (Gardner and Sutherland, 2004). Additional uncertain prognosis cases were a case of low-level mosaicism for trisomy 21 (case 13), one case of mosaicism for monosomy X (case 14) and a complex, potentially balanced rearrangement (case 15). The mosaic trisomy 21 case and the complex rearrangement can be considered viable pregnancies that would continue to term. Viability of the mosaic monosomy X case is uncertain.

Two cases had a poor prognosis: one case of trisomy 5 (case 16) and one case of a *de novo* interstitial deletion of chromosome 12 (case 17). Trisomy 5 pregnancies are non-viable and therefore this case would likely

have spontaneously aborted soon after CV sampling. Microdeletions of 12q14 have recently been reported to be associated with developmental delay and short stature (Menten *et al.*, 2007; Mari *et al.*, 2009; Buysse *et al.*, 2009). This chromosome 12 deletion is therefore the only viable abnormality with a poor prognosis within this group.

Abnormal postnatal karyotype following normal prenatal QF-PCR only result

All pregnancies detailed here have proceeded to term. Newborns with phenotype indicating possible chromosome abnormality were expected to be referred to one of the regional London laboratories. Results from these cases were collated.

Table 3 details six cases which were all referred prenatally with increased serum screening risks. All received normal QF-PCR results for chromosomes 13, 18 and 21 and subsequently presented with abnormal phenotype postnatally; karyotype analysis was then undertaken.

Applying the same categorisation as those described for the prenatal samples in the previous section, one of these postnatal cases had a good prognosis, a paternally inherited inversion (case 18) which was presumably not associated with the mildly dysmorphic postnatal phenotype.

All the other five cases had chromosome abnormalities that were considered likely to be the cause of abnormal phenotype. Case 19 had a postnatal diagnosis of interstitial deletion of chromosome 13, and had been received as a CV sample at 12 weeks' gestation with a 1 : 92 adjusted risk of trisomy 21. A 20-week ultrasound scan revealed coarctation of the aorta and short long bones; further invasive prenatal testing was declined by the couple. The interstitial deletion of chromosome 13 in this case has previously been reported to be associated with mildly dysmorphic features (as seen in this

Table 2—Details of prenatal cases referred with no indication for karyotyping, subsequently found to have an abnormal karyotype

Case	Referral	Sample type	Karyotype	Viability of fetus	Prognosis
1	Serum screening	AF	46,XY,inv(6)(q21q25.1)mat	Viable	Good
2	Maternal age	AF	46,XY,inv(6)(q15q16.2)pat	Viable	Good
3	Maternal age	AF	46,XY,inv(6)(q15q16.2)pat	Viable	Good
4	Serum screening	CV	46,XY,inv(19)(q13.2q13.3)mat	Viable	Good
5	Serum screening	AF	45,XX,der(13;14)(q10;q10)pat	Viable	Good
6	Serum screening	AF	45,XY,der(13;14)(q10;q10)dn	Viable	Good
7	Serum screening	CV	47,XXX	Viable	Good
8	Serum screening	CV	47,XXX[36]/46,XX[2]	Viable	Good
9	Serum screening	CV	46,XX,t(11;19)(q10;q10)[22]/46,XX[24]	Viable	Good
10	Myotonic dystrophy	CV	46,XX,t(1;4)(q21;p16)dn	Viable	Uncertain
11	Sickle cell	CV	46,XX,t(2;17)(q21;q25)[6]/46,XX[9]	Viable	Uncertain
12	Serum screening	AF	46,XY,inv(2)(p14p21),t(2;4)(p21;q13)dn	Viable	Uncertain
13	Serum screening	CV	47,XY,+21[3]/46,XY[46]	Viable	Uncertain
14	Serum screening	CV	45,X[36]/46,XX[4]	Uncertain	Uncertain
15	Thalassaemia	CV	46,XY,der(18)?t(18;21)(q2?1;q2?2),der(21)? t(18;21)(q2?1;q2?2)[4]/46,XY[36]	Viable	Uncertain
16	Serum screening	CV	47,XX,+5	Non-viable	Poor
17	Serum screening	AF	46,XX,del(12)(q14q14)dn	Viable	Poor

Table 3—Details of abnormal karyotypes detected postnatally in cases which were not clinically indicated for prenatal karyotyping

Case	Postnatal phenotype	Postnatal karyotype	Likely role of karyotype
18	Webbing of fingers and toes, almond-shaped eyes and depressed nasal bridge	46, XY,inv(2)(p13q24.2)pat	Not causative
19	Mildly dysmorphic features and possible heart defects	46,XX,del(13)(q14.3q22)[a]	Causative
20	Truncal hypotonia, bilateral ventricular dilatation and abnormal basal ganglia	46,XY,dup(11)(q?22.2q?23.3)	Causative
21	Vertical talus and narrow ear canals	46,XY,del(18)(q22)	Causative
22	Ambiguous genitalia	mos,45,X[76]/46,X,idic(Y)(q11.2)[20]	Causative
23	Small and floppy	UPD 14 (mos 47,XX,+mar[28]/48,XX,+14,+mar[2])	Causative

[a] Although this karyotype was detected postnatally, abnormal phenotype had been found on anomaly scan, and further testing was offered at this point, but declined by the couple.

child) and increased stature (van Bon *et al*., 2007). It is unclear whether the heart abnormalities seen prenatally remained postnatally and/or are related to the chromosome abnormality.

Case 20 had an interstitial duplication of chromosome 11. The majority of cases of partial trisomy 11q arise from a translocation with chromosome 22q. Phenotypic features include growth and mental retardation, hypotonia and congenital heart defects, some of which features were observed in case 20. It has been proposed that the region 11q21-23.2 may be associated with certain upper airway malformations (Zhao *et al*., 2003). Inheritance status is unknown at the time of writing.

The three remaining cases comprised one case of interstitial deletion of the chromosome 18 long arm (case 21), one case of mosaicism for an isodicentric Y chromosome and monosomy X (case 22) and one case of uniparental disomy for chromosome 14 (case 23).

Samples with NT 3–3.9 mm

It has been reported (Chitty *et al*., 2006) that abnormal non-trisomy karyotypes have a low prevalence in samples with NT < 4 mm at <14 weeks' gestation. For the period of our study, karyotype analysis was carried out on all samples with NT > 3 mm at <14 weeks' gestation. Four-hundred and fifty-three samples (4.7% of total samples) were referred with an NT measurement between 3 and 4 mm. Three-hundred and seventy-two of these samples (82%) received a normal result for QF-PCR, 81 samples (18% of this group) received an abnormal QF-PCR result, of which 65 were trisomy 21. All abnormal QF-PCR results were concordant with the karyotype.

Seven samples within this referral category had an abnormal karyotype (excluding inherited rearrangements) not detected by QF-PCR: one case of monosomy X, one case of mosaicism for monosomy X, one case of isochromosome Xq, one case of trisomy 20, one case of trisomy 22, one case of mosaicism for trisomy 22 which was normal on a subsequent AF sample, and one case of mosaicism for a marker chromosome. Applying the same criteria as for the prenatal and postnatally detected

abnormal karyotypes which were not clinically indicated for karyotyping, if samples with NT 3–3.9 mm were not karyotyped, the monosomy X and isochromosome Xq could be considered a poor prognosis. The mosaic cases would have an uncertain prognosis and the three trisomy cases would have been non-viable.

DISCUSSION

Our results show that QF-PCR as a stand-alone test for the 75% of samples which do not exhibit structural fetal abnormalities (or have a familial chromosome rearrangement) is a rapid and cost-efficient testing strategy which enables improved pregnancy management and is likely to significantly reduce parental anxiety (Leung *et al*., 2008).

However, limiting prenatal testing to QF-PCR only for the majority of samples resulted in fetuses with karyotype abnormalities proceeding to detection at anomaly scan or through to term, resulting in late termination, or the live birth of infants with congenital abnormalities, with the associated medical, emotional and financial consequences. The data presented here demonstrate that in the first 2 years of the QF-PCR stand-alone testing strategy in the London region, 25 samples (17 prenatal, six postnatal, two follow-up tests) had an abnormal karyotype result without having a clinical indication for karyotyping. This represents 0.3% of samples in this referral group. Of these cases, five were considered to have viable chromosome imbalances with a poor prognosis which were not identified by prenatal ultrasound (cases 17, 20, 21, 22 and 23). One of these cases (case 17) was detected prenatally, but would have been undetected if the privately funded karyotyping had not been carried out, and the remainder were detected postnatally. These five cases represent 0.069% of samples referred primarily for Down syndrome without fetal structural abnormalities. This figure is very close to that estimated by Ogilvie *et al*. (2005) as the <0.07% pregnancies referred primarily for Down syndrome testing that would proceed to term with a significant undetected chromosome abnormality. It is also similar to

the 0.056% of all liveborn children in the South East Thames region that presented with significant problems within their first 5 years of life and were found to have a non-trisomy chromosome abnormality (Ogilvie *et al.*, 2005). This would suggest that the prevalence of clinically significant non-trisomy chromosome abnormalities in pregnancies at raised risk of Down syndrome is only slightly higher than that found in the offspring of women who did not undergo invasive testing, therefore also suggesting that the presence of such abnormalities is not indicated by serum screening.

A 2004 study of >1500 pregnancies which received rapid aneuploidy detection (QF-PCR or FISH) as well as karyotyping (Leung *et al.*, 2004) predicted that restricting karyotype analysis to pregnancies exhibiting ultrasound abnormalities but performing QF-PCR on all samples would detect 95% of clinically significant chromosome abnormalities and reduce the number of karyotype analyses requested by 70%. Our data presented here from >9500 prenatal samples show that a QF-PCR stand-alone strategy reduced karyotyping by 75% and detected 89% of all chromosome abnormalities. This figure differs slightly from that predicted by Leung *et al.* as we have not removed the uncertain or good prognosis group from our total abnormal karyotype group. A 2005 retrospective audit of >140 000 pregnancies by the Association of Clinical Cytogeneticists (Caine *et al.*, 2005) found that QF-PCR for chromosomes 13, 18 and 21 detected approximately 70% of abnormal karyotypes (67% of abnormal AF and 78% of abnormal CV samples). The authors conclude that approximately 1 in 100 samples referred with a Down syndrome risk which received QF-PCR only would have an undetected autosomal chromosome abnormality and that 33% of these would have a substantial risk of serious phenotypic consequences. The latter figure is considerably higher than those suggested by other audits (Thein *et al.*, 2000; Lewin *et al.*, 2000; Ryall *et al.*, 2001; Ogilvie *et al.*, 2005), and higher than that suggested by the data presented here. Combining retrospective audit data and numbers from our 909 karyotyped samples, it is possible to estimate the incidence of undetected chromosome abnormality in our non-karyotyped group. Caine *et al.* reported a total of 111 510 samples referred with a raised risk from Down syndrome screening of which 1333 had a chromosome abnormality that would not be detected by QF-PCR. Incorporating our data (17 of 909 samples with an undetected chromosome abnormality) gives an incidence of 1.20%; this would predict the presence of undetected chromosome abnormality, mostly without clinical significance, in 87 of our non-karyotyped group (7284). However, it is recognised that within our data set there are infants that may yet present with abnormalities leading to the discovery of chromosome imbalance, particularly those with developmental delay. Most infants with serious or multiple congenital abnormalities indicating chromosome abnormality are likely to be identified soon after birth, referred for karyotype analysis and thus included in this data set. Rarely, other laboratories may be involved in postnatal follow-up. However, we would expect the regional laboratory that undertook the prenatal diagnosis to be informed in the event of an abnormal karyotype. In summary, the figure of 0.069% may be slightly lower than the true prevalence.

Whilst it is clearly important to detect abnormalities with poor prognoses, diagnosis of an abnormality associated with a good or uncertain prognosis causes counselling issues and anxiety for the parents whilst inheritance studies are carried out, anxiety which may continue into the early life of the infant. In the case of *de novo* translocations, a risk of 5–6% of abnormal phenotype is quoted (Gardner and Sutherland, 2004); the retrospective review of >32 000 pregnancies by Ogilvie *et al.* (2005) found that 3 out of 98 pregnancies with a good prognosis were terminated apparently based on this risk.

Chitty *et al.* (2006) reported that abnormal non-aneuploidy karyotypes have a low prevalence in samples with NT < 4 mm at <14 weeks' gestation. Over our 2-year period, 453 samples (4.7%) fell into this referral category. There were no clinically significant, viable autosomal abnormalities detected in this group by karyotyping that were not detected by QF-PCR. Three cases of X chromosome imbalance were detected by karyotyping, all of which would have been detected by sex chromosome QF-PCR had it been performed: one case of isochromosome Xq, one of monosomy X and one of mosaic monosomy X. It could therefore be proposed that full karyotype analysis of this referral group be replaced by QF-PCR sex chromosome testing.

The cost implications of the QF-PCR stand-alone strategy described here are significant; 25% of samples within our 2-year period had a full karyotype analysis, representing a significant saving in terms of staff costs, consumables, etc. As described in Ogilvie *et al.* (2005), required staffing levels are substantially higher for a full karyotype service compared to a QF-PCR only service.

In conclusion, there has been much discussion about the implications of ceasing karyotype analysis for a subset of prenatal samples, mainly focussing on the risks of missing clinically significant chromosome abnormalities. Non-invasive prenatal diagnosis is likely to be available for all pregnant women within the next 5 years, initially for trisomy 21, then potentially for trisomies 13 and 18. This methodology will also result in undetected chromosome abnormality, predicted to be at the same prevalence as that described here. Until then, the data presented in this paper show that QF-PCR stand-alone testing for pregnancies presenting without fetal structural abnormalities or relevant family history is a strategy that leads to the detection of any clinically significant chromosome abnormalities in 99.9% of pregnancies, while minimising parental anxiety and unnecessary terminations.

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