Glasgow Royal Infirmary, who performed the radioimmunoassays, and the nursing staff of the Burns Unit, Glasgow Royal Infirmary, for their cooperation.

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(Accepted 26 May 1987)

Chorionic villi sampling: cytogenetic and clinical findings in 500 pregnancies

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Abstract

The cytogenetic findings were analysed in a series of 500 pregnancies in which chorionic villi sampling was performed. In all cases a direct method was used, karyotyping being successful in 481 cases (96.2%). The main indication for sampling was

maternal age over 36 (412 cases; 82.4%). Abnormal laboratory findings resulted in 24 terminations of pregnancy (4.8%); in addition five unexpected balanced chromosome rearrangements were detected. Twelve of 15 cytogenetic discrepancies were detected at amniocentesis, two after termination, and one at spontaneous abortion. Complete follow up data were available for the first 250 patients, among whom nine pregnancies (3.6%) ended in spontaneous abortion before the 20th week. There were no false negative findings. Seventy additional chromosome studies were performed because of failure of chorionic villi sampling or equivocal results, or for confirmation.

Counselling before chorionic villi sampling should include the possibility that subsequent amniocentesis may be needed should mosaicism or other unexpected abnormalities be found. The success rate and accuracy of karyotyping chorionic villi samples by the direct method are acceptable but distinctly less than those of karyotyping cultured amniotic fluid cells.

Introduction

Simoni *et al* initially described chorionic villi sampling in the prenatal diagnosis of fetal chromosome abnormalities,¹ and their report was followed by two collaborative studies (M Mikkelsen and S Ayme, unpublished)² and only a few large one centre studies.^{3,4} We began chorionic villi sampling for prenatal chromosome studies

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in January 1985. This paper analyses our cytogenetic and clinical findings in the first 500 patients, among whom complete follow up data were available for the first 250.

Materials and methods

Transcervical chorionic villi sampling was carried out with a Portex cannula, Braun cannula, silver cannula, or Wolf biopsy forceps (3 mm diameter) between eight and 11 weeks of gestation. Gynaecologists from three university departments contributed samples (Amsterdam 211, Leiden 162, Utrecht 100), and a further 27 samples were received from a gynaecologist in a non-academic hospital in Arnhem.

chromosome translocation (eight), and a few miscellaneous conditions (eight). Successful karyotyping was possible in 481 cases (96.2%), samples from the remaining 19 patients containing insufficient cytogenetic material for a reliable diagnosis. Eight of the failures occurred among the first 250 patients and 11 among the second 250. The distribution of inadequate samples did not differ substantially among the participating centres (Amsterdam 6/211 (2.8%); Leiden 8/162 (4.9%); Utrecht 5/100). In 18 of these 19 cases a normal fetal karyotype was found after amniocentesis. In one case fetal sex was determined by DNA study alone.

Table I shows the abnormal laboratory findings resulting in the 24 terminations of pregnancy (4.8%). Attempts were made to confirm abnormal cytogenetic findings by culturing and karyotyping fetal or placental cells, or both, after abortion. These cell cultures were successful in 15 of 17 cases. In two—both of trisomy 21—there was doubt about the fetal origin of the

TABLE I—Abnormal laboratory findings resulting in termination of pregnancy (n=24)

Indication for chorionic villi sampling	Abnormal laboratory finding	No (%) of cases
(A) Maternal age ≥ 36 (n=412)*	Trisomy 21† Trisomy 18 Trisomy 13 47,XX,+mar‡	7 3 2 1 13 (3.2)
(B) Previous child with mutant chromosome aberration (n=42)	Trisomy 21	1 (2.4)
(C) X Linked disease (n=30)	46,XY (DNA: affected) 46,XY (without DNA study) 47,XX,+21 46,XYq+§ DNA studies only	2 4 1 1 1 9 (30.0)
(D) Parental chromosome translocation (n=8)	46,XY,-21,+t(16;21) p(11;q22)mat	1 (12.5)
(E) Miscellaneous (n=8)		

*In five patients combined indication (A+B) was tabulated under A.

†Twice only maternal material received after termination (see text).

‡Diagnosis confirmed in placenta after abortion (see table II).

§Diagnosis not confirmed after abortion (see table II).

TABLE II—Cytogenetic discrepancies (n=15)

Case No	Karyotype at chorionic villi sampling	Results after amniocentesis	Outcome
47	47,XX,+mar		Therapeutic abortion: placenta 47,XX,+mar; fetus 46,XX
103	46,XYq+		Therapeutic abortion: 46,XY*
113	46,XX/47,XX,+12 (7:5)	46,XX	Normal girl (46,XX)
130	46,XX/47,XX,+5 (8:7)	46,XX	Normal girl (46,XX)
162	46,XX/45,X/45,X,+mar	46,XX	Normal girl
210	92,XXYY (n=23)	46,XY	Normal boy
222	45,X/46,XX (8:1)	46,XX	Normal girl
258	46,XX/47,XX,+16 (21:9)		Spontaneous abortion 15 weeks: placenta 46,XX; fetus failed
268	46,XY/92,XXYY (8:8)	46,XY	Normal boy
272	46,XY/47,XY,+p19 (15:2)	46,XY	Normal boy
277	46,XX/47,XY,+mar (9:3)	46,XX	Normal girl
378	47,XX,+11 (n=16)	46,XX	Normal girl
411	46,XY/50,XY+2,+3,+7,+15 (28:5)	46,XY	Normal boy (46,XY)
428	46,XY/47,XY+7 (12:3)	46,XY	Normal boy (46,XY)
495	46,XX/46,XX,5p+ (14:2)	46,XX	Normal girl (46,XX)

*Indication X linked mental retardation.

In all cases the direct method was used,¹ with small modifications; all samples were stored overnight at -20°C after fixation. Specimens from Leiden, Utrecht, and Arnhem were processed up to fixation in the respective centres. Villi fragments were transported to Amsterdam after completion of the methanol-acetic acid fixation step. The average journey time was about one hour. Chromosomes were studied by Q banding and a minimum of 10 metaphases analysed in each case. Studies of deoxyribonucleic acid (DNA) for Duchenne muscular dystrophy and haemophilia A and B were performed at the department of human genetics in Leiden and will be the subject of a separate report.

The outcome of pregnancy in the first 250 patients was known.

Results

The main indication for chorionic villi sampling was a maternal age of 36 or over (412 cases; 82.4%); other indications were a previous child with a mutant chromosome aberration (42 cases), X linked disease (30), parental

TABLE III—Outcomes of pregnancy in first 250 patients

	No of pregnancies	No of children
Therapeutic abortion	13	13
Spontaneous abortion up to 20 weeks	9*	9*
Preterm delivery between 20 and 27 weeks; stillbirths	2	2
Preterm delivery (28-36 weeks):		
Stillbirths		
Death in first week		
Alive after first week	9	10
Birth at term (37 weeks):		
Stillbirths		
Death in first week	1†	1†
Alive after first week	216	216
Total	250	251

*Including cases 181 (46,XY,-18,+der(18)) and 245 (47,XX,+18).

†Congenital heart disease.

tissue; in one case (47,XX,+21) only 46,XX cells were found in material which was diagnosed as "maternal" by the pathologist, and in the other case (47,XY,+21) we also found only 46,XX (maternal) cells after the abortion. In all but two (cases 47 and 103) of the 15 cases in which cell cultures were successful chromosome aberrations were confirmed after the abortion (see tables I and II). A further patient (not listed in table I) in whom a fetus with trisomy 18 was detected decided to continue her pregnancy. At term she delivered a girl, and trisomy 18 syndrome was confirmed by karyotyping lymphocytes from the baby. The flow diagram shows the diagnostic procedures and outcome of investigation in our series.

In the group with X linked disease as the indication for chorionic villi sampling six pregnancies were continued after the initial finding of a male

abnormalities. In addition, there were two stillbirths between 20 and 27 weeks. Nine pregnancies ended in the preterm birth of 10 liveborn children (one twin pregnancy). Of the 217 term births, one child died of congenital heart disease within the first week, two children were growth retarded, and one child had a congenital hip dysplasia.

Perinatal mortality from the 28th week of gestation till the end of the first week of life was 0.4% (one child out of 227).

Three women in the second group of 250 were admitted to hospital after chorionic villi sampling with signs of intrauterine infection; in one case this was accompanied by the clinical syndrome of septic shock. All three pregnancies ended in abortion before the 20th week. The details of these cases will be published separately.

Flow diagram showing diagnostic procedures and outcome of investigations.

karyotype; the decision to continue was based on normal DNA appearances (five cases) or normal factor VIII activity in fetal blood sampled at fetoscopy (one). In one case with a female karyotype trisomy 21 was found.

Besides one unbalanced chromosome pattern in the group with parental chromosome translocation, three balanced chromosome translocations were detected— $t(11;22)(q23q11)mat$, $t(21q22q)mat$, and $t(14q21q)mat$. In this series of 500 pregnancies five unexpected chromosome rearrangements were diagnosed— $t(1;5)(q25q33)$, $t(1;8)(p31q24)$, $t(13q14q)$, $inv(1)(p11q12)$, and $22p+$. Parental karyotyping as a consequence of these findings showed all cases to be familial; the translocations and inversion had a maternal origin, whereas the father was shown to be the carrier of the $22p+$ chromosome.

Table II lists the cytogenetic discrepancies between chorionic villi cells and other fetal tissues. There were seven discrepancies among the first 250 patients and eight discrepancies among the second 250; the total incidence of initial false positive findings was therefore 3.0%. The eight pregnancies with cytogenetic discrepancies which were continued and for which the outcome was known resulted in normal children born at term and of appropriate development for gestational age.

Table III gives the complete follow up data on the first 250 pregnancies. There were no false negative findings in this group. Nine pregnancies (3.8%) ended in spontaneous abortion before the 20th week, and two of these fetuses (cases 181 and 245; not listed in table I) showed chromosome

Discussion

In 481 (96.2%) of the 500 pregnancies in this series chorionic villi cells were successfully karyotyped by using the direct method only. In 18 pregnancies amniocentesis had to be done because the material was insufficient for cytogenetic diagnosis. In one patient fetal sex was determined by DNA study. Though the number of failures was a little higher in Leiden and Utrecht than in the Amsterdam centre, we have shown that successful karyotyping of transported chorionic villi is possible after initial processing elsewhere.

In 16 cases the pregnancy was terminated because of a chromosome aberration (table I); one additional pregnancy (case 103) was terminated because of a male karyotype. In all but four cases the diagnosis could be confirmed after the abortion. In two of these only maternal material was received after termination, and two others (cases 47 and 103) should probably be considered as discrepancies. Hence among the 15 cases of cytogenetic discrepancy (3.0%) recorded in the series (table II) accurate interpretation of indications to continue or terminate pregnancy was possible in all but one case; in case 47 the diagnosis was confirmed in placenta cells only.

Twelve of the 15 discrepancies were detected at amniocentesis, two after termination of pregnancy, and one after spontaneous abortion. Hogge *et al* in a series of 1000 patients reported eight discrepancies after amniocentesis and five after termination of pregnancy (1.3%).⁴ Simoni *et al*, also reporting on 1000 diagnoses, found two discrepancies after amniocentesis and six at termination (0.8%).³ Plainly we all learnt from the early difficulties experienced by Simoni *et al*.⁵ We therefore offer amniocentesis to all patients in whom mosaicism is found (or suspected) at chorionic villi sampling.

Our overall incidence of spontaneous abortions before the 20th week of gestation (9/237 pregnancies; 3.8%) was lower than that reported by Hogge *et al* (48/941; 5.1%) but identical with that in a subgroup of pregnancies sampled by Hogge *et al* between 9.0 and 11.6 weeks (3.8%).⁴ Simoni *et al* reported on 39 pregnancies which ended before 24 weeks' gestation (5.8%).³ No definite conclusions are possible about the chance of a spontaneous abortion based on the results of chorionic villi sampling. Though we have tried to convince patients of the need for randomised studies as proposed, for example, from Oxford (I Chalmers, personal communication), participation in the Netherlands has been little to none. The Canadian collaborative study now in progress may answer some of the questions in this respect in more detail.

Apart from karyotyping the 500 chorionic villi samples a total of 70 additional chromosome studies were performed in this series. This inevitably meant extra workload for the laboratory, more expense for the health insurance companies, and considerable emotional stress for the couples concerned. Nevertheless, we believe that these extra analyses are necessary to maintain optimal reliability of this new method.

We conclude that the success rate and accuracy of karyotyping chorionic villi samples by the "direct" method are acceptable but distinctly less than those of karyotyping cultured amniotic fluid cells.⁶ Counselling before chorionic villi sampling should include information on the failure rate and the possibility that subsequent

amniocentesis may be needed should mosaicism or some other unexpected abnormality be detected. The possible risks of the procedures for the pregnancy should also be emphasised.

As analysis of chorionic villi is more time consuming and less accurate than culturing and karyotyping amniotic fluid cells, efforts should be made to improve the cytogenetic accuracy of chorionic villi sampling and the identification of patients at risk of abortion or infection after the procedure. Meanwhile areas with limited cytogenetic resources should reserve chorionic villi sampling for high risk cases only.

We thank Dr M J Kloosterman, of Arnhem, for chorionic villi samples; Professor P L Pearson, of Leiden, for DNA studies for Duchenne muscular dystrophy and haemophilia A and B; and Dr K H Nicolaides, of London, for fetal blood sampling and factor VIII estimation. We also thank Ms M Stam-Johan for secretarial work and Professor P E Treffers for his comments on the manuscript.

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(Accepted 5 June 1987)

100 YEARS AGO

The announcement made last autumn that Sir James Paget had consented to take the office of President of the Pathological Society of London was hailed with universal satisfaction. Everyone must have been glad to learn that an eminently scientific, yet at the same time essentially medical institution, was about to obtain the leadership and active patronage of so great a medical scientist as Sir James Paget. The presidency of the leader of English surgery could not but be as useful as it is honourable to the Society, and it is not surprising that the Society's meeting-room in Berners Street was crowded on Tuesday night, when the illustrious President took the chair for the first time, and delivered a short address. He has long enjoyed the reputation of possessing great skill in selecting and submitting to his audience abstract questions of a kind which, on his own demonstration, are no mere intellectual luxuries. His address on this occasion showed this kind of skill developed to perfection. Sir James Paget admitted that he had for many years given up active work on the details of pathology. He spent most of the time devoted to the address in stating his convictions as to the practical value of that science, to which he had been led by an experience of over a quarter of a century in clinical work. He concluded that pathology should be cultivated and encouraged by all possible means. He especially insisted on the advantages which the private practitioner possesses for the study of clinical pathology, and scouted the prevalent opinion that practice and science were antagonistic. What practice might be in any particular case depended, he said, upon the individual practitioner, and not upon the subject-matter. The discoverer of vaccination was a country doctor. We know that Jenner must have laboured under innumerable disadvantages, which no living rural practitioner can bring forward as an excuse for neglecting scientific study. True, he was a disciple of Hunter, but, on the other hand, few living medical men in this country can deny that they received a fair education in the rudiments of medical science at the medical school where they worked before taking their qualification.

Again, we know that the country medical man's time is much occupied in the duties of practice; but there is nothing that relieves the mind of a hard-worked man so much as scientific observation. The notion that the confidence of the public in a practitioner is materially affected when he gains

the reputation of being given to scientific study, is a common fallacy; such study can always be carried on without detriment to other duties, just as many industrious and successful practitioners spend some of their time very profitably in the cultivation of social accomplishments and artistic pursuits.

Sir James Paget held up for imitation the example of Jenner, as that of a scientific practitioner. He further indicated the direction of pathological work in the future. He dwelt for a little on the subject of specifics, which he said in reality were diagnostic tests of disease. The forms of epilepsy which could be cured by bromides must, he declared, be different from the varieties of epilepsy not amenable to the action of these drugs.

Another rich field for clinical pathology, the especial department of the practitioner, was the study of the sequels of disease. There must be some definite reason why certain local affections were sequels of particular constitutional diseases, and that reason should be discovered. The true character of mixed disorders also deserved patient study.

Sir James Paget concluded by a reference to some more definite and concrete questions, pertinent rather to experienced pathologists than to practitioners. He spoke of the microscope, and pointed out that it must be made to reveal far more minute structures than it had yet discovered. Protoplasm, or indifferent tissue, whose component parts could not yet be detected by microscopy, must differ greatly in different organisms, and improvements in microscopic research might discover the difference. He also spoke of the examinations of tissues whilst still living—that is, directly after their removal from the body, in contradistinction to their inspection after hardening, staining, and mounting. These, however, we believe to be questions which only experts, like some of the habitual workers at the Pathological Society, can attempt to solve. Sir James Paget spoke of the value of the labours of such workers, and declared, most truly, as all must admit, that he had no senile objection to novelties. He concluded with the question and reply which will eternally run through science. The pupil and the ignorant multitude will ever ask: Where are we to stop? The master in science, or rather any person inspired with the true scientific spirit, will ever answer: We must not stop where we are.

(*British Medical Journal* 1887;i:164.)