PRENATAL DIAGNOSIS
Prenat Diagn 2007; **27**: 34–39.
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI:** 10.1002/pd.1615

Ultrasound findings before amniocentesis in selecting the method of analysing the sample

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Objective To determine if the primary method of cytogenetic analysis in pregnant women undergoing amniocentesis should be quantitative fluorescent polymerase chain reaction (qf-PCR), with karyotyping being performed only on those with abnormal ultrasound findings.

Methods Amniocentesis was performed in 3854 cases. The median maternal age was 36 years and median gestational age was 18 weeks. The indication for karyotyping was an increased risk for aneuploidy in the absence or presence of sonographic abnormalities detected at the scan before amniocentesis. All samples were analysed by qf-PCR and full karyotyping. For each detectable fetal defect, the positive or negative likelihood ratio for aneuploidy was determined.

Outcome Measure Detection rate of clinically significant chromosomal abnormalities.

Results The karyotype was normal in 3617 (93.9%) cases. In 237 (6.1%) cases, the karyotype was abnormal and the detection rate by qf-PCR was 92.4%. A policy of performing qf-PCR in all cases and karyotyping in only those with combined likelihood ratios of >1, >3, and >5 would detect 98.3, 96.6, and 95.4% of all chromosomal abnormalities and would require karyotyping in 16.1, 8.0, and 5.4% of the cases, respectively.

Conclusions More than 95% of the aneuploidies can be detected if karyotyping is performed in addition to qf-PCR in about 15% of the cases selected on the basis of ultrasound findings before amniocentesis.

KEY WORDS: ultrasound; amniocentesis; karyotype; qf-PCR

INTRODUCTION

Traditional cytogenetic analysis of amniotic fluid has the advantage of diagnosing most of the clinically significant chromosomal abnormalities. However, culture and analysis of fetal cells is time consuming, with the results being available only in about two weeks after invasive testing, and is labour intensive, with one technician being able to handle only about 250 samples per year. Furthermore, it is uncertain whether screening for trisomy 21, which is the commonest indication for amniocentesis, should be followed by an invasive test for the diagnosis of all chromosomal abnormalities rather than just for the most common chromosomal abnormalities, such as trisomies 21, 18, and 13, and sex chromosome aneuploidies.

It is now possible to analyse samples by quantitative fluorescent polymerase chain reaction (qf-PCR), which can provide the diagnosis of common autosomal aneuploidies such as trisomy 21, 18, and 13, and all sex chromosome aneuploidies and triploidy within two days of sampling (Pertl *et al*., 1994; Verma *et al*., 1998; Levett *et al*., 2001; Mann *et al*., 2001). Furthermore, one technician can examine up to 5000 samples per year. However, a shift in prenatal diagnosis from full karyotyping to qf-PCR would be associated with failure to diagnose some significant abnormalities (Evans *et al*., 1999). An emerging policy in many centres is to undertake dual testing in all cases, with its benefit of both speedy and accurate results (Grimshaw *et al*., 2003). However, such a policy inevitably results in a substantial increase in the cost of screening and perpetuates the policy of testing for conditions that have not been screened for.

In this paper we report on our experience of dual testing of amniotic fluid and examine the percentage of clinically significant chromosomal rearrangements that were detected by qf-PCR, depending on the indication for amniocentesis. The aim is to determine whether it is necessary to carry out dual testing in all cases or whether the main method of analysis should be qf-PCR, with full karyotyping being reserved only for those cases with abnormal findings in the ultrasound scan undertaken before amniocentesis.

METHODS

At King's College Hospital and the Fetal Medicine Centre, London, all amniotic fluid samples began to be analysed by both qf-PCR and full karyotyping (TDL Genetics, London, UK) from September 1998. A systematic ultrasound examination was carried out for the diagnosis of any abnormalities or markers of chromosomal defects immediately before amniocentesis for fetal karyotyping. We searched the databases to identify all cases

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Received: 6 July 2006
Revised: 11 October 2006
Accepted: 18 October 2006

examined until December 2005 to determine the indication for amniocentesis, the sonographic findings, the results of qf-PCR, and full karyotype. We excluded cases undergoing amniocentesis as a secondary investigation of uncertain results following first-trimester karyotyping by chorionic villous sampling, because in these cases it was necessary to carry out full karyotyping.

The karyotypes obtained from the amniotic fluid cultures were classified as either normal or abnormal and according to whether they were detected by qf-PCR. In the normal karyotype group we included chromosomal abnormalities that had no or very low risk of adverse outcome, such as *de novo* balanced rearrangements. In the abnormal karyotype group, we included only those with a risk of clinically significant adverse outcome.

In each karyotype group, we examined the distribution of sonographically detectable fetal defects. The incidence of each defect in the chromosomally normal and abnormal fetuses was determined and the positive or negative likelihood ratio for chromosomal abnormalities was defined. The combined likelihood ratio for each case was calculated by multiplying the positive or negative likelihood ratio according to the presence or absence of each defect. We then estimated the potential impact, in terms of prenatal diagnosis of clinically significant chromosomal abnormalities, of a policy in which the main method of analysing amniotic fluid samples is qf-PCR, with full karyotyping being reserved for cases with a combined likelihood ratio of more than 1.0, 3.0, and 5.0.

RESULTS

Amniocentesis was carried out in 3854 cases. The median maternal age was 36 (range, 16–50) years and the median gestational age was 18 (range 16–25) weeks. The indication for fetal karyotyping by amniocentesis was increased risk for aneuploidy in the absence ($n = 2438$) or presence of sonographic abnormalities detected or confirmed at the scan carried out before amniocentesis ($n = 1416$). The abnormalities included major defects such as diaphragmatic hernia or exomphalos, and markers such as choroid plexus cysts or cardiac echogenic focus.

The amniotic fluid karyotype was normal in 3604 and abnormal in 250 of the 3854 cases. Thirteen cases with a clinically chromosomal abnormality with no or low risk of adverse outcome were classified as normal (two inherited markers, six inherited balanced translocations, two *de novo* balanced translocations, and three inherited inversions). Thus, there were 3617 (93.9%) cases with a normal and 237 (6.1%) cases with an abnormal result. The qf-PCR was normal in all cases with a normal karyotype. In the 237 cases with an abnormal karyotype, the abnormality was detected by qf-PCR in 219 cases (92.4%) and was not detected in 18 (7.6%) (Table 1). Therefore, in 3854 total cases, the qf-PCR was concordant with the karyotype result in 3836 (99.5%) cases and discordant in 18 (0.5%).

The ultrasound examination carried out before amniocentesis demonstrated defects in 1416 (36.7%) of the 3854 cases, including 1193 (33.0%) of the 3617 with normal karyotype, 207 (94.5%) of the 219 with abnormal karyotype and abnormal qf-PCR, and 16 (88.8%) of the 18 with abnormal karyotype and normal qf-PCR (Table 2). Therefore, in the 1416 fetuses with detectable defects, 92.8% (207 of 223) chromosomal abnormalities were detected by qf-PCR, whereas in the 2438 fetuses with no detectable defects, 85.7% (12 of 14) chromosomal abnormalities were detected by qf-PCR.

Table 1—Concordance in results from full karyotyping and qf-PCR

Karyotype result	*N*	Concordant qf-PCR result
Normal	3617	3617 (100%)
Abnormal	237	219 (92.4%)
All detected by PCR		
Trisomy 21	110	110 (100%)
Trisomy 18	55	55 (100%)
Trisomy 13	20	20 (100%)
Turner	8	8 (100%)
Other sex aneuploidies	6	6 (100%)
Triploidy	17	17 (100%)
Some detected by PCR		
Mosaic trisomies 13, 18, 21	4	3 (75%)
None detected by PCR		
Duplications and deletions including mosaics	12	0
Marker chromosomes including mosaics	4	0
Trisomy 9	1	0

The abnormalities that were diagnosed during the systematic ultrasound examination before amniocentesis are listed in Table 3. Also listed are the incidence of these abnormalities in the chromosomally normal and abnormal fetuses and the positive and negative likelihood ratios, respectively. The median combined likelihood ratio was 0.1 for those cases with a normal karyotype, 10.5 for those with a PCR-detected chromosomal abnormality, and 5.0 for those with a PCR-undetected chromosomal abnormality. Table 2 shows the combined likelihood ratio of these cases, which would have been missed by a policy that relies only on qf-PCR.

The detection rate of chromosomal abnormalities in a policy that relies entirely on qf-PCR would have been 92.4% (219 of 237). This detection rate would increase if, in addition to qf-PCR being carried out in all cases, full karyotyping was performed in fetuses with sonographically detectable defects. Table 4 reports the detection rate and the rate of full karyotyping in our population for different cut-offs with the combined likelihood ratio derived from the observed fetal defects as shown in Table 3. For example, if full karyotyping was carried out only in those cases with defects resulting in a combined likelihood ratio of 1.0 or more, the rate of full karyotyping would be 16.1% and the detection rate would be 98.3%.

DOI: 10.1002/pd

Table 2—Chromosomal abnormalities, ultrasound findings, and the combined likelihood ratio of cases with a normal qf-PCR result

Karyotype	Ultrasound findings	Combined likelihood ratio
Trisomy 9	Growth restriction, micrognathia, cardiac defect, dysplastic kidneys	>1000
Trisomy 9p	Ventriculomegaly	0.28
Trisomy 9p	Ventriculomegaly, cardiac defect, short limbs	44.70
Trisomy 9p	Ventriculomegaly, nuchal edema	1.67
Trisomy 6p	Hydronephrosis, talipes, clenched hands	53.65
Addition Xp	Nuchal edema, clinodactyly, short limbs	125.14
Deletion 4p	Cardiac defect, spina bifida, echogenic bowel, short limbs	10.72
Deletion 5p	Hypoplastic nose, hydronephrosis	8.13
Deletion 5p	Cardiac defect	2.33
Deletion 12q	Ventriculomegaly, hydronephrosis	1.30
Deletion Xq	None	0.12
Cat-eye syndrome Der22	Nuchal edema, micrognathia, echogenic bowel	24.64
Duplication chromosome 15	None	0.12
Mosaic trisomy 21	Cardiac defect, cardiac echogenic focus	6.12
Mosaic deletion 18q	Cardiac echogenic focus	0.31
Mosaic isochrome 12p	Nuchal edema, diaphragmatic hernia	3.78
Mosaic isochrome 12p	Cardiac echogenic focus, hydronephrosis, short limbs	11.18
Mosaic ring chromosome 4	Cardiac defect	2.33

Table 3—Incidence of sonographically detected fetal defects in chromosomally normal and abnormal fetuses and positive and negative likelihood ratios, respectively

Sonographically detected fetal defects	Normal karyotype		Abnormal karyotype		Likelihood ratio	
	$n = 3617$	%	$n = 237$	%	Positive	Negative
Absent corpus callosum	13	0.36	6	2.53	7.03	0.98
Dandy–Walker malformation	10	0.28	7	2.95	10.54	0.97
Holoprosencephaly	6	0.17	5	2.11	12.41	0.98
Ventriculomegaly	168	4.64	25	10.55	2.27	0.94
Choroid plexus cysts	142	3.93	36	15.19	3.87	0.88
Nuchal edema/cystic hygroma	133	3.68	44	18.57	5.05	0.85
Ascites, pericardial or pleural effusion	73	2.02	18	7.59	3.76	0.94
Facial cleft	35	0.97	13	5.49	5.66	0.95
Micrognathia	17	0.47	25	10.55	22.45	0.90
Nasal bone hypoplasia	48	1.33	40	16.88	12.69	0.84
Cardiac defect	138	3.82	105	44.30	11.60	0.58
Intracardiac echogenic focus	335	9.26	50	21.10	2.28	0.87
Diaphragmatic hernia	29	0.80	10	4.22	5.28	0.97
Exomphalos	21	0.58	10	4.22	7.28	0.96
Collapsed stomach	21	0.58	18	7.59	13.09	0.93
Duodenal atresia	7	0.19	5	2.11	11.11	0.98
Hyperechogenic bowel	119	3.29	11	4.64	1.41	0.99
Renal cystic dysplasia	16	0.44	7	2.95	6.70	0.97
Hydronephrosis	114	3.15	31	13.08	4.15	0.90
Spinal abnormality	19	0.53	4	1.69	3.19	0.99
Mild shortening of long bones	22	0.61	11	4.64	7.61	0.96
Abnormality of the hands	44	1.22	52	21.94	17.98	0.79
Talipes	55	1.52	15	6.33	4.16	0.95
No defect	2424	67.0	14	5.9	0.09	2.85

DISCUSSION

The findings of this study demonstrate that more than 90% of fetal chromosomal abnormalities identified by amniocentesis can be detected by qf-PCR without the need for full karyotyping, which is time consuming and expensive. Furthermore, the data indicate that the detection of chromosomal abnormalities is improved more than 95% if, in addition to qf-PCR, full karyotyping is carried out in those cases demonstrating certain fetal defects detected by ultrasound examination carried out at the time of amniocentesis.

Our finding that more than 90% of chromosomal abnormalities can be detected by qf-PCR is compatible with previous reports. A major multicentre study in the United Kingdom, involving 98 166 pregnancies

DOI: 10.1002/pd

Table 4—Consequences of different policies on full karyotyping in the detection of chromosomal abnormalities

Policy on full karyotyping	Full karyotyping $n = 3854$ (%)	Detection rate of chromosomal abnormalities	
		Normal qf-PCR $n = 18$ (%)	Normal and abnormal qf-PCR $n = 237$ (%)
All cases	3854 (100)	18 (100)	237 (100)
None of the cases	0 (0)	0 (0)	219 (92.4)
Cases with defects and combined LR of:			
Any defect	1408 (36.5)	16 (88.9)	235 (99.2)
LR $\geq$ 1.0	621 (16.1)	14 (77.8)	233 (98.3)
LR $\geq$ 3.0	309 (8.0)	10 (55.6)	229 (96.6)
LR $\geq$ 5.0	208 (5.4)	7 (38.9)	226 (95.4)

undergoing amniocentesis, reported that rapid aneuploidy testing for chromosomes 13, 18, and 21 would have detected only 70% of fetal chromosomal abnormalities diagnosed by full karyotyping (Caine *et al*., 2005). However, the detection rate would have increased to more than 90% had the authors included chromosomes X and Y in addition to 13, 18, and 21, in rapid aneuploidy testing, and had they excluded from the abnormal group the balanced rearrangements with no clinical significance (Leung and Lao, 2005). An observational study of 27 964 invasive tests, mainly amniocenteses in which both full karyotyping and qf-PCR for chromosomes X, Y, 13, 18, and 21 were carried out, reported that qf-PCR identified 95% (1058 of 1110) of the clinically significant chromosomal abnormalities (Cirigliano *et al*., 2005).

We have previously suggested that in women undergoing chorionic villous sampling, all samples should be analysed by qf-PCR and full karyotyping should be reserved for those with substantially increased fetal nuchal translucency thickness (Chitty *et al*., 2006). Similarly, in the case of amniocentesis, it was suggested that rapid aneuploidy testing could be the main method of analysing the samples, with full karyotyping being reserved only for cases with sonographically detectable fetal defects. In two studies with a combined total of 3235 cases, there were 177 clinically significant chromosomal abnormalities and 157 (88.7%) of these were detectable by FISH or qf-PCR (Leung *et al*., 2004; Thein *et al*., 2000). A policy of rapid aneuploidy testing in all cases and, in addition, full karyotyping in the 22% of cases with sonographically detectable fetal defects would have resulted in the detection of 96.0% (170) of all chromosomal abnormalities. In our study, 36.5% of the fetuses had sonographically detectable defects, and a policy of qf-PCR in all cases, with full karyotyping in those with a fetal defect, identified 99% of the chromosomal abnormalities.

The incidence of sonographically detectable fetal defects in women undergoing amniocentesis and, therefore, the need for full karyotyping essentially depends on two factors. Firstly, the nature of the centre performing amniocentesis; a higher proportion of sonographic defects will be seen in specialist fetal medicine units where pregnancies with such defects are referred for further assessment, compared to hospitals providing routine care where amniocentesis is primarily performed because of an increased risk following screening for trisomy 21. Secondly, the incidence of defects depends on whether minor deviations from normality, such as the presence of an intracardiac echogenic focus or mild shortening of the limbs, are included in addition to major fetal structural defects. In our study, 36.5% of fetuses had sonographically detectable defects and 92.8% of the chromosomal abnormalities in this group were detected by qf-PCR, whereas in the fetuses with no detectable defects, 85.7% of the chromosomal abnormalities were detected by qf-PCR. Therefore, the proportion of chromosomal abnormalities that are detectable by qf-PCR in a given prenatal diagnosis service will vary with the proportion of cases with fetal defects.

In general, each clinically significant chromosomal abnormality has it own syndromal pattern of multiple sonographically detectable defects (Nicolaides *et al*., 1992; Snidjers and Nicolaides, 1996). Furthermore, the importance of each defect, in terms of its association with chromosomal abnormalities, depends on the commonality of the defect in chromosomally abnormal fetuses compared to the chromosomally normal ones, rather than its anticipated degree of associated handicap. In this respect, a clenched hand or polydactyly is more important than diaphragmatic hernia, exomphalos, or holoprosencephaly. We have therefore suggested an approach in which the likelihood for chromosomal abnormalities for each fetus is calculated by multiplying the positive and negative likelihood ratios, depending on the presence or absence of a series of defects. Our results demonstrate that the detection rate of chromosomal abnormalities depends on the cut-off in the combined likelihood ratio selected for performing full karyotyping. The aim of our study was to propose the methodology for such an approach rather than to provide accurate likelihood ratios for each defect. The latter can only be established by major population-based prospective studies rather than studies on a pre-selected group of patients undergoing amniocentesis in one fetal medicine unit.

DOI: 10.1002/pd

It could be argued, on economic grounds, that full fetal karyotyping should be carried out in all women having amniocentesis and will involve additional cost. The lifetime cost for the provision of care to people with undetected chromosomal abnormalities may be greater than any saving from avoiding full karyotyping, even if most estimates are confined to trisomy 21, which is in any case detectable by qf-PCR (Waitzman *et al*., 1994). Indeed, this economic argument could also be advanced in support of analysing all samples for a rapidly expanding list of genetic disorders and identifiable susceptibilities to postnatal disease, which can now be diagnosed by molecular biology techniques. However, the ethical and consent issues associated with such policies would be considerable.

The alternative view is that analysis of the sample following invasive testing should be confined to the diagnosis of the condition for which screening and pre-test counselling was carried out in the first place. This issue was extensively discussed in the 1980s, when maternal serum AFP was widely used in screening for neural tube defects and amniocentesis was performed to measure acetyl cholinesterase in patients with high AFP. On the issue of whether karyotyping should additionally be carried out, the prevailing view was that karyotyping samples with a low yield of chromosomal abnormalities was an unnecessary cost at the expense of other health care provisions (Gosden *et al*., 1981; Thiagarajah *et al*., 1995). The same is also true in the case of amniocentesis for other indications, such as infection or haemoglobinopathies, where the samples are not automatically subjected to full karyotyping. An alternative argument was that it is unfair for patients being subjected to the risk of invasive testing to miss out on the opportunity to exclude chromosomal defects as well as spina bifida of ß-thalassaemia.

In the case of chromosomal abnormalities, screening is essentially directed towards the identification of pregnancies at risk of trisomy 21. In historical terms, the basis of offering amniocentesis to women older than 35 was because of the realisation that the risk for this specific abnormality increases with maternal age. Subsequently, the introduction of second-trimester maternal serum biochemical screening focussed on the development of algorithms for the calculation of risk for trisomy 21. In screening by maternal age or serum biochemistry, invasive testing and full karyotyping, which was until recently the only method of analysing the samples, occasionally resulted in the diagnosis of other chromosomal abnormalities in addition to trisomy 21. Although this may be considered to be a beneficial side effect of screening for trisomy 21, in the case of certain conditions, such as sex chromosome aneuploidies, a new controversy was created. In a recent survey of obstetricians in the United Kingdom to learn what they considered to be essential testing, in addition to trisomy 21, after amniocentesis following screening for trisomy 21, about 50% responded positively for trisomies 18 and 13 but less than 10% did so for the sex chromosome aneuploidies (Grimshaw *et al*., 2003). The fact that it is now possible to analyse the samples by qf-PCR for the specific condition for which they were obtained should not be considered a disadvantage compared to the previous method of full karyotyping, which by default could also diagnose other incidental abnormalities.

In the last 15 years, ultrasonographic examination of the fetus has acquired an increasingly important role in identifying fetuses with a wide variety of chromosomal abnormalities. In contrast to maternal age or serum biochemical screening for trisomy 21, the detection of sonographic defects created a new challenge in counselling the parents as to the likely prognosis, which often depended on whether the defect is isolated or associated with other abnormalities. For example, the parents of fetuses with hyperechogenic bowel would be counselled that the prognosis is likely to be normal unless there is an underlying congenital infection, cystic fibrosis or a wide range of chromosomal abnormalities. Consequently, if the parents choose to have an amniocentesis, they do so with the clear understanding that the sample would be tested for all these conditions, including full karyotyping as some of the expected chromosomal abnormalities are not detectable by qf-PCR.

This disease-oriented approach in defining the necessary investigations following the diagnosis of a fetal defect is clearly different from testing for all chromosomal abnormalities because a screening test indicates increased risk for trisomy 21. In this study, we propose an approach to identify the group of patients among those undergoing amniocentesis that would require full karyotyping. This approach is based on the findings of the ultrasound examination, which is in any case performed before amniocentesis.

ACKNOWLEDGEMENT

This study was supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116)

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DOI: 10.1002/pd