countries,[3,4,6,14] this possibility cannot be excluded. In a family which showed no linkage to the above markers owing to such heterogeneity, an unexpected number of recombinants might be found. Thus, the available set of linked markers will be useful also for identifying genetic heterogeneity even in small families.

These results will hasten the search for and eventual isolation of the FAP gene sequences. The information on the localisation of YN5.48 on the other side of the FAP locus from Pi227 opens a direct approach with a powerful set of DNA markers to accurate diagnosis of FAP in family members at risk.

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REFERENCES

1. Bussey HJR. Familial polyposis coli. Baltimore: Johns Hopkins University Press, 1975.
2. Herrera L, Kakati S, Gibas S, et al. Gardner syndrome in a man with an interstitial deletion of 5q. *Am J Med Genet* 1986; **25:** 473–76.
3. Bodmer WF, Baily CJ, Bodmer J, et al. Localization of the gene for familial adenomatous polyposis on chromosome 5. *Nature* 1987; **328:** 614–16.
4. Nakamura Y, Lathrop M, Leppert M, et al. Localization of the genetic defect in familial adenomatous polyposis within a small region of chromosome 5 *Am J Hum Genet* 1988; **43:** 638–44.
5. Leppert M, Dobbs M, Scambler P, et al. The gene for familial polyposis coli maps to the long arm of chromosome 5. *Science* 1987; **238:** 1411–13.
6. Meera Khan P, Tops CMJ, Broek Mvd, et al. Close linkage of a highly polymorphic marker (D5S37) to familial adenomatous polyposis (FAP) and confirmation of FAP localization on chromosome 5q21–22. *Hum Genet* 1988; **79:** 183–85.
7. Vasen HFA, Griffioen G, Offerhaus GJA, et al. The value of screening and central registration of families with familial adenomatous polyposis. *Dis Colon Rectum* (in press).
8. Hofker MH, Wapenaar MC, Goor N, et al. Isolation of probes detecting restriction fragment length polymorphisms from X chromosome-specific libraries: potential use for diagnosis of Duchenne muscular dystrophy. *Hum Genet* 1985; **70:** 148–56.
9. Stewart GD, Bruns GAP, Wasmuth JJ, Kurnitt DM. An anonymous DNA segment (Pi227) maps to the long arm of chromosome 5 and identifies a BstX I polymorphism (D5S37). *Nucl Acid Res* 1987; **15:** 3939.
10. Lathrop GM, Lalouel JM. Easy calculations of lodscores and genetic risks on small computers. *Am J Hum Genet* 1984; **36:** 460–65.
11. Conneally PM, Edwards JH, Kidd KK, et al. Report of the committee on methods of linkage and reporting. 8th International Workshops on Human Gene Mapping. *Cytogenet Cell Genet* 1985; **40:** 356–59.
12. Ott J. In: Analysis of human genetic linkage. Baltimore: Johns Hopkins University Press, 1985.
13. Botstein D, White LW, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *Am J Hum Genet* 1980; **32:** 314–31.
14. Dunlop MG, Steel CM, Wyllie AH, et al. Linkage analysis in familial adenomatous polyposis: Order of C11p11 (D5S71) and Pi227 (D5S37) loci at the apc gene. *Genomics* 1989; **5:** 350–53.
15. Tops CMJ, Breukel C, Wijnen JTh, et al. Linkage relationships between APC, D5S37 (Probe pi227) and D5S71 (probe C11p11) in the Dutch polyposis families. *Cytogenet Cell Genet* (in press).
16. Meera Khan P, Tops CMJ, Breukel C, et al. Closely linked DNA markers for preclinical prediction of familial adenomatous polyposis. In: Herrera L, ed. Familial adenomatous polyposis. New York: Alan R Liss, 1989: 389–92.

Preliminary Communication

PRENATAL SEX DETERMINATION BY DNA AMPLIFICATION FROM MATERNAL PERIPHERAL BLOOD

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Summary The polymerase chain reaction was used to amplify a Y-specific repeat sequence in peripheral blood DNA samples from 19 pregnant women who had a gestational age of 9 to 41 weeks. Y-specific sequences were amplified from all 12 women who bore a male fetus but in none of 7 women who bore a female fetus. With stringent precautions against contamination, this technique may assist prenatal diagnosis of sex-linked genetic disorders.

INTRODUCTION

FETAL cells in peripheral maternal blood are, in theory, an alternative source of tissue to specimens obtained by invasive techniques such as amniocentesis and chorionic villus biopsy. In 1969, by examination of metaphase chromosomes, Walknowska et al[1] found cells with a probable male karyotype in the peripheral circulation of pregnant women who later gave birth to boys. This method, however, is time-consuming and such cells may also be found in the blood of normal women who are not pregnant.[2] Quinacrine staining has also been used to detect cells that contain Y bodies in the maternal circulation, as a guide to fetal sex,[3,4] but drawbacks of this technique include non-uniform criteria for Y-body detection between laboratories,[5] the occurrence of false positives,[3,4] and failure of others to reproduce the results.[6] Herzenberg et al[7] used flow cytometry to detect cells of probable fetal origin, by immunogenetic and cytogenic criteria, from peripheral maternal blood. By a combination of flow cytometry and monoclonal antibody technology, Covone et al found cells that react with the monoclonal antibody H315 in the peripheral blood of pregnant women.[8] H315 identifies a glycoprotein expressed on the surface of human syncytiotrophoblasts,[9] but most H315-positive cells in the maternal circulation do not contain Y-chromosome-derived DNA when the fetus is male, and H315-negative cells can adsorb H315 antigen in vitro[10]—observations that cast doubt on the meaning of earlier results. An air-culture technique has also been developed to enrich for fetal cells in cultures of maternal blood.[11]

The polymerase chain reaction (PCR)[12,13] offers an alternative way to detect the occasional fetal cell amongst numerous maternal cells in maternal venous blood. However, a previous attempt to detect circulating male fetal cells by PCR was unsuccessful.[14] We describe use of PCR to detect male fetal cells from maternal peripheral circulation —a relatively non-invasive method for sex determination. We believe this report to be the first to describe molecular genetic analysis of the fetus by use of maternal blood.

PATIENTS AND METHODS

7–10 ml blood were taken from the antecubital veins of 19 pregnant women at various stages of gestation. Early pregnancy

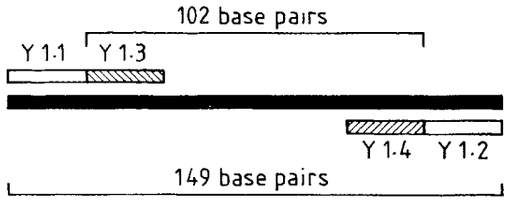

Fig 1—Relative locations of amplification primers.

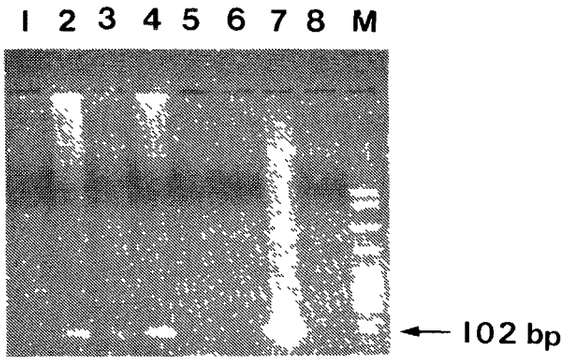

Fig 2—Dual amplification system for detection of male fetal cells in maternal circulation in early pregnancy.

1 = female control; 2–4 = DNA from pregnant women with male fetus (all 9 weeks' gestation); 5,6 = DNA from pregnant woman with female fetus at 10 and 11 weeks' gestation, respectively; 7 = male control; 8 = no DNA; M = pBR322 digested with *Msp*I (marker).

Results after 2 rounds of amplification (40 followed by 20 cycles).

samples (9–11 weeks) were obtained from 10 women under investigation for prenatal diagnosis of possible genetic disorders at the University of Milan. Blood samples were taken before chorionic villus biopsy and the sex as determined by PCR was compared with that obtained from cytogenetic analysis of chorionic villus culture. Late pregnancy samples (32–41 weeks) were obtained, with informed consent, from 9 women who attended an antenatal clinic at the John Radcliffe Hospital, Oxford; fetal sex as predicted by PCR was compared with the sex of the baby at delivery. Samples were also obtained from 3 female volunteers, who were not pregnant, as negative controls (DNA from one of these women was prepared before introduction of the stringent anti-contamination protocol described below).

DNA was extracted from maternal blood by standard techniques,[15] though under class II containment conditions. Each DNA extract was vortexed for 30 s to partially fragment long DNA strands and to allow DNA from fetal cells to mix thoroughly with maternal DNA before samples were taken for PCR. 2 μg DNA were subjected to 40 cycles of PCR with the primers Y1.1 and Y1.2, which flank a 149 base-pair fragment of a Y-specific repeat sequence.[16] Each cycle involved thermal denaturation on an

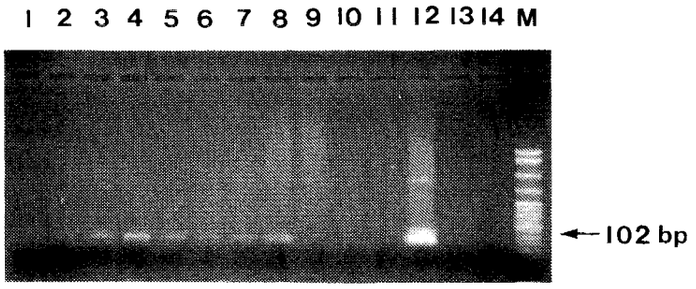

Fig 3—Dual amplification system for detection of male fetal cells in maternal circulation in late pregnancy.

1,2 = female controls; 3–8 = DNA from pregnant woman with male fetus at 32 (lane 3), 37 (4), 38 (5), 41 (6), 35 (7), and 38 (8) weeks' gestation; 9–11 = DNA from woman with female fetus at 37 (lane 9), 37 (10), and 41 (11) weeks' gestation; 12 = male control; 13 = no DNA; 14 = empty track; M = pBR322 digested by *Msp*I (marker).

Results after 2 rounds of amplification (40 followed by 17 cycles).

PCR AMPLIFICATION OF Y-SPECIFIC SEQUENCES FROM MATERNAL BLOOD

Cycle combination (external + internal)	Positive for Y sequences by PCR		
	Female controls	Mothers with female fetus	Mothers with male fetus
Early pregnancy			
40 + 0	0/1	0/4	0/6
40 + 15	0/1	0/4	2/6
40 + 17	0/1	0/4	2/6
40 + 20	0/1	0/4	6/6
Late pregnancy			
40 + 0	0/2	0/3	0/6
40 + 15	0/2	0/3	2/6
40 + 15*	0/2	0/3	2/6
40 + 17	0/2	0/3	6/6
40 + 17*	1/2†	0/3	6/6
All pregnancies	1/3†	0/7	12/12

*Repeat experiments.
†DNA not extracted under class II containment conditions.

'Intelligent heating block' (Hybaid, Teddington, Middlesex) at 94°C for 75 s, annealing at 55°C for 90 s, and extension at 72°C for 150 s. After 40 cycles, 2 μl of the PCR product was re-amplified with fresh reagents and primers Y1.3 (ATTACACTACATTCCCTTCCA) and Y1.4 (AGTGAAA-TTGTATGCAGTAGA), which flank a 102 base-pair fragment internal to Y1.1 and Y1.2, and were designed from sequence data.[17] The relative positions of these primers are illustrated in fig 1. The second amplification consisted of 15–20 cycles with conditions as before. 10 μl of the final PCR product were then analysed on ethidium gel. Reagents, which included *Taq* polymerase (5 units/100 μl reaction), were obtained from a DNA amplification reagent kit ('Gene amp', Perkin-Elmer Cetus, Norwalk, Connecticut, USA).

Dual amplification increases the risk of contamination. We therefore followed all previous guidelines to minimise the risk of false-positive results,[18,19] and also routinely incubated PCR reagents (including *Taq* polymerase) with *Eco*RI (30 units/100 μl PCR reagents) for 2 h at 37°C immediately before amplification. *Eco*RI was chosen because it cleaves inside the 149 base-pair region flanked by the Y1.1 and Y1.2 primers.[20] The restriction enzyme was then destroyed by heating at 94°C for 10 min before addition of DNA samples. As a further precaution we extracted no DNA samples from men whilst these experiments were in progress, and all blood samples were taken and subsequently handled by women.

RESULTS

10-fold serial dilutions of male DNA in female DNA were performed to determine the sensitivity of the dual amplification system. After the first round of amplification, positive bands were present at reciprocal dilutions of 10^4–10^5. After the second round of PCR with internal primers Y1.3 and Y1.4 (15 cycles), the detection limit was extended to 1 in 10^7. The number of cycles used in the second amplification was varied to obtain a system with maximum sensitivity and specificity (see table). We obtained correct prenatal sex determination in all 19 cases tested after internal amplification of 17 cycles for late pregnancy samples and 20 cycles for early pregnancy samples.

DISCUSSION

We used PCR to determine fetal sex from maternal peripheral blood, with the correct identification in all pregnancies, at 9–41 weeks' gestation. The second round of amplification gave the added sensitivity essential for success. Use of extracted DNA, rather than whole cells, may also increase sensitivity because the Y-specific sequence chosen

is present as a tandem array of 800–5000 subunits,[17] which is broken up into much smaller subunits and dispersed into solution and thoroughly mixed during the extraction procedure. A very small number of fetal cells in the original blood sample may therefore be detected by this system, even though only a tiny proportion of the total DNA sample is amplified in a single test.

The main drawback of the dual amplification system is its susceptibility to contamination. Even with our stringent experimental conditions, internal amplification beyond 20 cycles consistently yielded false-positive results. We found a "window" between 15 and 20 cycles in second amplification during which DNA samples from a mother who carries a male fetus will be positive whereas those from a mother who carries a female fetus are negative. The source of this "background" contamination remains unknown. The single positive case in a non-pregnant woman was obtained from a sample extracted before the start of our stringent anti-contamination protocol and illustrates the importance of careful preparation of samples for PCR. Although false-positives were obtained at more than 20 cycles of internal amplification, this can be compared with the results of Handyside et al[20] who used a single round of amplification with primers Y1.1 and Y1.2, and observed a faint band of PCR product in some of their female samples. Enrichment of fetal cells from maternal blood may overcome this problem of a low signal-to-noise ratio.

The technique we describe needs further development before any routine clinical application can be envisaged, but our early results indicate that some types of fetal genetic analysis from maternal blood samples may be feasible. Accurate determination of fetal sex may have implications for prenatal diagnosis of sex-linked conditions—for a female fetus, for example, further genetic investigation could be deferred until after delivery. For a male fetus, chorionic villus biopsy or amniocentesis would still be necessary to determine the presence or absence of an abnormal sex-linked gene. With further refinement, single gene analysis of amplified paternal base sequences may be possible; a similar technique may also be useful in veterinary medicine.

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REFERENCES

1. Walknowska J, Conte FA, Grumbach MM Practical and theoretical implications of fetal/maternal lymphocyte transfer. *Lancet* 1969; i: 1119–22.
2. Jacobs PA, Smith PG Practical and theoretical implications of fetal/maternal lymphocyte transfer. *Lancet* 1969; ii: 745.
3. Schroder J, de la Chapelle A. Fetal lymphocytes in the maternal blood. *Blood* 1972; **39:** 153–62.
4 Grosset L, Barrelet V, Odartchenko N. Antenatal fetal sex determination from maternal blood during early pregnancy. *Am J Obstet Gynecol* 1974; **120:** 60–63.
5. Schroder J. Transplacental passage of blood cells. *J Med Genet* 1975, **12:** 230–42
6 Zimmerman A, Schmickel R Fluorescent bodies in maternal circulation *Lancet* 1971; i: 1305.
7 Herzenberg LA, Bianchi DW, Schroder J, Cann HM, Iverson GM. Fetal cells in the blood of pregnant women detection and enrichment by fluorescence-activated cell sorting. *Proc Natl Acad Sci USA* 1979; **76:** 1453–55.
8 Covone AE, Mutton D, Johnson PM, Adinolfi M Trophoblast cells in peripheral blood from pregnant women. *Lancet* 1984; ii: 841–43.
9 Johnson PM, Cheng HM, Molloy CM, Stern CMM, Slade MB. Human trophoblast-specific surface antigen identified using monoclonal antibodies. *Am J Reprod Immunol* 1981; **1:** 246–54.
10. Covone AE, Kozma R, Johnson PM, Latt SA, Adinolfi M. Analysis of peripheral maternal blood samples for the presence of placenta-derived cells using Y-specific probes and McAb H315. *Prenat Diagn* 1988; **8:** 591–607.

References continued at foot of next column

Reviews of Books

Life Events and Illness

Edited by George W. Brown and Tirril O. Harris. London: Unwin Hyman. 1989. Pp 496. £35.00. ISBN 0-044454260.

George Brown and Tirril Harris are the doyens of life-event research and any book to which they contribute is certain of achieving wide influence. *Life Events and Illness* is very much their creation since, although there are fifteen other contributors, almost every page refers to their seminal work on the subject. The two editors begin with a scholarly review of the measurement of life events and give some insights into the difficulties. These are considerable: although it is fairly easy to quantify events externally, this does not help to predict who will suffer through experiencing them. There follows a series of chapters describing life events in psychiatric and physical disorders. Most of these record life events with the Bedford College Life Events and Difficulties Schedule (LEDS), an instrument developed by the editors in 1978. The results are impressive, since not only depression, anxiety, and schizophrenia but also myocardial infarction, appendicectomy and other gastrointestinal conditions, menstrual disturbance, and functional dysphonia are shown to be influenced by the experience of life events. Sometimes the influence can be construed as a trigger, and at other times as causal. The editors complete a *tour de force* by reviewing and revising their own work in a final 120 pages and they are probably justified in concluding that their work and that of others with LEDS "may have served to open up a whole range of hypotheses about psychological and psychosomatic functioning".

Despite these achievements life-events research has several unsatisfactory aspects. Nearly half of this book is preoccupied with methodology. The fact that this remains an important issue after 25 years of research experience suggests that there is a fundamental invalidity somewhere. This preoccupation leads to introspective examinations of different approaches that conjure up the word academic in its pejorative sense, and may also account for a reluctance to look at other elements (particularly personal predisposition to illness) that may be independent of the specific contribution of life events. Any researcher who gets hold of a

Y-M D LO AND OTHERS· REFERENCES—*continued*

11. Selypes A, Lorencz R. A noninvasive method for determination of the sex and karyotype of the fetus from the maternal blood. *Hum Genet* 1988; **79:** 357–59.
12. Saiki RK, Scharf S, Faloona F, et al. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* 1985, **230:** 1350–54
13. Saiki RK, Gelfand D, Stoffel S, et al Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; **239:** 487–91
14. Adinolfi M, Camporese C, Carr T. Gene amplification to detect fetal nucleated cells in pregnant women. *Lancet* 1989; ii: 328–29.
15. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning a laboratory manual. New York. Cold Spring Harbor Laboratory, 1982
16. Kogan KC, Doherty M, Gitschier J. An improved method for prenatal diagnosis of genetic diseases by analysis of amplified DNA sequences. *N Engl J Med* 1987; **317:** 985–90.
17. Nakahori Y, Mitani K, Yamada M, Nakagome Y A human Y-chromosome specific repeated DNA family (DYZ1) consists of a tandem array of pentanucleotides. *Nucleic Acids Res* 1986; **14:** 7569–80.
18. Lo Y-MD, Mehal WZ, Fleming KA. False-positive results and the polymerase chain reaction. *Lancet* 1988; ii: 679
19. Kwok S, Higuchi R. Avoiding false positives with PCR. *Nature* 1989; **339:** 237–38.
20. Handyside AH, Pattinson JK, Penketh RJA, Delhanty JDA, Winston RML, Tuddenham EGD Biopsy of human preimplantation embryos and sexing by DNA amplification *Lancet* 1989; i 347–49.