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Pre-implantation Diagnosis of Inherited Predisposition to Cancer

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INTRODUCTION

SCIENCE AND medicine often advance when two fields unite. One current convergence is between the areas of pre-implantation diagnosis of human genetic disorders after *in vitro* fertilisation (IVF) and the growing body of knowledge of genes which predispose to various types of cancer.

Most approaches to cancer prevention are population-based, and require not only the identification of usually rather weak risk factors, but the prospect of behavioural engineering of large numbers of individuals to diminish risk exposure. Although the benefits are potentially large, the costs involved are proportionately high, both in the size of the scientific studies needed to test the hypothesis and in the subsequent implementation of health education measures. In addition, the time scale for both is generally measured in decades. It is now clear that in some individuals a variety of inherited mutations greatly increase the risk of cancer arising at predictable sites. The penetrance of these genes may approach 100%. Progressively larger numbers of individuals will be diagnosed as gene carriers after presentation at cancer family clinics, and the nature of the particular mutation they carry will be defined. However, few measures are available clinically to prevent cancers occurring, and those that are, such as oophorectomy together with prophylactic bilateral mastectomy (for carriers of the *BRCA1* gene), are considered by many as only the lesser of two evils. Until effective methods of prevention or rapid cure are developed such individuals will live with the knowledge that they have a high probability of developing life-threatening cancer. In many instances, however, this will be predicted to occur in mid-life after the normal age of parenthood.

By extrapolation from the present situation for families affected by genetic diseases, such as muscular dystrophy and cystic fibrosis, we believe that it is increasingly likely that couples in which one partner is a carrier of a cancer predisposing gene mutation will seek prenatal diagnosis to prevent passing the mutant gene on to their children. Terminating a pregnancy after diagnosis by conventional means, for example by chorion villus sampling (CVS), would be controversial and may be unacceptable to many couples since the baby should otherwise be normal. In recent years, methods for diagnosing genetic defects in early embryos before implantation (pre-implantation diagnosis) have been developed allowing selection and transfer of unaffected embryos, thereby avoiding the possibility of a termination after pregnancy is established [1]. This approach may provide a more acceptable alternative for these couples. So far, pre-implantation diagnosis has only been used for prevention of inherited disease. The first pregnancies were in couples at risk of a variety of X-linked recessive diseases only affecting boys [2]. In these cases, the general strategy of identifying the sex of each embryo and selecting females for transfer was used. Already, however, specific diagnosis of the predominant mutation causing cystic fibrosis has been achieved [3], and there are an increasing number of reports of success with other conditions occurring throughout the genome including Lesch-Nyhan syndrome, Tay-Sachs, haemophilia A and Duchenne muscular dystrophy (DMD) [4]. This article considers the present limits on single cell analysis for pre-implantation diagnosis, the types of cancer predisposing genes that could be analysed and their associated risks, and opens the area for discussion of the ethical issues.

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INHERITED CANCER SYNDROMES

Cancer predisposing genes fall essentially into three categories: those where inheritance is inferred from familial patterns of inheritance of the phenotype of site-specific cancer, but without linkage to a particular chromosomal locus (for instance prostate cancer); those where linkage exists, but the gene is not yet identified (such as the *BRCA2* gene in breast cancer); and finally those where the gene at the locus has been identified, and individual mutations affecting families are known (such as the *APC* gene in familial adenomatous polyposis). A list comprising the latter two categories updated from a recent review by Knudson [5] is shown in Table 1. Clearly, genes need to have been identified and well characterised for pre-implantation diagnosis to be feasible, but at the current rate of progress one can predict that most, if not all of those listed as mapped but not yet identified, will be isolated within a few months to a few years time.

PRE-IMPLANTATION DIAGNOSIS OF CANCER PREDISPOSING GENES

A combination of factors will determine whether pre-implantation diagnosis is used for preventing transmission of germline mutations predisposing to cancer. These include the acceptability of assisted reproduction techniques as an integral part of the process, the feasibility of detecting these mutations, the cost versus the benefit gained, and (not least), the ethical considerations arising from screening embryos for a genetic defect, which in itself is not immediately deleterious.

In the future, it may be possible to flush embryos from the uterus relatively non-invasively after normal conception *in vivo* and to test for the presence of specific genes prior to reintroduction. Currently, however, the dangers of potentially affected

Table 1. *Inherited cancer genes (revised from Knudson* [5])

	Gene	Location
Identified genes		
Retinoblastoma	*RB1*	13q14
Wilms' tumour	*WT1*	11p13
Li-Fraumeni syndrome	*TP53*	17p13
Familial adenomatous polyposis	*APC*	5q21
Neurofibromatosis type 1	*NF1*	17q11
Neurofibromatosis type 2	*NF2*	22q12
Von Hippel-Lindau syndrome	*VHL*	3p25
Multiple endocrine neoplasia type 2A	*RET*	10q11
Multiple endocrine neoplasia type 2B	*RET*	10q11
Lynch cancer family syndrome 2	*hMSH2*	2p16
Lynch cancer family syndrome 2	*hMLH1*	3p21
Male breast cancer	*AR*	X
Tuberous sclerosis 2	*TSC2*	16p13
Familial breast/ovarian cancer	*BRCA-1*	17q21
Mapped genes		
Multiple endocrine neoplasia type 1	*MEN1*	11q13
Familial breast cancer	*BRCA2*	13q12
Familial melanoma	*MLM*	9p21
Neuroblastoma	*NB*	1p36
Basal cell nevus syndrome	*BCNS*	9q31
Beckwith-Wiedemann syndrome	*BWS*	11p15
Renal cell carcinoma	*RCC*	3p14
Tuberous sclerosis 1	*TSC1*	9q34

embryos remaining in the uterus after flushing and the possibility of causing ectopic pregnancies prevent this. Therefore, couples electing for pre-implantation diagnosis undergo IVF treatment in the same way as for infertile couples. The woman is superovulated, and multiple eggs are collected, by aspirating ovarian follicles through a needle passed transvaginally, and fertilised *in vitro* with the husband's prepared sperm. One or two cells are then biopsied from each normally fertilised embryo at about the 8-cell stage, early on the third day after insemination for genetic analysis (Figure 1), and embryos, diagnosed as having an unaffected genotype, are selected for transfer to the uterus.

Pre-implantation diagnosis may be an attractive option for couples at risk of transmitting inherited diseases who have had unsatisfactory experiences with conventional prenatal diagnosis. Nevertheless, IVF is physically and emotionally demanding, and it remains to be seen how attractive it will be to carriers of cancer predisposing genes. One difficulty with fertile couples is their perception of IVF as having a low success rate. In reality, cumulative pregnancy rates after IVF now exceed normal conception rates. Although it should be stressed that clinical experience with pre-implantation diagnosis is still very limited, with a total of 32 pregnancies and 26 babies born to date, pregnancy rates are currently between 25 and 35% per treatment [4]. However, many of these women were in their mid to late thirties when IVF is much less successful. For women in their twenties, rates should be higher and many may get pregnant after a single attempt.

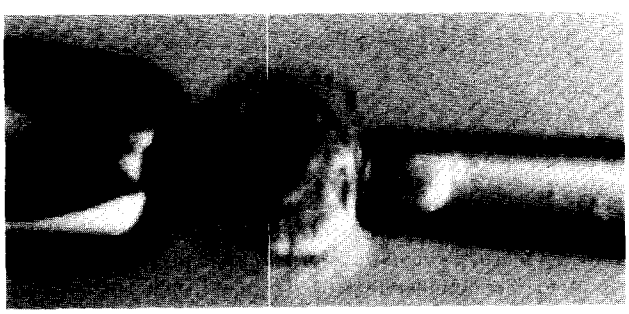

Figure 1. Video image of micromanipulation of a human eight cell embryo to remove a single cell (we are grateful to Dr Asangla Ao for this picture).

Diagnosis of genetic defects is based on rapid analysis of the one or two cells biopsied from each embryo. The aim is to have an answer later the same day so that selected embryos can be transferred to the uterus no later than they would be in a routine IVF cycle with consistent pregnancy rates. This has been made possible by the advent of two techniques, the polymerase chain reaction (PCR), and for chromosomal abnormalities, fluorescent detection of *in situ* hybridisation (FISH). For example, it is now possible to identify the sex of an embryo in 2 h using dual colour FISH with directly labelled X and Y chromosome specific probes [6]. Although only one of the cancer predisposing genes identified so far is X-linked (Table 1), selection of male embryos could already be offered to carriers of *BRCA1* since only females appear to be at risk of breast cancer from defects in this gene (although males may have an excess risk of prostate and other cancers, this has not yet been conclusively proved).

Specific mutation detection requires an increase in the sensitivity of PCR to the single cell level. This has been achieved by two rounds of PCR using nested primers (primers annealing to sequences internal to the first pair) for further amplification of an aliquot of the initial product. Various means, including rapid detection of heteroduplex formation for small deletions, have then been used to determine whether or not the mutation is present in the amplified fragment. In principle, the same strategies could be applied to mutations in cancer predisposing genes where the nature of the mutation has been identified in detail. As with many single gene defects, carrier status may be based only on linkage information. Efforts are now being directed towards developing strategies that will allow analysis of combinations of closely linked and intragenic markers, and be applicable in any couple at risk at a particular locus. One approach is to use multiplex PCR, i.e. the use of multiple primer pairs in the same reaction, in combination with incorporation of fluorescent nucleotides in primers or amplified product and the use of sensitive automated analysis. Another is a modification of PCR using random primers which results in amplification of fragments from throughout the whole genome of the single cell [7]. Aliquots of this initial product can then be used for amplification of specific sequences by conventional PCR. Application of whole genome amplification for detection of deleted exons of the dystrophin gene in DMD has recently been reported [8].

Technically, therefore, there seems no reason to suppose that the detection of mutations in cancer predisposing genes will not be feasible. However, as mutations in tumour suppressor genes act as dominants extra care will be needed in assessing the accuracy of detection. So far only diagnosis of recessive single gene defects has been attempted. In these cases, failure to amplify one allele or sampling of an aneuploid cell may lead to misdiagnosis of the genotype of the embryo as a whole, but should not result in the transfer of affected embryos. With a dominant effect, however, transfer of affected embryos under these circumstances would be likely.

The cost/benefit considerations are mainly the cost involved

in possibly multiple IVF treatments relative to the economic gain from avoiding unnecessary screening of high risk individuals and treatment of subsequent cancers (if these cannot be prevented). This is clearly conditioned by accuracy of the diagnosis (in cases where the mutation is inferred from linkage only), the penetrance of the gene (i.e. how many carriers develop cancer) and how life threatening the cancer is. With common mutations, such as the breast cancer gene *BRCA1*, perhaps we should be screening the thousands of couples undergoing IVF each year for infertility and offering embryo diagnosis to carriers. Although labour intensive, the biopsy and diagnostic procedures are relatively inexpensive and cost/benefit analysis in these couples may well be favourable.

Ethical issues include the morality of embryo selection for traits which are not immediately life-threatening versus the gain in cancer prevention affecting the well-being of both parents and children. The knowledge of the high probability of the development of cancer in gene carriers (quite different in magnitude to that present in most cancer prevention options) will be an important factor in individual decisions. Similar ethical issues are already being faced for late onset diseases such as Huntington's Chorea. A potential advantage of pre-implantation over later prenatal diagnosis in such diseases is that couples known to be at risk because of affected relatives, but not genotyped themselves, do not have to be told of their carrier status but simply reassured that if necessary non-carrier embryos would be selected for transfer. In addition, blood group incompatibilities, such as rhesus incompatibility, can now be identified, and may be justifiable in women who have been previously sensitised and may require repeated blood transfusions *in utero* with the associated risk of miscarriage.

Currently, there is no European consensus on the use of IVF for pre-implantation diagnosis. In the U.K., it is permitted by law and regulated by the Human Fertilisation and Embryology Authority who have, for example, banned the use of these techniques to enable couples to choose the sex of their children, except in cases of X-linked disease. In France, initial legislation outlawing pre-implantation diagnosis may now be relaxed in cases of severe inherited disease. In Germany, pre-implantation diagnosis is effectively banned altogether. Nevertheless, there is little doubt that these issues will have to be addressed in the near future.

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Gene Replacement Strategies for the Prevention and Therapy of Cancer

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THE IDENTIFICATION of specific genes that contribute to the development of the cancer cell presents an opportunity to use these genes and their products as targets for treatment and perhaps prevention of the disease. The gene families implicated in carcinogenesis include dominant oncogenes and tumour suppressor genes [1, 2] and a dynamic interplay exists within the cell between dominant oncogenes and genes that constrain cell proliferation. Proto-oncogenes (normal cellular homologues of oncogenes) participate in critical cell functions, including signal transduction and transcription. Only a single mutant allele is required for malignant transformation, and primary modification to the dominant oncogenes that confer transforming ability include point mutation, amplification, chromosomal translocation, and rearrangement (Figure 1). Loss of tumour suppressor gene function may involve either mutation, deletion, or a combination of these. Some tumour suppressor genes appear

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