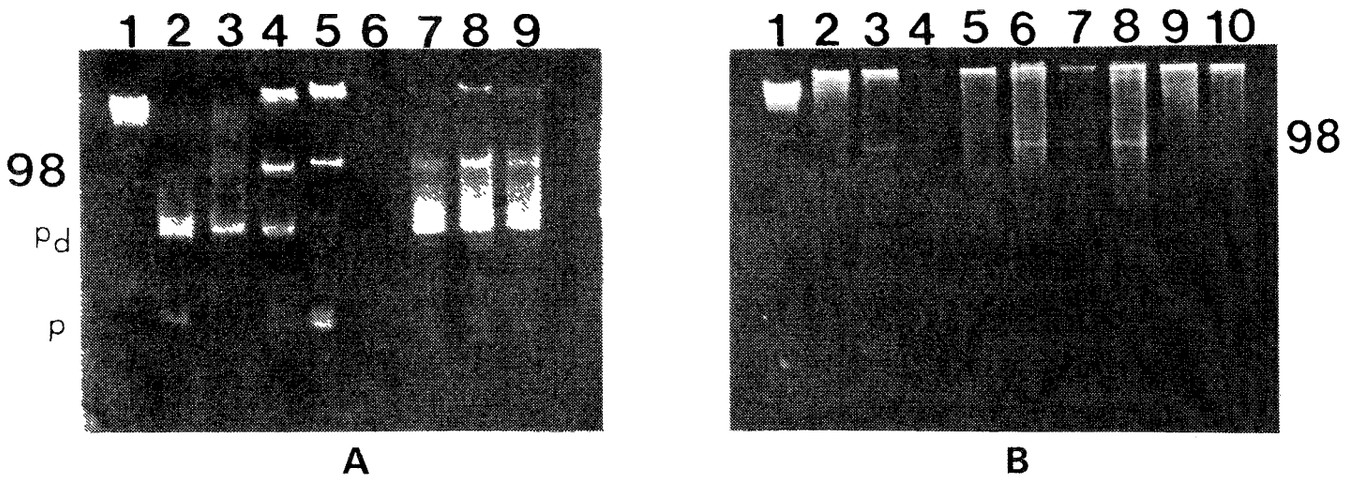
LETTERS to the EDITOR

Preconception genetic diagnosis of cystic fibrosis

SIR,—Preconception genetic diagnosis by polar body removal has been developed for couples at high risk for conceiving genetically abnormal fetuses.[1] The procedure involves retrieval of oocytes under ultrasound guidance, removal of the first polar body before fertilisation, and genetic analysis of the polar body using the polymerase chain reaction (PCR). In women heterozygous for a genetic disease, the polar body will, in the absence of crossing-over, be homozygous for the gene not contained in the oocyte from which it was removed. If crossing over has occurred, the polar body will be heterozygous, and it will not be possible to determine the genotype of the oocyte in this way. Pregnancies have been established following blastomere biopsy, demonstrating the feasibility of preimplantation genetic analysis.[2]

The $\triangle F_{508}$ mutation is the most common mutation causing cystic fibrosis (CF).[3] We have developed a method to identify consistently the presence of the $\triangle F_{508}$ mutation in single cells, using decontamination of buffers and reagents by restriction digestion with *Mse*I followed by heat inactivation before PCR. After PCR, the amplified products are allocated to three tubes. PCR products from a homozygous normal individual are added to one tube, and products from a CF patient homozygous for $\triangle F_{508}$ are added to the second tube. These tubes are heated to 95°C for 5 min and allowed to reanneal at 68°C for 15 min. This procedure permits heteroduplexes to form between $\triangle F_{508}$ and normal sequences.[4] The third tube is untreated. The DNA from the three tubes is then analysed by polyacrylamide gel electrophoresis. Polar body DNA that is homozygous for the normal allele will show two bands when PCR products of $\triangle F_{508}$ DNA are added but only one when homozygous normal DNA is added. Conversely, polar body DNA homozygous for the $\triangle F_{508}$ mutation will show two bands when normal DNA is added and only one when $\triangle F_{508}$ DNA is added. Heterozygous DNA will show two bands in untreated DNA and when either normal or $\triangle F_{508}$ DNA is added.[4] For every experiment we include controls for contamination of reagents and for reliable amplification of DNA from individuals with known genotype. Using this technique we successfully determined the genotype, in respect of the $\triangle F_{508}$ mutation, of 61 single cells of CF patients and controls. All procedures were approved by our institutional review board.

This technique has been applied to a couple who are both carriers of $\triangle F_{508}$. An oocyte found to be abnormal because of homozygosity for the normal gene in the polar body (figure A, lanes 7–9) was inseminated and the pre-embryo developed normally. In our programme, all oocytes found to contain the mutant allele and all oocytes with heterozygous polar bodies are inseminated and pre-embryo biopsy performed to determine the diploid genotype. One blastomere was removed at the six-cell stage and PCR analysis



PCR analysis of polar body (A) and blastomere (B) DNA.

Single cells placed in decontaminated PCR buffer and lysed by freezing at −70°C for 20 min followed by 95°C for 7 min After 40 cycles of PCR[3] products were mixed with homozygous control PCR products.

Lane	*A*	*B*
1	Size standards	Size standards
2	Buffer alone (no added DNA)	Buffer alone (no DNA added)
3	10 pg control DNA	Normal control DNA
4	Homozygous $\triangle F_{508}$ CF patient	Empty
5	Homozygous normal control	Buffer alone
6	Empty	Blastomere alone
7	Polar body DNA	Blastomere + homozygous $\triangle F_{508}$ DNA
8	Polar body DNA + normal control DNA	Blastomere + homozygous normal DNA
9	Polar body DNA + homozygous $\triangle F_{508}$ DNA	Buffer alone
10	..	Buffer alone

revealed homozygosity for $\triangle F_{508}$ (B, lanes 6–8) thus confirming the accuracy of the polar body diagnosis. Despite the manipulations, this affected pre-embryo developed to the blastocyst stage and was frozen.

This study demonstrates the feasibility of preconception and preimplantation diagnosis for couples carrying the $\triangle F_{508}$ mutation of CF.

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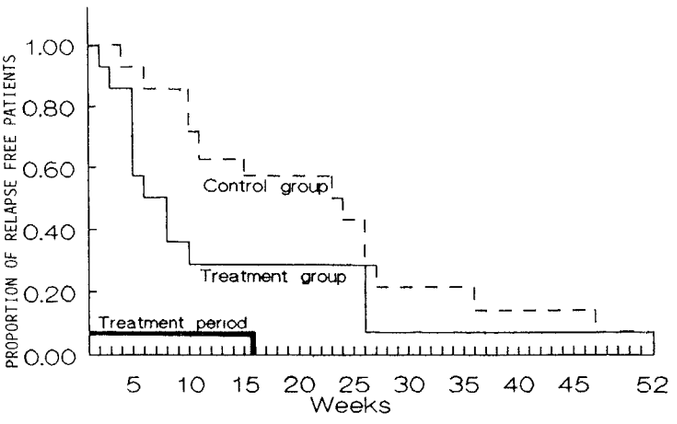
Increased relapse of duodenal ulcers in patients treated with cognitive psychotherapy

SIR,—In a study of cognitive psychotherapy[1] in patients with duodenal ulcer and non-ulcer dyspepsia with erosive prepyloric changes,[2] we had to interrupt treatment in the ulcer patients because of a significant increase in relapse rate. Recurrence is a major problem in duodenal ulcer disease, the relapse rate being 85% during the first year after healing.[3] Løøf et al[4] found no significant effect from psychological counselling but Broocks and Richardson[5] noted improvements, including a reduced recurrence rate, from an emotional skills training programme.

In our study the patients were recruited mainly from the section of gastroenterology in the medical department, Haukeland Hospital, a university hospital serving about 200 000 people. Duodenal ulcer was confirmed endoscopically and the patients were given an H_2-blocker or omeprazole. Provided there was endoscopically confirmed healing and in the absence of specified exclusion criteria, the patients were then randomly allocated to the control or treatment group. The two groups did not differ in respect of factors (age, sex, smoking) that might influence outome. The initial interview included general questions about history, application of the comprehensive psychopathological rating scale, and questions about the illness and psychological factors.

The patients in the treatment group received individual cognitive psychotherapy, modified from the scheme of Beck and colleagues[1] and consisting of ten sessions over 3–4 months. The two therapists (T. T. H. and I. W.) had received training in cognitive therapy from Arthur Freeman.[6] Therapy focused on "target complaints", defined by the patients, such as pain, marital difficulties, depression, anxiety, or phobias. Cognitive techniques were used to illuminate irrational beliefs and automatic thoughts and to help patients to a cognitive restructuring. The control group received no treatment.

We planned to include 100 patients. After 11 months we had recruited 28, 14 in the treatment group and 14 controls. Relapse was frequent in both groups, but happened earlier in the treatment group (figure). At first we thought that reports reaching us from the controls might be less reliable. However, careful examinations of patients in the control group did not alter the pattern. Statistical analysis[7] on week 10 (Mantel-Cox, log-rank test) was significant ($p = 0{\cdot}015$):[7] only 28% of the patients in the treatment group were relapse-free at week 10 compared with 71% of controls. After 11 months no more patients were included in this programme. The



Estimated proportion of relapse-free patients in treatment and control groups.

ultimate frequency of relapse is the same in both groups (figure) but relapses happened earlier in the treatment group. Sifneos[8] has suggested that dynamic psychotherapy might be contraindicated for many patients with psychosomatic diseases. Our patients were given cognitive treatment in a drug-free period, and we think that our experience indicates the need for caution in introducing psychotherapy without pharmacological support. Our research programme continues, but cognitive therapy is now given with omeprazole (20 mg in the evening for 8 weeks).

It seems that psychological factors may indeed have a role in peptic ulcer disease. However, the direction of our results suggests that the relation is a complex one. An important part of this psychotherapy is increased awareness of dysfunctional cognitions. The initial phase of the therapy may therefore be provocative and imply a re-evaluation of many of the coping strategies used by the patients. This may in turn lead to an increase in psychobiological activation[9] and in the ulcerogenic processes.[10]

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Antihypertensive activity of sinorphan

SIR,—Atrial natriuretic factor (ANF) is a cardiac polypeptide hormone exerting potent cardiovascular and renal effects, but poor intestinal absorption and rapid inactivation have hampered therapeutic development.[1] However, inhibition of endogenous ANF metabolism by intravenous[2] or oral[3–5] administration of inhibitors of membrane metalloendopeptidase (atriopeptidase, enkephalinase, EC 3.4.24.11) results in enhanced plasma