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Research

Fetal nuchal translucency scan and early prenatal diagnosis of chromosomal abnormalities by rapid aneuploidy screening: observational study

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Abstract

Objective To investigate an approach for the analysis of samples obtained in screening for trisomy 21 that retains the advantages of quantitative fluorescent polymerase chain reaction (qf-PCR) over full karyotyping and maximises the detection of clinically significant abnormalities.
Design Observational study.
Setting Tertiary referral centre.
Subjects 17 446 pregnancies, from which chorionic villous samples had been taken after assessment of risk for trisomy 21 by measurement of fetal nuchal translucency (NT) thickness at 11 to 13^{+6} weeks of gestation.
Interventions Analysis of chorionic villous samples by full karyotyping and by qf-PCR for chromosomes 13, 18, 21, X, and Y.
Main outcome measure Detection of clinically significant chromosomal abnormalities.
Results The fetal karyotype was normal in 15 548 (89.1%) cases and abnormal in 1898 (10.9%) cases, including 1722 with a likely clinically significant adverse outcome. Karyotyping all cases would lead to the diagnosis of all clinically significant abnormalities, and a policy of relying entirely on qf-PCR would lead to the diagnosis of 97.9% of abnormalities. An alternative strategy whereby qf-PCR is the main method of analysis and full karyotyping is reserved for those cases with a minimum fetal NT thickness of 4 mm would require full karyotyping in 10.1% of the cases, would identify 99.0% of the significant abnormalities, and would cost 60% less than full karyotyping for all.
Conclusions In the diagnosis of chromosomal abnormalities after first trimester screening for trisomy 21, a policy of qf-PCR for all samples and karyotyping only if the fetal NT thickness is increased would reduce the economic costs, provide rapid delivery of results, and identify 99% of the clinically significant chromosomal abnormalities.

Introduction

Prenatal diagnosis of chromosome abnormalities has traditionally been done after culture of amniotic fluid or chorionic villous cells. This approach leads to the detection of aneuploidy, such as trisomy 21; sex chromosome anomalies; and chromosome rearrangements, which may be balanced (and will often be familial) or unbalanced. Culture and analysis of fetal cells is time consuming (the results are available about two weeks after invasive testing) and labour intensive (one technician can handle about 250 samples a year).

Advances in molecular technology have led to the introduction of quantitative fluorescent polymerase chain reaction (qf-PCR), which can provide diagnosis of the common autosomal aneuploidies—trisomies 21, 18, and 13; all sex chromosome aneuploidies; and triploidy within two days of sampling.[1–4] Furthermore, one technician can examine up to 5000 samples a year. The disadvantage of qf-PCR is that it cannot detect all chromosomal rearrangements.[5] Although many of the defects that cannot be detected are of little or no clinical significance, a shift in prenatal diagnosis from full karyotyping to qf-PCR would be associated with failure to diagnose some significant abnormalities. It therefore can be argued that, in order to comply with the recommendation of the white paper on genetic services that all prenatal results should be available within three days,[6] rapid testing by qf-PCR should always be followed by full karyotyping.[7]

Three major arguments can be made against double testing in all cases. Firstly, in the United Kingdom, universal screening and counselling of the patients is done for trisomy 21 and not for all chromosomal abnormalities. Secondly, the initial diagnosis of chromosomal rearrangements often needs further investigations, some of which are invasive, to distinguish between those that are clinically significant and those that are not. Thirdly, a policy of double testing would substantially increase the cost of screening.

Traditionally, screening for trisomy 21 was based on maternal age and biochemical testing of second trimester maternal serum. However, it can now be provided effectively by a combination of the ultrasonographic measurement of fetal nuchal translucency (NT) thickness and biochemical testing at 11 weeks to 13 weeks and six days (13^{+6} weeks) of gestation.[8] Increased NT thickness is found not only in fetuses with trisomy 21 but also in a high proportion of those with other clinically significant chromosomal abnormalities.[9]

We examined the association between fetal NT thickness and chromosomal rearrangements and the likely impact of a policy whereby qf-PCR is the main method of analysis and full karyotyping is reserved for those cases with increased fetal NT thickness in the early pregnancy scan.

Methods

At King's College Hospital and the Fetal Medicine Centre, London, chorionic villous sampling for fetal karyotyping is done at the parents' request after screening for Down's syndrome by

ultrasound examination with or without maternal serum biochemistry at 11-13[+6] weeks, at which the fetal crown-rump length and NT are measured.[8] Since September 1997 all chorionic villous samples have been analysed by both qf-PCR and full karyotyping (TDL Genetics, London). We searched our databases to identify all cases examined up to January 2005 to determine the fetal crown-rump length; NT thickness; the results of qf-PCR, full karyotype, and any further investigations, including amniocentesis, detailed ultrasound, disomy studies, and parental karyotyping; and pregnancy outcome.

The karyotypes obtained from cultured chorionic villi were classified as normal or abnormal. In the abnormal group, some karyotypes conferred a high risk of adverse outcome whereas others needed further investigations to define the prognosis accurately and were thus initially classified as uncertain prognosis. On the basis of the chorionic villous sampling karyotype and the results of further investigations, we further classified the cases into those with and those without a risk of clinically significant adverse outcome and according to whether they were detected by qf-PCR. We then examined the distribution of fetal NT thickness in each group and estimated the potential impact, in terms of prenatal diagnosis of clinically significant chromosomal abnormalities, of a policy whereby the main method of analysing chorionic villous samples is qf-PCR and full karyotyping is reserved for cases with increased fetal NT thickness.

Results

Chorionic villous sampling was done in 17 479 cases. The median maternal age was 37 (range 15-49) years, the median gestation was 12 (11-13[+6]) weeks, and the median fetal NT thickness was 2.1 (0.5-24.0) mm.

The chorionic villous sampling karyotype was normal in 15 548 cases and abnormal in 1931. In some of the 1931 cases with abnormal karyotype, further investigations were needed to ascertain if the fetus would have a clinically significant adverse outcome. In 33 such cases these necessary investigations were not done, either because the parents did not want them or because the pregnancies ended in miscarriage or termination before the investigations could be done. We did not include these 33 cases in the further analysis of the data. We subdivided the remaining 1898 cases into 1722 cases with a likely clinically significant adverse outcome and 176 with no risk or very low risk of adverse outcome. Therefore, in total, we had 15 724 cases at no or low risk and 1722 at high risk of clinically significant adverse outcome (table 1).

In the 15 724 cases at no risk or low risk of clinically significant abnormal karyotype, the qf-PCR was normal in 15 718 (99.96%) and abnormal in 6 (0.04%). In these six cases the chorionic villous sampling culture was mosaic for chromosomes 13, 21, or X and amniocentesis showed a normal fetal karyotype. In total, the chorionic villous sampling karyotype showed mosaicism in 91 cases, but subsequent investigations showed that in 77 (84.6%) cases the fetal karyotype was normal and therefore the mosaicism was confined to the placenta.

In the 1722 cases at high risk of clinically significant adverse outcome, qf-PCR detected the abnormality in 1685 (97.9%) cases and did not detect it in 37 (2.1%). Table 2 summarises the distribution of fetal karyotypes according to fetal NT thickness. A policy of relying entirely on qf-PCR would lead to the diagnosis of 1685 (97.9%) of the 1722 clinically significant chromosomal abnormalities without the need for further investigations. An alternative policy, whereby qf-PCR is the main method of analysis and full karyotyping is reserved only for those cases with a minimum fetal NT of 4 mm, would identify 1704 (99.0%) of the clinically significant chromosomal abnormalities. With this approach 1767 (10.1%) of all 17 446 cases would need full karyotyping. However, 18 chromosome abnormalities conferring a significant risk of adverse outcome (0.1% of the 17 446 fetuses karyotyped or 1.0% of the 1722 clinically significant abnormalities) would still remain undetected (table 3). These 18 cases included six in which major fetal defects were identified by ultrasound examination and three in which the pregnancies ended with a miscarriage.

A health technology assessment study on the evaluation of molecular tests for prenatal diagnosis of chromosome abnormalities reported that in the UK the cost for full karyotyping is about £150 ($264; €220) per sample and estimated that, in a laboratory examining a minimum of 5000 samples a year, the cost for qf-PCR would be £42, which is 72% cheaper.[10] On the basis of these estimates, the cost of analysing the samples in this

Table 1 Findings* in pregnancies that had fetal karyotyping by chorionic villous sampling (CVS)

Final karyotype after CVS and further investigation	Total (n=17 446)	CVS qf-PCR result	
		Abnormal (n=1691)	Normal (n=15 755)
No risk or low risk of adverse outcome:	15 724 (90.1%)	6 (0.04%)	15 718 (99.96%)
Normal karyotype 46,XY or 46,XX	15 548	–	15 548
Abnormal karyotype:	176	6	170
Normal variants	8	–	8
Duplications and deletions (with good outcome)	6	–	6
Marker chromosomes (inherited)	2	–	2
Balanced rearrangements (inherited)	50	–	50
Balanced rearrangements (de novo)	15	–	15
Mosaic balanced rearrangements	2	–	2
CPM trisomies 2, 4, 6, 7, 8, 9, 10, 12, 15, 16, 19, 20, 22	18	–	18
CPM mosaic trisomies 2, 4, 6, 7, 8, 9, 10, 12, 15, 16, 19, 20, 22	46	–	46
CPM mosaic trisomies 13, 18, 21	12	3	9
CPM mosaic duplications or deletions	6	–	6
CPM mosaic marker chromosomes	2	–	2
CPM mosaic sex aneuploidies	9	3	6
High risk of adverse outcome:	1722 (9.9%)	1685 (97.9%)	37 (2.1%)
Group A—all detected by qf-PCR:	1667	1667	0
Trisomy 13	138	138	–
Trisomy 18	336	336	–
Trisomy 21	936	936	–
Turner	134	134	–
Other sex aneuploidies	53	53	–
Monosomy 21	1	1	–
Triploidy	69	69	–
Group B—some detected by qf-PCR:	30	18	12
Mosaic trisomies 13, 18, 21	3	2	1
Mosaic sex aneuploidies	5	1	4
Unbalanced rearrangements	22	15	7
Group C—none detected by qf-PCR:	25	0	25
Trisomies 8, 9, 15, 16, 20, 22	5	–	5
Duplications and deletions	13	–	13
Marker chromosomes (de novo)	1	–	1
Mosaic trisomies 8, 9, 20, 22	1	–	1
Mosaic duplications or deletions	4	–	4
Mosaic marker chromosomes (de novo)	1	–	1

CPM=confined placental mosaicism; qf-PCR=quantitative fluorescent polymerase chain reaction.
*First two columns show result of fetal karyotype, which in some cases required additional investigations after initial CVS. Last two columns show CVS qf-PCR result. 33 cases in which necessary further investigations were not done are not listed.

Table 2 Consequences of three policies on full karyotyping in detection of cases at high risk of adverse outcome. Values are numbers (percentages)

Policy on full karyotyping*	Need for full karyotyping	Pregnancies at high risk of adverse outcome	
		Detected	Not detected
In all cases	17 446 (100)	1722 (100)	0
In no cases	–	1685 (97.9)	37 (2.1)
In cases with increased fetal NT:			
≥6.5 mm	629 (3.6)	1693 (98.3)	29 (1.7)
≥5.5 mm	901 (5.2)	1697 (98.5)	25 (1.5)
≥4.5 mm	1350 (7.7)	1700 (98.7)	22 (1.3)
≥4.0 mm	1767 (10.1)	1704 (99.0)	18 (1.0)
≥3.5 mm	2508 (14.4)	1705 (99.0)	17 (1.0)
≥2.5 mm	6476 (37.1)	1712 (99.4)	10 (0.6)

*Firstly, full karyotyping in all cases; secondly, full karyotyping in no cases; thirdly, full karyotyping in cases with increased fetal nuchal translucency (NT) thickness.

study would be £2 616 900 for the policy of full karyotyping for all, £732 732 for the policy of qf-PCR for all, and £997 782 for the policy of qf-PCR for all followed by full karyotyping for those with a minimum fetal NT thickness of 4 mm.

Discussion

Our findings show that in a population who have had chorionic villous sampling for fetal karyotyping after first trimester screening for trisomy 21 by measurement of fetal NT thickness, all cases of trisomy 21 and 97.9% of all other clinically significant chromosomal abnormalities can be diagnosed by qf-PCR alone. The distribution of chromosomal abnormalities in this study is similar to that in a multicentre study that screened for trisomy 21 by maternal age and fetal NT thickness in 96 127 pregnancies and is therefore likely to be representative of the whole population.[8]

Table 3 18 cases with clinically significant abnormal fetal karyotype and nuchal translucency (NT) thickness <4 mm

Chorionic villous sampling karyotype	Amniocentesis karyotype	Fetal NT thickness (mm)	Fetal abnormalities	Outcome
46,XX, add(18)(p11.2)	–	3.6		Fetal death
47,XX +9	–	1.6		Fetal death
46,XX,del(13)(q22)	–	1.1		Fetal death
46,XY,del(3)(q23q26.1)	–	3.3	Major cardiac defect	Termination
mos 46,XX,t(9;19)(q10;q10), i(18)(q10)[19]/46,XX,t(9;19)(q10:q10)[13]	46,XX, t(9;19)(q10;q10) de novo, del(18)(p10) de novo [13]	3.3	Spina bifida, cardiac defect	Termination
46,XX,del(4)(p15.2)	–	3.0	Facial cleft	Termination
46,XY,del(5)(q32q32)	–	2.5	Major cardiac defect	Neonatal death
46,XY,add(21)(p11.1)	–	2.3	Growth restriction, exomphalos	Termination
mos 47,XX,+idic(9)/46,XX		2.9	Growth restriction	Termination
mos 47,XXX[4]/46,XX [29]	mos 47,XXX (29%)/46,XX	2.9		Termination
mos 45,X[14]/46,XX[29]	mos 45,X (20%)/46,XX	2.7		Live birth
mos 47,XY+marker(17%)/46,XY	mos 47,XY + r(10)/46,XY	2.4		Termination
mos 47,XX,+mar.ish inv dup (15)q(13)(D15Z1++,SNRPN++)[59]/46,XX[15]	mos 47,XX, +inv dup(15) (q13)(76%)/46,XX	2.3		Termination
47, XY,+mar.ish der(22)	–	2.1	Cat eye syndrome	Termination
47,XX,+16	46,XX, UPD16	1.8		Live birth
47,XX,+9	–	1.8		Termination
46,XY,del(17)(p11.2p11.2)	–	1.8		Termination
47,XY,+15	mos 47,XY +15/46,XY, UPD 15	1.3		Termination

Advantages of replacing full karyotyping with qf-PCR

Our findings provide supportive evidence for the recommendation of the UK National Screening Committee that, in newly established screening programmes for trisomy 21, rapid aneuploidy diagnosis alone may be offered. An advantage of replacing full karyotyping by qf-PCR is the substantially lower economic cost.[10] Additionally, such a policy can have considerable benefits to women and their partners, as early availability of results reduces anxiety and allows for earlier decision making in cases in which the fetus has a significant abnormality.[11] Furthermore, the need for further investigations caused by detecting chromosomal rearrangements of little or no clinical significance can be avoided, as can the anxiety this causes. In some of our cases the degree of anxiety was high enough for the parents to elect to terminate the pregnancy without waiting for the results of further investigations, which may have shown a normal karyotype. As shown in our study, about 10% of cases with an abnormal chorionic villous sampling karyotype result have no risk or only a small risk of a clinically significant fetal abnormality.

Disadvantages of relying on qf-PCR alone

Three disadvantages of replacing full karyotyping with qf-PCR exist. Firstly, some cases at significant risk of adverse outcome would go undetected, and some parents would be denied the option of terminating such a pregnancy. Secondly, in some cases with normal fetal karyotype and confined placental mosaicism for chromosomes 13, 18, 21, or X, the qf-PCR could lead to the erroneous diagnosis of fetal abnormality. This problem, observed in six of our cases, can be reduced by independently assaying two separately identified fronds of villi from the same sample and doing full karyotyping in any case in which mosaicism is suspected on qf-PCR. Consideration will also need to be given to provision of back-up culture and full karyotyping where there is a positive initial result but no ultrasonographic abnormality. Thirdly, some parents who are carriers of a balanced rearrangement and could therefore in a subsequent pregnancy produce offspring with an unbalanced chromosomal abnormality may remain undetected. However, data from a large US study imply that in the absence of definite family history, most carriers are at very low risk of an unbalanced abnormal outcome at term.[12] Additionally, qf-PCR could identify a large proportion of the unbalanced rearrangements in the ongoing and subsequent pregnancies.

Value of fetal NT thickness in deciding if full karyotyping is needed

Fetal NT thickness of 4 mm or more is seen in about 0.5% of the general population.[8] This is associated with a high risk of a whole range of fetal malformations and genetic syndromes, so the management of such pregnancies includes a series of investigations in addition to fetal karyotyping.[9] Our data suggest that if this small group of pregnancies is investigated by full karyotyping and all others by qf-PCR alone, full karyotyping would be necessary in about 10% of cases that have invasive testing and about 99% of all clinically significant chromosomal

What is already known on this topic

Measurement of fetal nuchal translucency (NT) thickness provides effective first trimester screening for trisomy 21

Subsequent analysis of those at high risk by full karyotyping of chorionic villous samples detects all clinically significant chromosomal abnormalities

Analysis by quantitative fluorescent polymerase chain reaction (qf-PCR) is cheaper and faster but does not detect all significant abnormalities

What this study adds

Analysis of samples by qf-PCR in all cases and full karyotyping only in the 10% of cases with fetal NT of 4 mm or more identifies 99% of significant chromosomal abnormalities

This strategy costs 60% less than full karyotyping for all

abnormalities would be identified. Indeed, the observation of failed diagnosis in about 1% of cases may be an overestimate because, as shown in table 3, about half of these cases either miscarried spontaneously or had major abnormalities that were detected by the routine second trimester scan.

In summary, a policy of initially doing rapid aneuploidy exclusion for chromosomes 13, 18, 21, X, and Y in the first trimester, with full karyotyping only if the fetal NT thickness is increased, would reduce the economic costs of a first trimester screening programme for trisomy 21, allowing for rapid delivery of results and minimising parental anxiety about ambiguous results and the risk of unnecessary termination of pregnancy. However, in up to 1 in 1000 fetuses having chorionic villous sampling a chromosomal rearrangement conferring a high risk of adverse outcome will go undetected.

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Ethical approval: King's College Hospital Ethical Committee approved the study.

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