Prenatal screening for cystic fibrosis

Moira E. Mennie Annette Gilfillan Mary Compton
Lucy Curtis W. A. Liston I. Pullen Dorothy A. Whyte
D. J. H. Brock

Screening for carriers of CF (cystic fibrosis) is now possible but the best way of delivering such a service is unknown. In one model 4348 women attending antenatal clinics in an Edinburgh maternity hospital were invited to participate in a trial of prenatal screening. Mouthwash samples were tested for six CF alleles (85% of mutant genes) and when a woman was found to be a CF carrier her partner was also tested. Heterozygous couples were offered prenatal diagnosis.

609 (14%) women declined to enter the trial and another 574 (13%) were not screened, usually because of late booking. Among the remaining 3165 women there were 111 carriers of a CF gene (1 in 29). 4 of these 111 had carrier partners and these couples opted for prenatal diagnosis, the 1 pregnancy with an affected fetus being terminated. The psychological impact of screening was assessed by the general health questionnaire. There was a significant increase in stress at the time of the test result among women identified as carriers. However, this disappeared when their male partners tested normal and did not reappear later in the pregnancy.

By providing time for couples to discuss the possibility of screening and by offering the test at a point (the antenatal booking clinic) at which most pregnant women are seen, this approach has advantages, provided that counselling is readily available.


Introduction

The cloning of the cystic fibrosis (CF) gene in 1989 and the demonstration that 70% or so of mutations are the AF508 allele, made it possible to contemplate prenatal screening. In the UK the Cystic Fibrosis Research Trust solicited bids for trial projects aimed at delivering screening either through community health services (family-planning clinics or general practitioners) or in antenatal clinics. Although there are some advantages in focusing testing on individuals or couples before conception experience in screening for other autosomal recessive genetic disorders suggests that testing during pregnancy is more effective.

In our trial project we assessed screening in the antenatal clinic of Edinburgh's largest maternity hospital, the Simpson Memorial Maternity Pavilion, which has about 5000 deliveries a year. Although couple screening has recently been proposed we used the more conventional two-step model. Women were offered testing at their first antenatal clinic visit; if they were negative no further action was taken but if they were positive the partner was tested. When both parents carried CF alleles—ie, there was a 1-in-4 risk of an affected child—prenatal diagnosis was offered. If the father was negative the residual risk was explained to the couple in a counselling session, but no further action was taken.

The difficulty with this and any other method of screening lies in the molecular heterogeneity of CF. Over one hundred and fifty mutant CF alleles have been described (International CF Genetics Analysis Consortium, personal communication), many very rare. It is only possible to test for the more prevalent mutations in a specific population and to calculate residual risks on the basis of known allele frequencies. We have looked for six mutations representing some 85% of those found in Scotland. Residual risks are outlined in fig 1.

Methods

Recruitment

Before the full trial 180 women were sent an information leaflet and questionnaire to test their reactions. On the basis of the responses received (81%), a printed leaflet was designed describing the main features of CF and the methodology of the screening trial. This leaflet was sent to all women with their booking clinic appointment; they were asked to discuss it with their partners and were invited to join the trial by signing a consent form. The leaflet emphasised that screening is imperfect; it reduces but does not abolish the risk of an affected child. Women were advised not to participate if they could not identify the baby's father. Other exclusions are shown in the table.

Counselling

At the clinic the midwife responsible for booking asked the patient whether she had read the leaflet, understood it, and wished to join the trial. Women who had not read the leaflet (often because of reading difficulties) or found it too complex were counselled with visual aids by a genetics nurse. Women carrying a CF allele were

Addresses: Human Genetics Unit (M. E. Mennie, HV, A. Gilfillan, BSc, M. Compton, NM, L. Curtis, BSc, Prof D. J. H. Brock, PhD); and Departments of Obstetrics and Gynaecology (W. A. Liston, MB); Psychiatry (I. M. Pullen, MBB); and Nursing Studies (D. A. Whyte, PhD), University of Edinburgh, Edinburgh, UK. Correspondence to Prof D. J. H. Brock, Human Genetics Unit, Western General Hospital, Edinburgh EH4 2XL, UK.
later seen with their partners for further counselling by the nurse. If both had CF alleles they were referred to a consultant obstetrician for discussion of possible prenatal diagnosis.

**Psychological status**

All women entering the trial were asked to complete a twelve-item general health questionnaire (GHQ) before testing (threshold GHQ). Carriers filled in a GHQ at the time of their test result (GHQ1), at the time of their partner’s test result (GHQ2), and again 6 weeks later (GHQ3). For every carrier 2 control women of the same parity who had received negative results were selected from the same clinic and tested at comparable times.

**Laboratory analyses**

In the early part of the trial blood samples were collected from women and mouthwash samples from their partners. However, mouthwash samples had fewer difficulties and all participants were subsequently asked to rinse their mouths briefly with 10-15 ml tap-water which was transferred to a Universal container. Buccal cells were pelleted by centrifugation, suspended in 50 mmol/1 sodium hydroxide, heated in a boiling water bath for 20 min, neutralised, and centrifuged.

Two assay systems were used, with overlap in the mutant alleles detected. In the in-house assay exons 10 and 11 were simultaneously amplified by the polymerase chain reaction; the AF508 and Δ507 alleles in exon 10 were detected by electrophoresis on polyacrylamide gels, whereas the G551D and R553X alleles in exon 11 were detected by differential restriction enzyme digestion. The commercial assay (courtesy of Cellmark) used the amplification refractory mutation system (ARMS) to detect AF508, G551D, G542X, and 621+1G→T. The in-house assay detected 79% and the ARMS system 83% of mutations, in combination they allowed scanning for 85% of mutations.

**Reporting of results**

Participants were told that initial testing would take 7 days and that at that time they could assume that no mutant alleles had been detected. Women who tested positive were informed by telephone if possible or by letter otherwise and an appointment was made for counselling, together with their partner. Partner’s samples were tested as quickly as possible and the results communicated by telephone. All results were recorded in the obstetric notes; positive results were also communicated by letter to the woman, her general practitioner, and the consultant obstetrician.

**Results**

Screening was introduced in October, 1990, the offer being made initially in just one of nine weekly antenatal clinics. By April, 1992, 4348 women in 431 clinics had received the information leaflet and an invitation to participate (table). Most of the 609 women who refused to take part gave their reason as non-acceptance of the possibility of termination in the event of an affected fetus. A further 574 women were not screened because they were already over 18 weeks’ gestation (430), because of abnormal pregnancies (mainly blighted ova) (73), or because their partner would not participate (56). Other reasons are shown in the table.

Among the 3165 women tested there were 111 CF heterozygotes, giving a carrier rate of 1 in 29. Since the detection rate of CF alleles was 85%, the true heterozygote frequency is 1 in 24. Partners of 110 of these 111 carriers were screened and 4 (3 AF508, 1 G551D) were found to be positive. All 4 of these couples opted for prenatal diagnosis, 3 via amniocentesis and 1 via a transabdominal chorionic villus biopsy. 1 woman was found to be carrying a AF508 homozygote and she decided on termination; the diagnosis was confirmed on fetal tissues. The other 3 women were carrying unaffected fetuses and are proceeding to term.

GHQs were regarded as positive if the score was 3 or more. At the time of the test result (GHQ1), 53% (95% confidence interval [CI] 41-66%) of carriers had positive results as against 31% (95% CI 21-41%) of controls.
(p < 0.01). This difference had disappeared by the time of the partner's negative test result (GHQ2) (fig 2).

Discussion

The cloning of the CF gene prompted considerable discussion about population heterozygote screening and the consensus was that trial projects of different modes of delivery, not necessarily mutually exclusive, were needed. One method is preconception screening, which provides carrier couples with several options (such as changing partners, artificial insemination by a screened donor, forgoing reproduction, or prenatal diagnosis) and time for reflection and an unpressured choice. However, it is difficult to see how delivery through general practices and family-planning clinics could reach a broad range of the population, and bias towards take-up by the educationally and socially advantaged seems unavoidable.

CF heterozygosity is of medical significance when a pregnancy is planned or in progress, so it makes sense to target screening close to pregnancy. Evidence from other recessively inherited disorders suggests that screening during pregnancy is the most practicable time. In a 1989 US report on screening for Tay-Sachs disease, in which participants could be tested before or during pregnancy, 80% of respondents were already pregnant when the test was sought. In the UK almost all pregnant women now attend hospital at some stage and the antenatal clinic is an effective way of ensuring that screening is offered to as many women as possible. Reducing the number of delivery points would be of great importance if screening for CF is to become routine, because of the need for back-up counselling at every point.

In the two-step model of delivery tested here, the take-up rate was high (86%) but because of late booking and other reasons the proportion of women screened was 73%. This may represent an effective upper limit for this type of programme. An earlier study showed that some 40% of women decided to enter the programme without reference to their partners but we found that only 1 of the 111 female carriers was unable (or unwilling) to persuade her partner to be tested. This suggests that the motivation of the woman is an important contributor to a good take-up rate—and that non-paternity is not likely to be an important source of error. We have also noted that first-degree relatives of 15 of the 111 carriers detected by screening have made appointments with the genetic counselling clinic to establish their carrier status.

One concern in this trial was stress in women identified as carriers. 20-30% of women had positive GHQ scores before they were tested, a result which accords with previous findings of 35% in pregnancy. Women identified as carriers were significantly more likely to record a positive GHQ than controls but this difference disappeared once their partners had been tested and found not to carry a detectable CF allele. Thus stress would appear to be of short duration in carrier women, despite their being warned that their residual risk of bearing a CF child was still 1 in 600 or so.

The model of prenatal screening for CF tested here has considerable merit. Information leaflets about CF and screening can be studied by women and their partners at home, since couples have 4 weeks or so before any decisions need to be made. Most of their questions can be answered by midwives during the booking-in procedure. More than 96% of screened women had no detectable CF alleles in our series and there is no evidence for an increase in stress amongst this negative group. For an allele detection rate of 85% only 1 in every 856 couples will need to be referred to a doctor for detailed discussion of prenatal diagnosis.

If about 73% of women offered testing are screened and if the detection rate for CF alleles was at 85% we would expect to identify about half (0.73 × 0.85 × 0.85) the 1-in-4 risk couples in our population. We do not know what proportion of these would opt for prenatal diagnosis and termination of pregnancy if the fetus were affected; all 4 of our couples indicated that this was their intention. However, even if only half the parents of an affected fetus chose termination, this would still lead to the reduction in live-born incidence of 1 case of CF for every 10 000 tests. The Royal College of Physicians estimates the cost of treating a CF child at least £5000 per year (1986 figures); over an expected life-span of 25 years this represents a sum of £125 000. Laboratory costs for 10 000 tests are about £30 000, while the total programme costs (including laboratory) are about £80 000. Thus a crude cost-benefit calculation suggests that screening of this kind represents good value. The major resource implicated is in the management of carrier women in the period between their test result and that of their partner. At least one counselling session is needed to answer questions, allay fears, and place risks in perspective. This can be done by informed non-medical personnel with good communication and counselling skills. In our experience a specialist genetics nurse is essential to the smooth delivery of this type of programme.

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REFERENCES