Table 2.

Disease	Location	Mutation analysis
Breast ovarian	17q21	Not yet available
von Hippel-Lindau	3p25	YES for *VHL*
Familial adenomatous polyposis	5q21	YES for *APC*
Gorlin's syndrome	9q	Not yet available
MEN Type II	10q11.2	YES for *RET*
MEN Type I	11q	Not yet available
Wilms' tumour	11p13	YES for *WT1*
Retinoblastoma	13q	YES for *RB1*
Li–Fraumeni	17q	YES for *TP53*
NF I	17q11.2	YES for *NF1*
NF II	22q11.2 to q12.1	YES for *NF2*
Lynch syndrome	2p22	YES for *hMSH2*
Lynch syndrome	3p21	YES for *hMLH1*

MEN, multiple endocrine neoplasia; NF, neurofibromatosis.

However, mutation analysis is more labour intensive. A sample is collected from an affected individual and mutation analysis is carried out. Once a mutation is identified, other members of the family can then be tested to see whether or not they carry the mutated gene. Linkage analysis and direct mutation analysis are now possible for many different cancer susceptibilities (Table 2).

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Pergamon

European Journal of Cancer Vol. 30A, No. 13, pp. 2015–2029, 1994
Elsevier Science Ltd
Printed in Great Britain
0959–8049/94 $7.00+0.00

0959-8049(94)00396-3

Screening for Cancer Predisposition

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INTRODUCTION

THE LAST 10 years have seen enormous strides in our understanding of events at the molecular level which underlie the development of malignancy. Many examples of potential opportunities for screening provided by these discoveries are presented in articles within this issue. The challenge which now faces us is how to translate this massive body of knowledge into appropriate screening programmes [1, 2] and this challenge is accentuated because the issues involved are enormously complex [3–5]. We will highlight the need for expansion of the academic disciplines contributing to screening for disease predisposition, and the attendant public health questions raised [6]. The need for research prior to provision of services of this type is always underestimated, and the resources required will always tend to be large because of the expense involved in most epidemiological studies [7]. Detailed economic assessment of health gain to be anticipated from cancer predisposition screening must be undertaken [8]. It is important that we clearly understand where cancer screening stands now, and how it might best be further developed in the future, in the most cost effective manner [9]. This whole area remains highly controversial, mainly because financial calculations are very soft and are not necessarily universally applicable. The additional diagnostic yield which may be possible, based on our new molecular knowledge, must be seen as something to add to and dovetail with current cancer screening programmes, and not as a separate entity in itself.

Whilst we focus here on problems in genetic screening for cancer predisposition [10–12], we should not lose sight of the fact that total population screening is likely to remain the major contributor to reducing cancer morbidity and mortality in industrialised populations [2]. Whilst the systems are by no means perfect, the probability is that screening will continue to be our major weapon in the fight against cancer indefinitely because the development of curative therapeutic modalities for advanced disease is highly unlikely in the foreseeable future. Even if such agents become available, the earliest possible diagnosis will remain desirable to reduce morbidity. Thus, our major hope for reducing the disease burden overall is in either preventing it happening in the first place or in finding and

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treating early disease, since in essentially all organ systems, the clinical outcomes are very significantly improved if the spectrum of disease is shifted to earlier stages. Given the vital importance of cancer screening, it is essential that systems are continuously reviewed and updated. For example, despite massive investment for many years and enormous experience, it would be naive to think that the definitive, most effective, systems are in place to screen for breast and cervical cancer [13, 14].

In this review, we initially discuss some of the issues involved in screening itself, including ethical and organisational questions. We outline briefly the types of screening procedures that are currently undertaken, so as to highlight existing opportunities at which samples could be obtained to undertake the types of testing that may be developed as a result of our increased genetic knowledge. We believe that an incremental approach is the most sensible way forward, where the new developments add to what is already accepted as good clinical practice [2] (Table 1). We then present some of the methodologies which are being developed for identifying disease-causing or disease-associated mutations in clinical samples for both inherited (germline-transmitted) cancers and sporadic (somatic) disease. Finally, we consider screening for cancer predisposition on a disease-specific basis, highlighting particular diagnostic opportunities which present themselves in specific anatomical sites. We have deliberately attempted to highlight some of the controversies which still surround many of the current population screening approaches. Whilst many of our remarks will be seen as naive by the expert screening community, we seek primarily in this review to bring some of these issues to the attention of those involved in studying the molecular genetics of cancer specifically, and those with an interest in developing efficient technologies for testing, which might allow large numbers of samples to be examined in cost effective ways. We believe that the real potential for advancement lies at the interface between the cancer molecular genetics field and the screening community at large.

GENERIC PROBLEMS WHEN SCREENING FOR CANCER

A number of important considerations relating to the process of screening itself have been widely debated and national guidelines are available in a number of countries. In the cancer area, these are almost invariably produced on a disease by disease basis. Major concerns include the ethics of screening and the legal responsibilities implicit in offering such a service. The question of who will actually provide screening is often contentious, and will again vary from disease to disease [15, 16]. The spectrum varies from tertiary referral centres through to primary health care providers to self-testing. It is likely that for several specific malignancies, services will initially be introduced (and hopefully assessed by cost-benefit analysis) in a hospital setting, prior to more widespread dissemination to the community. In the context of familial cancer, discussed in this issue by Dr Murday, provision of genetic counselling both pre- and post-diagnosis is essential (this issue pp. 2012–2015). Whilst a skills base exists to provide this information in the clinical genetics community, training needs must be met to offer a similar quality of counselling in the context of general oncology or surgical practice [17]. It is well known that the uptake of screening services is largely dependent on social class, and specific targeting of screening programmes so that they reach disadvantaged sections of society (who may well be at higher risk of certain cancers) requires further examination [18]. The successful implementation of any screening programme is likely to be dependent on an element of public education, which is currently undertaken extensively in the U.S.A., but for which there is little experience or tradition in the U.K. and much of Europe.

Any screening programme must address the issues of specificity and sensitivity. The acceptability of a false positive diagnosis on the one hand, or a missed diagnosis (false negative) on the other will vary from malignancy to malignancy. It will depend on the availability and accuracy of additional tests to confirm positive primary screening tests. The false negative rate will determine in some measure the frequency at which screening must be repeated on individuals, as will the natural history of the particular malignant disease. Here again the problems inherent in genetic screening for cancer predisposition are very different, depending on whether the test is for inherited or sporadic disease. In particular, sensitivity will tend to be less of a problem in the context of familial cancers (all the subject's nucleated cells should carry the mutation and potentially be available for testing), whereas detection of populations of mutated somatic cells will generally tend to be difficult at early stages because of the background of normal tissue [19]. Quality control and quality assurance in any screening programme are always vital. Examples of failures of both in traditional cancer screening programmes are legion, and would have a serious negative effect on the widespread introduction of genetic testing were they to continue to occur. In all cases, the availability of an efficacious treatment for a particular cancer will drive the screening programme forward. In the majority of instances, the aim of screening for cancer predisposition is to allow surgery at earlier stages, or indeed prophylactic surgery (oophorectomy, mastectomy) in some situations [20, 21]. Overall economic appraisal, cost-benefit analysis and quality of life (QUALY) assessment are difficult in circumstances where substantial quality of life improvements can be achieved, but at a high price for screening, surveillance and early therapeutic intervention [22].

Consideration of the many non-genetic screenable factors predisposing to malignancy is beyond the scope of this short review (e.g. hepatitis B or C infection leading to hepatocellular carcinoma; EBV infection leading to different malignancies in specific populations; *H. pylori* infection predisposing to gastric carcinoma or lymphoma and potentially amenable to screening for the presence of serum antibodies; PCR-based screening for the presence of HPV infection; screening of patients with ulcerative colitis for the development of colorectal carcinoma and/or sclerosing cholangitis followed by cholangiocarcinoma). There is currently much debate on the practicalities of introducing population-wide genetic testing programmes for non-malignant inherited diseases such as cystic fibrosis [23]. It is vital that generic lessons learned in these exercises are taken into account when genetic cancer-predisposition screening commences.

It is important to appreciate the clinical circumstances in which cancer screening is provided already. (Obviously screening programmes which identify colonic adenomas, dysplastic cervical epithelium or premalignant breast lesions are all "screens for cancer predisposition", but we must limit discussion in this article to screening programmes aimed at identifying genetic variants predisposing to malignancy, which are either inherited after germline transmission or which have occurred by somatic mutation.) The first opportunity to implement "screening for cancer predisposition" arises when a clinician takes a family history from patients newly presenting with cancer [24] but historically this has not been a major element of clinical management. It is vitally important that the message is rapidly disseminated to the clinical community that a detailed family history

Table 1. *Some current recommendations for cancer screening* [2]

	Organisation	Country	Protocol
Breast	American Cancer Society/ National Cancer Institute	U.S.A.	Annual mammograms in women: (i) over 50 years, (ii) aged 40–49 years if they, or mothers or sisters had breast cancer, (iii) aged 35–39 years if they have a personal history of breast cancer. Monthly breast self examination over 20 years old.
	(Forrest Report 1986) NHS Breast Screening Programme/European Society for Mastology Breast Screening Evaluation Committee	U.K./Europe	Mammography in women aged 50–64 years every 3 years. Mammography in women over 65 years old on request.
Cervix	DHSS Committee on Gynaecological Cytology (1982)/Royal College of Obstetricians and Gynaecologists (1987)/ ICRF Co-ordinating Committee on Cervical Screening (1984)	U.K.	Pap screening every 3 years to age 65 years.
	National Cancer Institute/ American College of Physicians	U.S.A.	Annual Pap screening for 3 years; 3 yearly screening if all normal. Recommendations vary over 65 years old—consensus probably 5 yearly.
Colorectum	WHO/American Cancer Society/American College of Gastroenterology/ National Cancer Institute/ American College of Physicians	U.S.A.	Annual stool occult blood testing Flexible sigmoidoscopy every 5 years over age 50 years for those at average risk.
	Atkin *et al.* (ICRF) [120]	U.K.	Single flexible sigmoidoscopy age 55–60 years. Surveillance colonoscopy in those with adenomas.
Stomach	Screening of high risk populations (Japan, radiological; China, radiological and/or faecal occult blood screening; follow-up gastroscopy on positives). Association of *H. pylori* seropositivity with increased risk of gastric cancer in many populations.		
Oesophagus	No specific screening programmes except in China where mortality rates are among the highest in the world and balloon-cytology screening produces a high diagnostic yield [101].		
Ovary	No population screening programmes in place. Assessment of transvaginal ultrasonography (TVS) and CA-125 serology in postmenopausal women reported from U.S.A., U.K.		
	National Institutes of Health	U.S.A.	TVS and CA-125 screening should be reserved for women with hereditary ovarian cancer syndromes.
Lung	No specific screening programme has been conclusively shown to improve mortality rates		
Uterus	No specific screening programmes in place. Emerging need for surveillance in hereditary non-polyposis colon cancer (HNPCC) family members. Patients with diabetes and hypertension possibly at increased risk of endometrial cancer. Transvaginal ultrasonography is thought to be effective. There will be a need to monitor women on long-term adjuvant tamoxifen therapy for breast cancer for the development of associated uterine cancer. It is conceivable that cryptic HNPCC carriers are at particular risk of this unfortunate side effect.		
Prostate	American Cancer Society (1993)	U.S.A.	Annual digital rectal examination in men over 50 years old plus a PSA assay.
Skin	No specific screening programmes in place. Surveillance essential of patients with dysplastic naevi and in pedigrees segregating familial dysplastic naevus syndrome and melanoma. UICC Melanoma Control Manual: 1992. NIH Consensus Statement on Diagnosis and Treatment of Early Melanoma: 1992.		

in newly presenting colorectal, breast, ovarian, uterine and potentially gastric, prostate and even lung cancer is essential, not only in identifying relatives for cascade genetic screening, but also to predict prognosis and to potentially determine the optimal treatment package for the individual. As our understanding develops further, it may be that treatment for breast cancer which arises as a result of mutation in the *BRCA1* gene [25], will be different from that arising subsequent to mutation in the *hMSH2* gene [26] or *hMLH1* gene [27, 28] as part of the Lynch type II syndrome. Several other cancers, such as bladder cancer, are associated with the defective DNA mismatch mutation repair phenomenon [29, 30]. It would not be surprising, therefore, if some bladder cancers were familial (and perhaps provoked by specific environmental influences). It has been reported that there is an autosomal dominantly inherited form of prostate cancer [31] analogous to familial polyposis (FAP). Anecdotal evidence suggests, however, that this familial form is not always associated with an earlier age of disease presentation, in contrast to the situation in FAP. Pedigrees segregating this disorder will only be identified by the most careful and astute clinical enquiry. Obviously individuals who carried the "prostate cancer gene" may have died from other causes if presentation was a late event, and the fact that the disease affects only males, but inheritance is not necessarily X-linked, adds a further complication. It is vital that urologists are aware of these questions, not least because of the enormous importance of pedigrees of this type in mapping prostate cancer susceptibility genes [32] in a manner analogous to that which was so successful for FAP. It may well be the case that the "family doctor/general practitioner" (depending on which particular country's health system is under consideration) is best able to elicit a full family history, and it is essential that the process of professional education concerning the genetic basis of much cancer is not focused entirely on specialist clinicians in referral centres, but is disseminated widely to primary healthcare professionals [33].

Again, in the context of taking a careful family history, we can envisage ethical problems which may arise. The hereditary non-polyposis colon cancer (HNPCC) situation is a good example. Vogelstein and his co-workers have estimated that some 1 in 200 members of the population carry mutations in this gene (or rather these genes), and are therefore at increased relative risk of a spectrum of malignancies [34]. Assuming that tests are available to detect such mutations (as discussed below these seem highly likely to become available), should we aim to undertake widespread population screening? To take the U.K. as an example, what would we do for the approximately 300 000 individuals who would be found to be carriers? They might reasonably expect to be provided with frequent colonoscopic surveillance (*hMSH2* and probably *hMLH1* mutations tend to be associated with right sided colonic tumours, and these appear to develop rapidly from adenomas so that the interval between screening ought to be short), mammography (the question of whether frequent mammography to detect early disease in individuals with a potential DNA repair deficiency is likely in itself to lead to mutation and tumour development needs to be addressed with urgency) and ultrasonography (to monitor for the presence of ovarian and uterine tumours). Health economists will note immediately that ironically these three screening modalities are among the most expensive in our repertoire. It will be interesting to discover whether cloning of the *BRCA1* gene or the imminent cloning of the *BRCA2* gene present similar dilemmas in breast and ovarian cancer [35, 35a].

Alternative strategies create different ethical problems. For example, most hospital pathology departments retain banks of tumour tissue processed for diagnostic assessment from clinical samples. It is relatively straightforward to isolate DNA from the tumour and, separately, from surrounding normal tissue from these samples. Tumours of the colon [36], breast, uterus [37], ovary or stomach [38], arising as a result of inherited or acquired loss of DNA mismatch mutation repair function, are characterised by a pathonemonic instability of their microsatellite repeats which can rapidly be analysed by simple polymerase chain reaction (PCR) [39]. Thus, we have the ability to look retrospectively at very large numbers of cancers, and identify individuals in whom Lynch type II HNPCC mechanisms were involved. Only a proportion of these individuals (20% seems a likely figure, but this will need further experimental confirmation) will have the inherited as opposed to the acquired condition. Whilst in the medium term, it should become possible to identify those in the former category by a careful study of normal tissue in archival samples (in these cases a single mutant allele would be found), the combined data that could easily be generated now provides a theoretical basis for cascade genetic screening in relatives of individuals identified as having Lynch type tumours. The ethical questions are profound. The individuals on whom these diagnostic tests were performed would not have provided informed consent or in many cases, patients will already have died of their disease. Generation of this type of data would mean that apparently healthy relatives of cancer victims "acquire" an increased relative risk of malignant disease, and although they may have no wish to know this, currently they are not in a position to give or withhold consent.

A comfortable ethical option is to say that such concepts are entirely unacceptable, but the possible consequences cannot be ignored. For example, individuals who have had a (curative) right hemicolectomy for what is, in fact, Lynch type II disease, and are not diagnosed by specific genetic screening may ultimately succumb unnecessarily to, for example, ovarian cancer, which would have been entirely predictable and preventable. Similarly, in possession of the full facts, many relatives of "carriers" of HNPCC genes would wish to establish their own genotypes in the expectation that they might then avail themselves of a full range of regular surveillance programmes if necessary. As public understanding of these complex issues increases, the comfortable option will be progressively undermined as some individuals demand genotyping. The implications, in the context of medical insurance and malpractice, are considerable and it will be most interesting to see how this debate develops, particularly in the U.S.A. Given that genetic screening for cancer predisposition seems likely to increase the need for follow-up surveillance screening in at-risk individuals, there are resource implications for current population based screening programmes. As funds will always be limited, the implication is that less will be available for population screening. How are we to make choices which might be between funding those with DNA mismatch repair mutations and provision of Papanicolou testing for cervical lesions in all women? The expectation in breast cancer screening programmes would be that diagnostic yields in population screening programmes will fall as more women with genetic predisposition are identified and moved into specific surveillance schemes. However, we cannot simply disenfranchise women who supposedly are not at "genetic" risk of breast cancer (at least via the mechanisms of which we are currently aware). This group will continue to be much larger than the former group, and overall will have much larger

numbers of "sporadic" breast cancers for detection by screening. There are no simple answers to this problem.

To return to the question of the mechanics of screening for cancer predisposition, a second very simple method is that of patient self-examination. Breast palpation is a vital component of the overall breast cancer screening effort [40]. The importance of self-examination of the skin as a method of ensuring early diagnosis of melanoma in populations at risk has been emphasised [41]. Self faecal occult blood screening is possible, and the simplicity of this as a preliminary screen will compensate in some measure for the low specificity and sensitivity of the method. Public education programmes, designed to increase participation in this type of activity, are a possible vehicle through which to increase public awareness of the potential and availability of genetic screening tests for cancer predisposition [42]. Whilst no technology is available at present for "self genotyping", it is not beyond the bounds of possibility that cheap, simple assay formats will be developed in the course of the next 10 years. They will allow analysis of DNA, derived for example from buccal swabs, urine samples or stool samples. It will be appreciated that the two different diagnostic challenges inherent in genetic screening for cancer predisposition (has the individual inherited a predisposing mutation which is present in all nucleated cells and for which screening can therefore be performed, or has the patient acquired a somatic mutation in a specific anatomical site, which therefore predisposes to the development of cancer at that site, and can only be diagnosed using patient material derived from that site) will probably require slightly different approaches. In the former situation, sensitivity may not be a limiting factor. Sensitivity is always likely to be a major consideration in the latter instance because, by implication, the greater the sensitivity, the higher the probability of detecting the emergence of a mutated clonal cell population at the earliest possible point to allow most effective treatment.

All other current screening modalities involve direct contact with healthcare professionals, and therefore provide an opportunity to introduce genetic predisposition screening. The list of examples includes physical examination (digital rectal examination, sigmoidoscopy, colonoscopy, bronchoscopy and even laparoscopy), imaging techniques (ultrasonography, mammography, CT, MRI and other X-ray investigations including barium contrast studies), biochemical studies (using a range of serological markers where clinical contact is required to obtain the blood or CSF sample), cytological studies (Papanicolou smears, fine needle aspirates or ascitic fluid), and histological studies (fine needle aspirates, needle biopsies or surgical resection specimens). All of these clinical interventions represent opportunities to obtain samples from which DNA and RNA or protein can be isolated for analysis and to provide detailed genetic counselling to patients.

TECHNOLOGIES FOR CANCER PREDISPOSITION SCREENING

In this section, we concentrate on generic methods either available, in development or foreseeable which might allow population-wide genetic screening for cancer predisposition. The major focus of our attention is diagnosis of major solid tumours. In the important although rarer inherited malignancies, many of which are discussed elsewhere in this issue, families will be clearly identified and screening can be delivered by highly expert individuals in the context of specialised clinics [24]. Such individuals will be well aware of the technological repertoire available to them, again as clearly exemplified by Murday (pp. 2030–2032), Gullick and Handyside in this issue (pp. 2012–2015). We will concern ourselves with generic technologies that may find application for a broad range of clinical samples (blood, buccal swabs, CSF, stool, urine, tumour tissue) and for a wide variety of often very different genes, where the challenge of finding a mutation is very different in every case (compare, for example, the problem of detecting one of the three common activating mutations in the *KRAS* oncogene [43] with the problem of detecting *a priori* one of the thousands of potential mutations which may occur in the *APC* gene [44]; analysis of either or both these phenomena may be required in screening for predisposition to colorectal cancer).

Research into the molecular genetic basis of cancer is mainly conducted by those whose primary expertise is in the analysis of nucleic acids. The tendency is, therefore, for tests to be initially developed based upon analysis of DNA (mainly involving PCR). In general, there is greater expertise in the diagnostic industry in the provision of assays and instrumentation based on immunological techniques [45]. Further development of these approaches is required, for example, in the analysis of proteins such as APC, which will often be present as truncated variants in individuals with inherited or acquired susceptibility to colon cancer [46–48]. In circumstances where potentially millions of individuals will require screening, the issue of automation (and price!) becomes all important. Further developments in this area for both DNA- and protein-based diagnostics are to be anticipated.

In practical terms, providing screening in obvious familial cancer will usually be straightforward because the gene responsible will usually be apparent, and there will be sufficient time to examine this gene in a proband in detail so as to precisely determine the germline mutation. A number of simple techniques allow segregation of this mutation to be precisely followed in the pedigree. If, for some reason, a mutation proves particularly difficult to identify (as might be the case in very large genes such as *DCC* [49] or *APC* or where the mutation creates a cryptic splice site within a large intron), it is still possible to analyse disease in families as highly polymorphic microsatellites can invariably be isolated close to the mutation. (These are already described [50] for most "cancer predisposition genes", and in those few instances where they are not already described, yeast artificial chromosomes for the genes of interest are available. These will on average contain several microsatellites which can be identified and formatted for analysis [51].) Screening in families will also not usually be compromised by the sample available for analysis. The mutation will be present in half the DNA molecules obtained from an individual's blood sample which does not represent a significant signal to noise problem. Similarly, it will be possible to obtain either mRNA/cDNA or protein for analysis. In the case of FAP or Li–Fraumeni syndrome, for example, it may eventually be simplest to look for truncated variants of *APC* (by comparing the binding of N-terminal and C-terminal specific antibodies) or *TP53* (comparing the binding of wildtype and mutant specific anti-*TP53* antibodies) rather than analysing DNA, as this may obviate the need for identification of specific mutations in new families. Furthermore, it may be possible to obtain biopsy material from those tissues most susceptible to a specific familial malignancy, if circumstances arise where the mutant protein is only expressed at that site.

In terms of reducing the overall population cancer burden, early detection of sporadic tumours arising as a result of somatic

mutations in "cancer predisposition genes" will have much more impact. Here, the vast majority of somatic cells will have two normal alleles of the gene in question. Diagnosis will rely on the ability to detect any new mutation or deletion occurring in any one of a number of large genes, to create a clone of heterozygous mutant cells, and to do so against a background of a massive excess of homozygous normal cells. Despite the apparent difficulty of this challenge, examples have been described where it has been successfully achieved.

These high sensitivity applications are heavily reliant on PCR. In 1991, Sidransky and colleagues showed that *TP53* mutations in bladder cancer cells could be detected using DNA which was isolated from shed cells collected in urine samples [52]. In these examples, the *TP53* mutation present was known *a priori*. However, as discussed below, it should soon be possible to detect any new mutation occurring in the *TP53* or other genes in the bladder transitional epithelium, and thus to conduct prospective screening for dysplastic change in the bladder. The clinical value of such testing would need to be carefully assessed. Haematuria is often a relatively early clinical sign of bladder cancer, and the disease incidence is age-related. The overall clinical value of population monitoring for early bladder cancer would need to be measured against the alternative of continuing with current approaches to management. Analysis of *TP53* mutations in shed urine cells may, in the long term, provide an alternative to check cytoscopy for the continuing surveillance of individuals who have had bladder tumours removed transurethrally. This will depend on our ability to develop semi-quantitative assays, and to show that a "mutant *TP53* signal" detectable prior to removal of the tumour either disappears or is greatly reduced after treatment, and that the reappearance of the "mutant *TP53* signal" correlates with the regrowth of bladder cancer. As discussed more recently by Sidransky and associates, it may also be appropriate in screening to monitor urine for the appearance of mutant variants of the chromosome 9q gene implicated in bladder tumour initiation, once it has been cloned [53]. Analysis of shed urine cells for the presence of *HRAS* mutations may be worthwhile as this oncogene is commonly activated in bladder tumours.

In a similar series of studies of even greater potential clinical significance, Sidransky and associates isolated DNA from patient stool samples, and searched for the occurrence of *KRAS* mutations after PCR amplification [54]. The results were unexpected at the time and extremely encouraging. In a series of 24 patients, 9 of whom proved to have tumours with *KRAS* mutations, the particular *KRAS* variant gene present was detected in 8 of the 9 positive patients. The particular advantage of *KRAS* mutations in this context is that only three sites seem to be involved (at codons 12, 13 and 61). This is in marked contrast to other genes implicated in the development of colorectal cancer (*APC*, *TP53*, *DCC*, *hMSH2*, *LMLH1*, *MCC* [55], E-cadherin, α and β catenin [56]) where a vast number of potential mutations can and do lead to loss of function and disease. Novel approaches to the detection of any sporadic mutation against a background of normal DNA are discussed further below. It may be that a relatively small number of *TP53* mutations occur commonly in colorectal tumours and, as in certain other cancers, this may provide a diagnostic opportunity [57]. A combination of screening stool for the three *KRAS* mutations and the three most common *TP53* mutations might detect up to 50% of pre-symptomatic colorectal cancers [19]. Allele-specific PCR approaches are particularly useful in detecting small numbers of mutant alleles against a background of an excess of normal alleles [58, 59].

Initially, Sidransky and associates were surprised at the relative ease with which they could detect *KRAS* mutations in stool, given the expected signal to noise problems [54]. They calculated that the colon contains 5×10^{10} epithelial cells with up to 10^{10} normal cells shed per day on the basis of colonic villus epithelial cell migration rates. A colonic tumour of 1 cm^3 contains of the order of 10^9 cells so that approximately 10% of the tumour bulk would need to be shed daily to produce a stool DNA sample in which mutated cells constituted 1% of the total. Hall and Lane in this issue point to a phenomenon which may indirectly explain why detection of mutant cells in stool was easier than anticipated (pp. 2001–2012). The growing impression is that colonic epithelial cells are not simply shed from the tips of villi, but undergo apoptosis and re-absorption to a significant degree. In biological terms, it obviously makes sense for an organism to conserve metabolites in this way. In contrast, it is now clear that one characteristic of the malignant cell is that it has lost the ability to undergo apoptosis. This is particularly the case in colon cancer where mutations in the *TP53* gene, a vital component of the apoptotic process, are common [60]. Thus, it is perhaps not so surprising that tumour cells are over-represented in stool samples. Whilst this phenomenon may mean that diagnosis based on the analysis of stool samples will be skewed, in that it will tend to detect those tumours which have undergone *TP53* mutation more efficiently, it should nevertheless encourage the further development of this approach to screening. Whether the relative over-representation of malignant cells in clinical specimens as a result of a lack of apoptosis will provide screening and diagnostic opportunities for other cancers at different anatomical sites remains to be explored.

Table 2 represents a brief summary of the genes which may currently be regarded as targets for genetic screening in the common cancers. Obviously, the list is not exhaustive. Table 3 highlights genes which appear to be the best candidates for genetic screening in specific cancers. It will be appreciated that the list of genes in Table 2, implicated in the development of common solid tumours, may well extend considerably as studies are undertaken of the role in other cancers of the tumour suppressor genes involved in MEN1, MEN2, neurofibromatosis and eventually familial melanoma, bladder cancer and prostate cancer [61, 62]. The generality of mutation in the *MTS1/p16* cell cycle regulator gene in malignancy may make it an additional candidate for population screening purposes [63]. Tables 2 and 3, in combination, hopefully give some insight into those cases where screening strategies might quickly be developed. In colorectal cancer, we are in a position to provide detailed molecular analysis in patients with a view to comprehensive family counselling. It should be possible to implement population screening for *hMSH2* and *hMLH1* mutations in the near future, either by cascade screening of relatives with HNPCC or using currently evolving PCR-based assays to screen these relatively large genes for mutation. In the context of screening stool to detect early premalignant changes, we can envision *KRAS* and *TP53* hotspot based allele-specific PCR [19] or antibody screens [45], and eventually the possibility of assays being developed to allow *APC*, *hMSH2* and *hMLH1* to be screened blind in their entirety from stool to detect early mutations. Similar opportunities present themselves for bladder cancer with urine as a substrate [52, 53], although the clinical advantages of this remain to be established. In breast cancer, the

Table 2. Genes frequently mutated in the development of common cancers

Gene	Chromosome localisation	Gene size	Spectrum of mutations	Antibody-based diagnosis	Cancers arising
TP53	17p12	Medium	Multiple (T,S,D) Possible hot spots	Yes	Essential all, at high frequency
RB1	13q14	Large	Multiple	?	Breast, prostate, lung, ovarian and others at lower frequency
KRAS	12p12	Medium	Simple (three only)	Theoretically	Colon, lung, bladder, upper GI, others at lower frequency
APC	5q21	Large	Multiple (T,S,D) hot spots	Yes	Colon, stomach, pancreas, ovary, others at variable frequency
DCC	18q21	Very, very large	Multiple	Theoretically	Colon, others at lower frequency
MCC	5q21	Large	Multiple (T,S,D)	?	Controversial
E-Cadherin	16q22	Large	?	?	Colon, prostate, breast, stomach, head and neck, hepatocellular etc.
α-Catenin	5q31	Large	?	?	Colon, oesophagus
β-Catenin	3p21	Large	?	?	Colon
hMSH2	2p15	Large	Multiple (T,S,D)	Theoretically	Colon, breast, uterus, stomach, ovary, bladder and probably others
hMLH1	3p21	Large	Multiple (T,S,D)	Theoretically	Colon, breast, uterus, stomach, ovary, bladder and probably others
BRCA1	17q21.1.	Large	Multiple (T, S, D)	Theoretically	Breast, ovary, colon, prostate
(*BRCA2* recently linked to chromosome 13)					

T, Truncation; S, amino acid substitutions; D, deletions.

situation is different to that in cancer of the colorectum. Whilst it should be relatively straightforward to screen for familial disease by monitoring *hMSH1*, *hMLH1* and eventually *BRCA1* and *BRAC2* in pedigrees [20], it is not yet clear what clinical material might be available on which to base genetic screening for early development of sporadic breast cancer. It remains to be seen whether malignant cells from the stomach survive in stool to the extent that screening for occult gastric cancer can be undertaken in this way. It has been shown that *H. pylori* infection of the upper GI tract can be diagnosed by PCR amplification from stool. This whole field is developing extremely rapidly and, without doubt, further opportunities for screening will emerge.

Emerging technologies for genetic screening for cancer predisposition are outlined in Table 4. Many of these are relatively familiar and will not be considered here in detail. The importance of continuing to keep the possibility of using antibodies in mind has been stressed recently [45]. Many of the assay formats discussed above, particularly those involving rapid scanning of whole genes or those involving sensitive detection of trace amounts of mutant cells in a normal background (e.g. stool, urine), require very extensive process development. This is not usually the kind of activity performed well in an academic environment. Given the dominant position of PCR in all these approaches, commercial process development of the type required is unlikely to be undertaken except by the owners of this intellectual property. Given the numbers of screens potentially required and their individual complexity, it seems unlikely that the "owners" of PCR will be capable of developing the wide range of tests required in a timely manner. In several of the examples discussed below, other parties have patent positions covering formats and adjunct methods for PCR assays. The commercial background to the provision of PCR-based cancer genetic tests is therefore complex in the extreme. Unfortunately, it seems inevitable that this will delay the development of many of the following concepts.

Two exciting recent discoveries point to the possibility of rapidly screening hundreds of kilobases for mutations, in simple assay formats. The first finding is that use of alternative thermostable DNA polymerases allows PCR products over 10 kb to be generated [65, 66]. The second finding, inspired by the dis-

Table 3. Candidate genes for screening in specific cancers

Cancer	Familial or sporadic	Gene	Test sample	Comments on potential formats and inherent problems
Colorectal	F (FAP) (Gardner syndrome)	*APC*	Blood	PCR or antibody Wide range of possible mutations in a large gene
Colorectal	S	*APC*	Stool (peritoneum)*	As above. Probably PCR essential for sensitivity
Colorectal	F (Li–Fraumeni)	*TP53*	Blood	PCR or antibody Wide range of possible mutations in a medium-sized gene
Colorectal	S	*TP53*	Stool (peritoneum)*	As above. Probably PCR essential for sensitivity
Colorectal	S	*KRAS*	Stool (peritoneum)*	Potential simple allele-specific PCR format for 3 mutation sites only
Colorectal	F	*hMSH2*	Blood	PCR, potentially antibody
Breast	(Lynch type I, II)	*hMSH2*	Blood	—
Ovary	(Lynch type I, II)	*hMSH2*	Blood	Wide range of possible
Uterus	(Lynch type I, II)	*hMSH2*	Blood	mutations in a large gene
Stomach (?)	(Lynch type I, II)	*hMSH2*	Blood	—
Bladder (?)	(Lynch type I, II)	*hMSH2*	Blood	Indirect PCR assay possible for microsatellite instability
Colorectal	S	*hMSH2* and *hMLH1*	Stool (peritoneum)*	PCR essential for sensitivity
Breast	S	*hMSH2*	Fine Needle Bx	Prognostic tests on
Ovary	S	*hMSH2*	Guided Bx	tumours found by
Uterus	S	*hMSH2*	D and C	population screening
Bladder	S	*hMSH2*	Urine	PCR essential for sensitivy
(Urine and stool testing is for actual mutations in the *hMSH2* and *hMLH1* genes; indirect assay of microsatellite instability difficult to configure because of the presence of excess normal cells.)				
Breast	F	*BRCA1* and *2*	Blood	PCR tests of linked microsatellites
Ovary	F	*BRCA1* and *2*	Blood	to identify at risk relatives of affected patients
Breast	S	*BRCA1* and *2*	-	Gene has just been identified [35a].
Ovary	S	*BRCA1* and *2*	-	Not yet apparent how to obtain clinical material by non-invasive procedures to allow simple screening
Bladder	S	*TP53*	Urine	As above
Bladder	S	*KRAS*	Urine	As above
Bladder	S	9q tumour suppressor	Urine	Not yet cloned
Melanoma and multiple other cancers	F and S	*MTS1/p16*	Blood/ Skin Bx/ Urine	Wide range of mutations in a small gene

*Samples labelled "peritoneum" might be obtained by peritoneal lavage post-operatively after colorectal cancer resection to screen for residual disease or recurrence. A *BRCA2* gene has recently been mapped to chromosome 13 [35b].
F, familial; S, sporadic; PCR, polymerase chain reaction; Bx, biopsy; D and C, cervical diletation and uterine curettage.

covery that these genes are responsible for HNPCC, was that DNA mismatch repair enzymes, when expressed and purified, are able to detect mutations in samples because mismatches are generated by the denaturing and re-annealing of PCR products [67–69]. The component of the DNA mismatch repair system responsible for recognising mismatches in the first place prior to repair is known in *E. coli*, yeast and now man [70, 71]. Although this remains to be fully reduced to practice, there is no theoretical reason why Mut S or its higher homologues should not recognise point mutations in large DNA fragments. This is precisely the situation it encounters *in vivo*. Thus, the combination of large PCR products and Mut S type DNA mismatch detection proteins promises to provide a powerful and general methodology for diagnosis. Successful implementation of this technology requires precise copying by the DNA polymerase used. It should be possible to develop very simple formats, given that the presence of mutation in the cancer cell and the corresponding mismatch in the PCR products arising after re-annealing will lead to binding of a Mut S or equivalent, which can be detected with an antibody to the protein. Alternatively, a 3′ exonuclease can be

Table 4. Techniques for screening genes to predict cancer predisposition

(a) MUTATION UNCHARACTERISED

(i) Detection of large deletions

- **Loss of heterozygosity** (LOH) studies using microsatellite PCR with automated quantification of alleles using an ABI Sequencer with GeneScan software.
- **Multiplex PCR** to produce fragments covering the whole gene or key exons (compare example of Duchenne muscular dystrophy deletion detection) [64]. Potential for automation as above. Recent advances allowing production of large (>10 kb) PCR products gives this technology much greater potential scope [65, 66].
- Use of N- and C-terminal **specific antibodies** to detect gross deletions at the protein level.

(ii) Detection of point mutations or small insertions/deletions

- **Direct sequencing**: automation with DNA sequencers and dedicated robotics for sample preparation. Possible for familial disease but too slow with current technology to screen for sporadic cancer.
- **Chemical cleavage**: denaturation/annealing of PCR products from samples heterozygous for a mutation, creates double-stranded DNA containing a mismatch, cleaved using reagents developed for Maxam–Gilbert DNA sequencing. Problems detecting *all* mutations giving possible false negative results. Possibility of further development as a result of advances, allowing production of large PCR products with high sequence fidelity. Cleavage theoretically independent of DNA size. Similar comments apply to RNase cleavage approach. If large genes (>200 kb) can be amplified from clinical samples as a multiplex of say 10 × 20 kb PCR products, it may be possible to identify a specific mutation in a single chemical cleavage reaction of this whole complex mixture [65, 66].
- **Single strand conformation polymorphism** (SSCP) and **denaturing gradient gel electrophoresis** (DGGE); electrophoretic techniques which allow mutations to be detected in relatively short PCR products on the basis of the conformational changes induced by sequence alterations. Currently, the most widely used technologies for identifying mutations in patient samples in a research setting. However, unlikely to be long term solution for population screening in sporadic cancer because not useful for analysing very large PCR products (where conformational differences become insignificant) so that rapid scanning of large genes may not be possible.
- **Controlled hybridisation assays**: based on the observation that, with carefully controlled hybridisation conditions, a perfectly base-paired hybrid will form in preference to one containing a mismatch. PCRs are performed with a "capture" moiety on one primer and a "signal" moiety on the other. Assay signal is measured after capture of PCR product. If careful annealing of denatured PCR products containing a mutation is conducted in the presence of a large excess of unlabelled "normal" PCR product, then persistance of positive signal points to the presence of the mutation. Very simple easily automated formats are possible. Again the technique will be limited by its inability to perform efficiently with very large PCR products [73, 74].
- **DNA mismatch repair enzyme-based assays**: stimulated by cloning of HNPCC genes. Similar long term potential to chemical cleavage with probable advantages of greater sensitivity and specificity. Uses enzymes responsible for finding mismatches *in vivo* to identify them in denatured and rapidly re-annealed PCR products. Variety of possible formats e.g. signal generation via labelled antibodies specific for repair enzyme; 3′-exonuclease partial protection of PCR product by the repair enzyme generates a signal from mutant DNA whereas samples with no mutations are completely destroyed. Again, potentially applicable to very large PCR products for rapid scanning of large genes for unknown new mutations [65–69].
- **Antibody-based assays**: in general, more useful in the familial than the sporadic context. N-terminal specific antibodies will identify truncated proteins generated by creation of premature termination codons either by point mutation or frameshift insertions/deletions. Wildtype or mutant-specific antibodies may be available (e.g. *TP53*). Western blot or ELISA-type formats (e.g. based on a relative loss of signal for a C-terminal specific antibody).

(b) KNOWN MUTATION (IN FAMILY STUDIES OR MONITORING DISEASE RECURRENCE)

- **Allele specific amplification/ARMS**: generally applicable technology based on the finding that mismatched residues at the 3′ end of PCR primers prevent amplification. Amenable to automation and can be multiplexed to allow simultaneous analysis of multiple mutations in several genes. Usual format is to perform two PCRs with normal or mutant specific primers, respectively. Possible unique value in screening for known mutations (e.g. *KRAS*) in sporadic tumours or to monitor for disease occurrence where it is possible to selectively amplify very low levels of mutated tumour cell DNA, even in the presence of massive excesses of normal cellular material.
- **Allele-specific oligonucleotide hybridisation**: conditions can be found where labelled oligonucleotides complementary to normal or mutant sequences will only bind to their exact counterparts. Has been used to detect mutant *KRAS* amplified from stool samples, but does not provide selection for the mutant allele. Complicated two-step PCR/hybridisation format necessary.
- PCR with primers introducing a restriction site into products dependent on the presence/absence of a mutation. Again requires two steps (**PCR and restriction digestion**) and, therefore, unlikely to form the basis of high throughput screens.
- **Antibody-based assays** (see above). If specific mutations are sufficiently frequent (e.g. *KRAS*, *TP53*) it will be worthwhile to raise unique monoclonals to detect that specific protein variant alone. This may provide sufficient sensitivity to allow screening for sporadic tumours in samples with a massive normal background.

used. PCR products without mismatches will be degraded. PCR products with mismatches and bound Mut S are protected from nuclease degradation beyond the mutation site, and two single-stranded DNA molecules are produced from those portions of the PCR product which lie 5′ to the region of mismatch. Again, these are relatively simple to quantify [69]. Availability of this type of generic technology moves genetic cancer diagnosis forward in many areas. An ideal scenario, for example to monitor stool for the presence of shed mutant cells, would be to perform multiplexed large-fragment PCRs so that the whole of the *APC*, *TP53*, *KRAS*, *hMSH2*, *hMLH1* and any other candidate genes could be amplified. After denaturation and re-annealing of these

mixtures of PCR products, binding of Mut S protein would indicate mutation(s) somewhere in them and suggest the possibility of developing malignancy. Colonoscopic examination could then be undertaken. Whether population polymorphism in these genes will mask the appearance of underlying mutation remains to be established. Use of a "normal" control from the same patient's blood DNA might allow polymorphism to be controlled for. Alternatively, common polymorphic variants in specific populations will be quickly identified in these programmes, and these can be eliminated from the analysis, for example by always designing PCR primers which remove polymorphism by virtue of siting them so that the variable base pairs are towards their 5′ ends. Thus, the stage is set for exciting developments in this area.

SCREENING FOR SPECIFIC CANCERS

Skin cancer

The potential value of visual examination by a dermatologist as a melanoma/skin cancer screening tool is well recognised, particularly in populations at high risk of melanoma [72]. It remains to be seen whether cloning of genes implicated in melanoma development on chromosomes 9p [63, 75], 1p [76] or 6q [77] provides opportunities for genetic screening. Without question these genetic advances would create a screening demand in familial melanoma and the dysplastic naevus syndrome [41, 78]. The epidemiology and public health issues surrounding melanoma have been reviewed recently [79–81]. Xeroderma pigmentosum is known to be associated with malignant melanoma [82]. Mutations in *TP53*, *RB1* and *NF1* have all been described in melanoma [80]. Patients with Li–Fraumeni syndrome are at increased risk of malignant melanoma [83]. Eventual cloning of the gene for Gorlin syndrome on chromosome 9q22.3, which is associated with naevoid basal cell carcinoma, may add additional insights into processes in cancers of the tegument [84]. A systemic genetic screening modality for skin cancer has been suggested by the observation that metastatic malignant melanoma cells can be detected by PCR in the circulation of patients [85]. Whether "premalignant" melanoma cells, which have early genetic mutations (possibly in the 9p gene) predisposing to progression to malignancy, appear in the circulation is not known. Much novel technology is being developed to purify fetal cells from the maternal circulation for antenatal diagnosis, and these approaches may eventually be applicable for the isolation of cancer cells. Thus, in the long term, simple blood tests may be possible to avoid the need for labour intensive clinical examination or a requirement for skin biopsy specimens.

Head and neck cancer

There are no definitive recommendations concerning screening for head and neck cancer [86]. Routine screening for laryngeal cancer using indirect laryngoscopy has not been shown to be beneficial. Regular examination of the oral cavity for squamous cell carcinoma has been suggested [87]. *TP53* mutations [88] and 3p deletions [89] appear common in most tumours of the aerodigestive tract, and the possibility of using saliva or sputum samples in screening programmes needs to be borne in mind. The technologies applicable would be the same as those appropriate for stool and urine samples. Screening programmes are less appropriate because of the association of these cancers with smoking and excess alcohol consumption. It is not clear whether cells from malignant brain tumours appear early in the cerebrospinal fluid (CSF). An increasing range of LOH or genes with mutations are now described in various brain tumours including *TP53*, chromosome 9p and 10q loci in glioblastoma; chromosome 19q loci in astrocytoma and oligodendroglioma; 22q in ependymomas, acoustic neuromas and meningiomas; neurofibromatosis types 1 and 2 mutations on chromosomes 17q and 22, respectively [90]. Routine screening of CSF is never likely to become accepted practice given the need for lumbar puncture to obtain it.

Lung cancer

Despite the obvious association of lung cancer with cigarette smoking, debate continues about possible approaches to screening because of the huge numbers of cases involved [91]. In the U.S.A., lung cancer represents 17% of male malignancy and 12% of female disease in terms of incidence, and 34% of male and 22% of female cancer mortality. Some 170 000 new cases were diagnosed in 1993 in the U.S.A., 70 000 of them women. 149 000 patients died including 56 000 women. Estimates of smoking association are between 85 and 90% of the disease load. Recent prospective randomised controlled trials of screening for lung cancer by large scale radiology or cytology did not show any significant reduction in the mortality rate [92, 93]. These studies are prone to lead time bias and length biased sampling. Thus, although a number of potential genetic approaches can be envisaged (as well as *TP53*, *RB1* and *KRAS*, cloning of 3p genes mutated in small cell lung cancer can be anticipated in the immediate future), it is difficult to see how these could be incorporated into clinical practice [94, 95]. Screenable variants in cytochrome P450 and glutathione S-transferase genes predispose to lung cancer by rapid oxidative activation of aromatic hydrocarbons in cigarette smoke [96]. It remains difficult to justify lung cancer screening when the cause of so many cases is avoidable [97].

Upper gastrointestinal cancer

Simple screening for predisposition to oesophageal cancer remains elusive. A clear link between Barrett's oesophagus and a 30-fold increase in risk of progression to columnar adenocarcinoma is well recognised [98]. The importance of the length of Barrett's oesophagus in this process has been demonstrated as has the need for regular surveillance of such patients when identified. The incidence of oesophageal cancer seems to be increasing. The occurrence of a familial syndrome, Tylosis with oesophageal cancer, recently linked to human chromosome 17q12-24, suggests that it could be possible to clone genes predisposing to this malignancy [99]. Known tumour suppressor genes have been implicated in oesophageal cancer (see Table 2) [100]. Cytological screening of some 13 000 individuals in China detected substantial numbers of patients with squamous or columnar dysplasia and cancer [101]. In another Chinese study, 233 825 people between the ages of 20 and 70 years were screened by faecal occult blood testing. 28 557 persons (12%) were positive, and gastroscopy of some 17 000 of these detected approximately 200 oesophageal, 200 gastric cardia and 200 gastric body cancers. Initial Chinese data suggest that lesions are being detected at an early stage, and that surgical intervention is producing a significant mortality reduction in upper GI tumours [102].

Screening programmes for gastric cancer have been implemented with considerable success in Japan in response to the high local incidence of the disease. Such screening has been undertaken since 1961. Twenty-five year follow-up studies suggest that two thirds of gastric cancer patients detected by screening were successfully cured of their disease surgically

[103]. This compares with a 20% 5-year survival rate still quoted in U.K. studies [104]. Clear association of *H. pylori* infection and gastric carcinoma is now well established [105]. *H. pylori* infection may be an important component of the development of gastric cancer in up to 55% of cases. However, most persons infected with *H. pylori* do not develop gastric carcinoma. By relating *H. pylori* infection rates in various populations with incidence of gastric cancer in the same populations, infection gives an estimated 6-fold increase in the risk of gastric cancer. The overall health gain anticipated from the elimination of *H. pylori* infection stands to be greater than might be achieved by any population-based screening programme for genetic mutations predisposing to this maligancy [106].

In terms of screening for pancreatic cancer, there are preliminary suggestions that ultrasonography alone may be useful [107]. It is not yet clear whether such screening would have any impact on overall mortality, given the morbidity associated with pancreatic surgery and the lack of other effective treatment modalities for the condition. There are some data to suggest that cells shed from pancreatic tumours are present and detectable in stool samples but this needs further investigation. It is difficult to envisage how other samples to allow presymptomatic screening for pancreatic cancer might be obtained.

Large intestine

The way in which colorectal cancer screening programmes have been evaluated and developed over the past 5 years is exemplary [108–116]. Those who seek to use novel genetic screening approaches in this context can learn a great deal by consideration of the scale and duration of the clinical studies required to demonstrate the essential outcome of a screening programme, reduction in overall mortality [117]. Despite many years' experience and hundreds of thousands of patients undergoing faecal occult blood screening, it is only recently that such a mortality reduction has been demonstrated [118]. The debate now centres on the role of flexible sigmoidoscopy [119] where the main issue is whether this should be offered every 3 to 5 years to individuals over 50 years or whether a single examination between the ages of 55 and 60 years will, in fact, detect a substantial proportion of occult early stage disease [120]. It may be that routine sigmoidoscopic screening could be performed by trained nurses [121, 122]. Current estimates of the cost of introducing a single flexible sigmoidoscopy examination for all 55-year-olds in the U.K. are some £30 million per annum for a total population of approximately 60 million. To put this in context, cervix cancer screening now costs £48 million per annum and breast cancer screening £25 million in the U.K. [120]. Given these enormous financial commitments, it is essential to establish that the proposed programmes are beneficial [123–126].

Controlled trials of the value of randomised faecal occult blood screening for colorectal cancer in Scandinavian, European, British and American populations began to report in the latter half of the 1980s [127–129]. In all cases, screening led to a significant increase in the detection of Duke's grade A and B cancers. The effect on disease survival was not a primary consideration in all these studies. The actual specific type of faecal occult blood test used was changed over time, and this affected sensitivity and specificity [130]. The lesson is that it is well worth ensuring that any genetic screening test for colorectal cancer using stool is very fully validated prior to commencing any large scale trial. The value of colonoscopic follow up of individuals found to have different sizes and types of adenomas has begun to be assessed [131–134]. There is now an extensive literature in this field, but encouragingly there is also a wide participation of health economists in study designs and the public debate. Thus, there are grounds for optimism that trials for any genetic colorectal cancer screen will be well designed and conducted [135–139]. A key issue (for which international guidelines are now urgently required) is the question of what to do about population screening for HNPCC carriers and what surveillance procedures are most appropriate for them. Given the high annual incidence of colorectal cancer (160 000 cases per annum in the U.S.A. with 60 000 deaths), the prospect of reducing overall mortality by 20% by simple FOB testing and flexible sigmoidoscopy is very exciting [120]. It is important to establish whether identification of individuals at risk for HNPCC would add significantly to this figure.

Breast cancer

An enormous literature has accumulated as a result of some 30 years' experience worldwide on mammographic screening. Frequent mammography after the age of 50 years is now widely accepted as desirable [140–153]. The U.S. incidence of breast cancer apparently rose by some 28% between 1973 and 1989, but this increasing incidence seems to have plateaued between 1989 and 1991 [154]. This increase has been compensated for by an increase in detection of early disease through screening so that overall mortality has remained constant. A number of aspects of breast cancer screening remain controversial [155–160]. Firstly, debate continues as to the merit of screening premenopausal women in the 40 to 49 year age group by mammography [161–165]. To date, the evidence of benefit is limited. The remaining concern and an area for further research is that of how to increase uptake of screening in the population at large so as to achieve maximal health gain [166–168]. A detailed review of this massive field is beyond the scope of this short article.

Obviously cloning of the *BRCA1* gene creates a considerable need for focused screening [35, 35a]. Estimates of overall risk of breast and ovarian cancer in *BRCA1* mutation carriers have been produced [20]. The estimated cumulative risk of breast cancer in gene carriers is 87% by the age of 70 years. The estimated cumulative risk of ovarian cancer in gene carriers is 44% by the age of 70 years. A significant increase in the incidence of colon and prostate cancer in carriers of *BRCA1* mutations has also been noted. Current estimates suggest that some 6% of all breast cancers may be the result of *BRCA1* mutation. The *BRCA2* gene has recently been mapped to chromosome 13 [35b]. The extent to which DNA mismatch repair gene mutations (HNPCC) cause breast cancer remains to be determined. The role of mutations in these various genes in the development of sporadic breast and ovarian tumours promises to be most interesting [21, 169]. The effect of repeated mammography on breast tissue in individuals carrying DNA mismatch repair enzyme mutations gives grounds for some concern as discussed above. Considerable adverse publicity was generated by the suggestion some time ago that the incidence of breast cancer in populations subjected to frequent mammography was in fact higher than that in unscreened populations [170, 171]. Perhaps the HNPCC phenomenon is the explanation for this.

Ovary

There is still no established consensus that population screening for ovarian cancer is worthwhile [172, 173]. Both CA-125 serology and transvaginal ultrasonography with or without

colourflow Doppler sonography have been extensively assessed, but no evidence is available to show that detection of these tumours reduces mortality [174–176]. In terms of genetic predisposition, similar comments apply to those above for breast cancer in carriers of *BRCA1*, *BRCA2* and HNPCC mutations [177–179]. It seems reasonable to predict that individuals identified with high risk genetic mutations will require frequent surveillance with CA-125 serology and ultrasonography prior to possible elective oophorectomy. Prophylactic surgery does not completely remove the need for continued surveillance as there are instances where peritoneal malignancy has developed even after removal of two apparently normal ovaries.

Cervix

Although again there is reasonable unanimity that Papanicolou cervical smear testing is a successful cancer prevention technique, there are still issues that require resolution [180–182]. These include the most efficient organisational systems to guarantee continued uptake of testing [183, 184] and the optimal screening service to offer to women over 65 years of age [185, 186].

Uterus

Population-wide screening for uterine cancer is not currently undertaken [187]. Again, a major consideration here will be surveillance of individuals found to carry HNPCC mutations. It is conceivable that regular abdominal ultrasonography will be required to monitor the appearance of ovarian, uterine and potentially rightsided colorectal tumours in Lynch syndrome type II. Obviously, ultrasound for detection of tumours of the large bowel is of limited sensitivity.

Prostate

Whilst the American Cancer Society has recommended that men over the age of 50 years have annual digital rectal examination and a prostate-specific antigen assay [188], no randomised controlled study of prostate cancer has ever demonstrated disease-specific mortality reduction from any test or procedure [189]. Whilst transrectal ultrasound will sometimes identify tumours, the technology is notoriously unreliable. Ultrasound-guided biopsies or specimens removed at transurethral resection for prostatic hypertrophy will often reveal occult malignancy, but the potential impact of this type of detection on overall mortality is far from clear [190]. A range of tumour suppressor genes are known to be frequently mutated in prostate carcinomas, but no non-invasive sampling techniques have been defined to allow screening for these. There has been some suggestion that a number of specific loci in the human genome are mutated in prostate cancers (7q, 8p, 10q and 16q), and this may allow additional tumour suppressor genes to be cloned. There is an autosomal dominant form of prostate cancer which is not apparently always associated with early presentation [31, 32]. Whether the retrospectoscope will eventually show that a substantial proportion of prostate cancer is, in fact, familial awaits further research. The role of *BRCA1* mutation in prostate cancer also requires further study [20].

CONCLUSIONS

Without question, we are now at the beginning of a new era in which extensive genetic screening will be possible for the effective detection and clinical management of several human cancers. Many of the technologies developed to allow these screening programmes will be relatively generic and applicable to cancers in different organs. It will be interesting to see how the introduction of such programmes is managed in different countries. There is a danger that in those countries where screening guidelines for specific cancers are issued by different professional bodies, overall co-ordination may be lacking. We can anticipate that the NCI and the American Cancer Society will take a leading role in the U.S.A., but it is not currently clear how the U.K. National Health Service will organise itself to deliver these kinds of services. Responsibility seems to lie somewhere between the Regional Genetic Testing Laboratories and traditional histopathology departments. Given the massive amounts of money which are inevitably consumed in any major screening programme, it seems prudent to set aside substantial resources to allow very careful assessment of the value of genetic screening for cancer predisposition in the very many different clinical circumstances in which it is likely to be attempted. Whilst the potential for health gain here is enormous, the scope to waste large quantities of public money is considerable.

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