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Comments and Critique

Predictive Testing for Germline Mutations in the p53 Gene: Are All the Questions Answered?

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THE EXPANDING FIELD OF THE MOLECULAR GENETICS OF FAMILIAL CANCER

CANCER IS a genetic disease at the cellular level; a series of genetic changes occur to produce the malignant phenotype. In familial cancer, the first of these steps can be inherited in the germline thereby predisposing carriers to an increased risk of certain cancers. This risk can be considerable, for example, an individual carrying an abnormal p53 gene may have a 90% risk of developing cancer by age 50. Cancer predisposition is often manifest as cancer at an earlier age than in sporadic cases of the disease [1] and multiple cancers, for example, bilateral as opposed to unilateral breast cancer [2].

As more of the human genome is cloned in the Human Genome Project, and the positions of disease genes are ascertained by genetic linkage studies in cancer families, increasing numbers of cancer-predisposing genes will be sequenced. This will open up the possibility of predictive genetic testing to identify individuals at higher risk of developing certain cancers than the general population.

The problems that arise in predictive testing for cancer-predisposing genes include the correct identification of a "cancer family", identifying the cancer families appropriate for predictive testing and the genes to test, estimating the risk of cancer development from molecular epidemiological studies, counselling about the implications of a positive genetic test, and the limitations of the test. Individuals at risk then need to be offered screening or preventative measures which are effective.

Predictive testing is not yet possible for most cancer families, since the genes responsible have yet to be cloned. One of the most important of these is the BrCa1 gene on chromosome 17q, which predisposes to breast and ovarian cancer. The cloning of this gene (which is likely within the next 2 years), may lead to more widespread genetic testing. Predictive testing of other genes is already possible; one of these is the cancer predisposition gene, p53. This comment article discusses the problems that have arisen in predictive p53 testing which illustrate many of the hurdles which will have to be overcome in the testing of each new cancer-predisposition gene as it is discovered.

THE p53 GENE IS A CANCER PREDISPOSITION GENE

The p53 gene is the most commonly altered gene in human cancer [3, 4]. It is a tumour suppressor gene which in its normal form codes for a 53-kD protein which binds to DNA and acts as a transcription factor to halt cells in the G1 to S transition in the cell cycle. Mutant forms lack this DNA binding activity. The gene consists of 11 exons and five conserved regions within which most, but not all, of the p53 mutations in tumours have been located.

The first clue that p53 is a cancer-predisposing gene came from the Li–Fraumeni familial cancer syndrome. This was first described as a clinical entity in 1969 by Li and Fraumeni [5], who noted the association between young onset sarcoma and other tumours in close relatives [6]. It consists of sarcoma in the index case at < 45 years, associated with sarcoma, breast cancer, primary brain tumour, leukaemia or adrenocortical tumour in a first degree relative at < 45 years and a cancer in another close relative at < 45 years of age or sarcoma at any age.

These kindreds are quite rare and often members are affected by cancer at a young age. Since these are associated with a high mortality, it has been difficult to perform linkage studies because of the paucity of large kindreds. However, in 1990, Malkin *et al.* [7] reported mutations in five Li–Fraumeni families in an area of the p53 gene (exon 7) which is mutated in a variety of sporadic tumours. Germline p53 mutations were subsequently found by other workers, mainly in Li–Fraumeni and Li–Fraumeni-like families. The mutations occurred throughout a large area of the gene, although they were usually found in the conserved regions. However, it is possible that only about half of all Li–Fraumeni families have p53 mutations (Li and Birch, personal communication), but the mutation screening techniques used could have missed some mutations.

Although the first p53 germline mutations were described in classical Li–Fraumeni families, they have subsequently been found in other familial types, which can be defined as Li–Fraumeni-like. These are families with some, but not all features of the classical Li–Fraumeni syndrome. We define these families as containing two close relatives (up to third degree with respect to each other) who each have different tumours, and which consist of the tumours listed in the Li–Fraumeni syndrome. Current research is aimed at finding the frequency of p53 mutations in these families and see if they are restricted to a certain family pattern. The published germline p53 mutation results are shown in Table 1 [8–19].

It is already known that germline p53 mutations are not a significant cause of breast cancer families [15, 16, 20, 21]. In those breast cancer families (< 1%) which have mutations in p53 all but one have a family history of Li–Fraumeni-type tumours.

Li–Fraumeni families also often contain members with multiple tumours. The most common tumour occurring in adult survivors of p53 carriers who have had a childhood tumour, is early onset breast cancer. Malkin [13] has shown that 7% of children with tumours who subsequently develop another

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Table 1. Germline p53 mutations published to date

	Number
Types of families	
Li–Fraumeni	14
Li–Fraumeni-like	14
Non-Li–Fraumeni	2
No family history of cancer	1
Mutations	
Exons	
4	2
5	4
6	1
7	15
8	8
9	1
Mutation Type	
Missense	28
Stop codons created	
Insertion	2
Deletion	1
Tumour types in tested carriers	
Sarcoma	32 (42%)
Breast	25 (32%)
Brain	7
Colon/gastric	4
AML	1
ALL	1
NHL	1
Hepatoblastoma	1
Neuroblastoma	1
Lung	1
Endometrial carcinoma	1
Choriocarcinoma	1
Thyroid	-
Asymptomatic carriers	16 (aged 4–74; two known to be > 50 years)

tumour have germline p53 mutations. Kindreds which are not classical Li–Fraumeni have been described in which p53 carriers have had a large number of tumours (e.g. [17]). The patient described had five primaries at the time of publication and has now had seven primaries). The p53 carrier rate in the population of patients with multiple independent primary tumours is unknown, and the necessity for a family history of Li–Fraumeni-like tumours to increase the chances of being a p53 carrier is also unknown.

WHAT ARE THE CONSEQUENCES OF THE PRESENCE OF A p53 MUTATION?

Follow-up of p53 carriers in Li–Fraumeni families has shown that they are at increased risk of all the cancers seen in the Li–Fraumeni syndrome (Table 2), particularly before the age of 45 [22]. However, since many of these cancers are rare, even a large increased risk [such as a relative risk (RR) of 111 for adrenocortical carcinoma], still results in a low absolute risk. The risk of early onset breast cancer is, however, significant; the overall penetrance of gene carriers in the Li–Fraumeni syndrome is 90% by age 50, and the majority of cancers after childhood are breast cancer.

One major area of uncertainty with regard to p53 mutations is whether all mutations give rise to equal cancer risks. At present, there is no evidence that there is allelic heterogeneity which would give rise to differential risks, but the number of mutants is too small to be certain. It is not clear why some families have a classical Li–Fraumeni pattern and others are Li–Fraumeni-like and if their cancer risks are equivalent.

WHO SHOULD BE TESTED?

The clinical decision about whom to test depends on the chances of finding a mutation and the ability to take action to prevent the cancer(s) caused.

To date, Li–Fraumeni families have the highest proportion of germline p53 mutations (present in a minimum of 50%) and, therefore, are a candidate population for testing. Families fulfilling the classical criteria are uncommon, but because the cancer risks are so high, testing would be justified in a restricted number of cancer centres. These should have close liason with those centres with a paediatric oncology unit, because of the high incidence of childhood tumours in these kindreds.

Problems arise when the definition of the classical Li–Fraumeni syndrome is modified. Some investigators do not restrict the age of tumour onset and further studies have suggested that testicular tumours [23], melanoma, and even pancreatic and prostate cancer, the latter occurring at older ages, could be included in the syndrome which, after all, is only a clinical definition. It is also unknown if Li–Fraumeni-like families are a discrete entity or represent a variation of the Li–Fraumeni syndrome with a different penetrance and manifestation of p53 mutations which may be due to epigenetic effects, or could just have a paucity of sarcomas due to chance. It is possible that, in the future, patients with p53 mutations will be classified as having the "p53 syndrome", which will include Li–Fraumeni families. When other genes are identified which cause the remaining Li–Fraumeni families which are not due to p53, these may then be reclassified genetically. The frequencies of p53 germline mutations in Li–Fraumeni-like families as defined earlier is not yet known, but preliminary results from our laboratory suggest that it lies between 5 and 30%.

Toguchida *et al.* [12] have found that about 4% of sarcoma patients carry germline p53 mutations, and 63% of these have a positive family history and so are members of Li–Fraumeni or Li–Fraumeni-like families. The majority of these patients had osteosarcoma, so osteosarcoma patients, again with a family history, are a candidate population for p53 screening. From Toguchida's data, the incidence of p53 mutations in such patients without a family history is low and does not warrant routine testing of all osteosarcoma patients. The incidence of p53 mutations in a series of other types of sarcoma such as rhabdomyosarcoma is unknown, and research is needed to define further candidate populations.

Table 2. Relative risk of tumour development in gene carriers in Li–Fraumeni kindreds

	Age of carrier	
	≤ 45	> 45
Tumour type	RR	RR
Breast	17.9	1.8
Sarcoma	27.8	2.1
Brain	25.5	3.6
Leukaemia	13.1	3.9
Adrenal cortex	111.1	—

RR, Relative risk.

The incidence of p53 mutations in pure breast cancer families is very low (< 1%), and the majority of mutations have been found in cases in Li–Fraumeni or Li–Fraumeni-like families, so testing should again be confined to this group.

THE ROLE OF THE FAMILIAL CANCER CLINIC

The concept of genetics clinics is not new, but cancer genetics clinics are a recent development. There are about 12 such clinics in the U.K., and throughout Europe the field of cancer genetics as a speciality is expanding. In France, a Cooperative Network has been formed, and in the U.K., the Cancer Family Study Group is currently drawing up guidelines to develop a coordinated service and research effort in this area.

The role of the familial cancer clinic is to identify families at increased cancer risk due to an inherited predisposition. Such families usually have several affected members, often with cancer at a younger age than is seen in sporadic cases, and the cancers can be multiple. Young age in this context is usually less than 50 years. The pattern of cancers is also important, as is illustrated by the Li–Fraumeni and Li–Fraumeni-like syndromes.

The second role of these clinics is counselling about the cancer risks, the possible genetic causes, and the options for predictive testing and screening. The ultimate aim is to reduce cancer incidence and mortality.

HOW SHOULD THE TEST BE PERFORMED?

After counselling, many clinics allow individuals the option of a "reflective" period of about 1 month whilst the implications are considered by the patient. Written consent to testing is recommended, and the procedures to avoid sample mislabelling are rigorous. Similar guidelines to those for testing for other genetic diseases are being developed by the cooperative groups mentioned above.

The testing must involve molecular genetic techniques. Immunohistochemistry has been shown to fail to stain normal cells carrying a p53 mutation [17], and conversely to stain normal cells in a cancer family in which the p53 gene has been shown to be normal [24].

Predictive testing for p53 mutations involves testing of the whole gene, as the mutations within it are widespread. Since the mutations are various and the majority are missense mutations, resulting in a change in one amino acid in the protein product, either the whole coding sequence has to be sequenced or techniques to rapidly screen parts of the gene for mutations have to be used. With the latter techniques, once the area containing the mutation is identified, it has to be confirmed by sequencing of that region. There are three commonly used mutation screening techniques: single strand conformational polymorphism (SSCP) [25], denaturing gel electrophoresis (DGGE or a variant, CDGE [26, 27]) and chemical mismatch (HOT [28]). The principles are as follows: in SSCP, single strands of DNA have a different secondary conformation dependent on their base composition; in DGGE, double stranded DNA denatures at different temperatures, or concentrations of denaturant, dependent upon the base pair composition. The HOT technique mixes normal DNA with test mutant and allows single strands from each sample to reanneal. At the site of a base mutation, a mismatch occurs and can be identified by a chemical which binds to the mismatch and acts as a cleavage site for piperidine. Each technique has its advantages and disadvantages; SSCP and CDGE are rapid, but each exon (or at the most, two exons together) of p53 has to be analysed separately. Both have a sensitivity of nearly 90%. HOT can analyse larger areas, but is laborious and uses hazardous chemicals. It had a higher sensitivity than the other methods in a blinded study of samples [29], but has been reported to miss G to T mutations. The gold standard is sequencing, and this will probably be the method of choice if patients wish to have a 100% assurance that their p53 gene is normal.

It is more important, however, to ensure that positive results are confirmed since the actions taken by patients may be considerable, such as prophylactic mastectomy. Mutations should always be sequenced, and also confirmed by an independent test on a separate blood sample from the patient. The mutation should be corroborated by at least one other technique, such as restriction enzyme digestion or allele-specific hybridisation, in addition to sequencing.

Once a mutation is identified, tests can show with 100% certainty whether a relative is a carrier or normal.

WHEN IS A MUTATION NOT A RARE POLYMORPHISM?

Rare variations of DNA sequence can be normal in the population. When a mutation is discovered, its association with cancer development in other members of the family indicates that it is probably causing the cancer prediposition. As more mutants are characterised, it will become clearer which are really rare polymorphisms. If there is any doubt, the mutation should not be present in 100 normal population controls. A functional assay of the p53 mutants is now available [30] if there is any doubt about the mutation found.

EARLY DETECTION AND PREVENTION

The screening measures for the cancers seen in the Li–Fraumeni syndrome are unproven. Within research protocols, blood screening has been proposed to screen for leukaemia and magnetic resonance scans for early detection of brain tumours and sarcoma. These are all unproven, but early detection of soft tissue sarcoma may certainly warrant investigation since T stage is an independent prognostic factor for survival in this tumour [31]. If this were to be investigated, then asymptomatic children would have to be tested, which some investigators currently exclude from predictive testing (see ethical issues). Mammographic screening has been shown to reduce breast cancer mortality in the over 50 year age group [32], but its efficacy in the under 50s is unknown. It has not been proven to be detrimental, and the studies have not concentrated on the high risk populations. If effective, the optimal screening interval is also not known. Those recommending mammography tend to advocate annual or biannual screens because breast cancer in younger women tends to have higher proliferative index [33] and a greater number of interval cancers would, therefore, be expected in a younger screened group. The safety of mammographic screening in p53 mutants is uncertain. There are some limited data that Li–Fraumeni fibroblasts are at the radioresistant end of the spectrum [34], however, they also transform more readily in culture [35, 36], and if Lane's model [37] that p53 is a "molecular policeman" is correct, they may be more mutable and accumulate radiation damage. This is an area for urgent research.

Other breast cancer prevention options include chemoprevention or prophylactic surgery. The former are experimental and include tamoxifen and retinoids. Tamoxifen reduces by 40% the incidence of a contralateral breast cancer in women who have already had one breast carcinoma [38], but its ability to reduce the *de novo* incidence of breast cancer in women at increased risk is unknown. A pilot study is in progress in the U.K., but the

lowest entry age is 35 years because of the risks of tamoxifen to the fetus. Women with a p53 mutation are at greatest risk of breast cancer at less than 45 years; many have breast cancer in their late twenties to early thirties and so this option for prophylaxis is unsuitable for them unless they forego childbearing. If the risk reduction is the same as in women with breast cancer, their risk would be reduced to about 10 times that of the population which is still 1 in 10 by the age of 40. Retinoids would be a candidate for prevention trials in this group.

Although a contentious issue, prophylactic subcutaneous mastectomy does provide a high chance of protection against breast cancer, and may be justified in very high risk women. At present these would be p53 and BrCa1 gene carriers which have a breast cancer risk of about 18 times that of the general population at age 50 [22, 39]. Careful counselling is needed prior to deciding to have surgery, and the role of genetic clinics is to provide the information to enable women to make decisions about health prevention. Studies of the uptake and psychological effects of this treatment are needed.

THE ETHICAL ISSUES

There are concerns that predictive testing for cancer-predisposing genes will increase patients' anxiety, resulting in low uptake of health preventative measures due to psychological avoidance mechanisms [40]. The effects on the family, and social and economic consequences also need to be considered. Only further research to characterise p53 mutations and their effects will help us better advise families [41]. Since there are no preventative measures for most of the tumours which p53 carriers are at risk of developing before the age of 18, we do not advocate testing unaffected children outside research protocols. The effect on life insurance premiums is unclear, but individuals with a strong family history would have weighted premiums and these would be reduced to normal if a relative of a person with a known mutation was shown not to carry it.

THE FUTURE

Testing for abnormalities in cancer-predisposition genes is in its infancy, but it is a rapidly expanding field which will become part of the routine oncology service over the next decade. The effects of the concept of being at risk on the psychology of the individual need to be carefully considered. The potential benefits in terms of reassurance of those not at risk and the reduction in mortality from effective screening and prophylactic measures could be considerable. At-risk individuals in cancer families accrue mortality at a young age with subsequent devastating effects upon young families. At present, there are as many questions as answers in the area of p53 cancer gene predisposition, in particular, the at-risk groups need to be better defined and follow-up of known carriers is needed. A reduction in cancer mortality resulting from the interface between the laboratory and the clinic would be a breakthrough.

1. Claus EB, Risch NJ, Thompson WD. Age at onset as an indicator of familial risk of breast cancer. *Am J Epidemiol* 1990, **131**, 961–972.
2. Ottman R, Pike MC, King M-C, Henderson BE. Practical guide for estimating risk for familial breast cancer. *Lancet* 1983, **ii**, 556–558.
3. Hollstein MC, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. *Science* 1991, **253**, 49–53.
4. de Fromentel CC, Soussi T. TP53 Tumour suppressor gene: a model for investigating human mutagenesis. *Genes, Chromosomes and Cancer* 1992, **4**, 1–15.
5. Li FP, Fraumeni JF Jr. Soft tissue sarcomas, breast cancer and other neoplasms: a familial cancer syndrome? *Ann Intern Med* 1969, **71**, 747–752.
6. Li FP, Fraumeni JF Jr, Mulvihill JJ, *et al.* A cancer family syndrome in twenty-four kindreds. *Cancer Res* 1988, **48**, 5358–5362.
7. Malkin D, Li FP, Strong LC, *et al.* Germline p53 mutations in a familial syndrome of breast cancer, sarcomas and other neoplasms. *Science* 1990, **250**, 1233–1238.
8. Srivastava S, Zou Z, Pirollo K, Blattner W, Chang EH. Germline transmission of a mutated p53 gene in a cancer-prone family with Li–Fraumeni syndrome. *Nature* 1990, **348**, 747–749.
9. Santibanez-Koref MF, Birch JM, Hartley AL, *et al.* p53 germline mutations in Li–Fraumeni syndrome. *Lancet* 1991, **ii**,1490–1491.
10. Metzger AK, Sheffield VC, Duyk G, Daneshvar L, Edwards MSB, Cogen PH. Identification of a germline mutation in the p53 gene in a patient with an intracranial ependymoma. *Proc Natl Acad Sci USA* 1991, **88**, 7825–7829.
11. Law JC, Strong LC,Chidambaram A, Ferrell RE. A germ line mutation in exon 5 of the p53 gene in an extended cancer family. *Cancer Res* 1991, **51**, 6385–6387.
12. Toguchida J, Yamaguchi T, Dayton SH, *et al.* Prevalence and spectrum of germline mutations of the p53 gene among patients with sarcoma. *N Engl Med* 1992, **326**, 1301–1308.
13. Malkin D, Jolly KW, Barbier N, *et al.* Germline mutations of the p53 tumour suppressor gene in children and young adults with second malignant neoplasms. *N Engl J Med* 1992 **326**, 1309–1315.
14. Prosser J, Porter D, Coles C, *et al.* Constitutional p53 mutation in a non Li–Fraumeni cancer family. *Br J Cancer* 1992, **65**, 527–528.
15. Borresen A-L, Andersen TI, Garber J, *et al.* Screening for germ line TP53 mutations in breast cancer patients. *Cancer Res* 1992, **52**, 3234–3236.
16. Sidransky D, Tokino T, Helzlsouer K, *et al.* Inherited p53 gene mutations in breast cancer. *Cancer Res* 1992, **52**, 2984–2986.
17. Eeles RA, Warren W, Knee G, *et al.* Constitutional mutation in exon 8 of the p53 gene in a patient with multiple independent primary tumours: molecular and immunohistochemical findings. *Oncogene* 1993, **8**, 1269–1276.
18. Brugieres L, Gardes M, Moutou C, *et al.* Screening for germ line p53 mutations in children with malignant tumours and a family history of cancer. *Cancer Res* 1993, **53**, 452–455.
19. Kovar H, Auinger A, Jug G, Muller T, Pillwein K. p53 mosaicism with an exon 8 germline mutation in the founder of a cancer-prone pedigree. *Oncogene* 1993, **7**, 2169–2173.
20. Prosser J, Elder PA, Condie A, MacFayden I, Steel CM, Evans HJ. Mutations in p53 do not account for heritable breast cancer: a study in five affected families. *Br J Cancer* 1991, **63**, 181–184.
21. Warren W, Eeles RA, Ponder BAJ, *et al.* No evidence for germline mutations in exons 5-9 of the p53 gene in 25 breast cancer families. *Oncogene* 1992, **7**, 1043–1046.
22. Garber JE, Goldstein AM, Kantor AF, Dreyfus MG, Fraumeni JF Jr, Li FP. Follow-up study of twenty-four families with Li–Fraumeni syndrome. *Cancer Res* 1991, **51**, 6094–6097.
23. Hartley AL, Birch JM, Kelsey AM, Marsden HB, Harris M, Teare MD. Are germ cell tumours part of the Li–Fraumeni cancer family syndrome? *Cancer Genet Cytogenet* 1989, **42**, 221–226.
24. Barnes DM, Hanby AM, Gillett CE, *et al.* Abnormal expression of wild type p53 protein in normal cells of a cancer family patient. *Lancet* 1992, **340**, 259–263.
25. Orita M, Suzuki Y, Sekiya T, Hayashi K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics* 1989, **5**, 874–879.
26. Fischer SG, Lerman LS. DNA fragments differing by single base-pair substitutions are separated in denaturing gradient gels: correspondence with melting theory. *Proc Natl Acad Sci USA* 1983, **80**, 1579–1583.
27. Borresen A-L, Hovig E, Smith-Sorensen B, *et al.* Constant denaturing gel electrophoresis as a rapid screening technique for p53 mutations. *Proc Natl Acad Sci USA* 1991, **88**, 8405–8409.
28. Montandon AJ, Green PM, Giannelli F, Bentley DR. Direct detection of point mutations by mismatch analysis: application to haemophilia B. *Nucl Acids Res* 1989, **17**, 3347–3357.
29. Condie A, Eeles RA, Borresen A-L, Coles C, Cooper CS, Prosser J. Detection of point mutations in the p53 gene. Comparison of SSCP, CDGE and HOT. *Human Mutation* 1993, **2**, 58–66.
30. Frebourg T, Barbier N, Kassel J, Ng Y-S, Romero P, Friend SH. A functional screen for germ line p53 mutations based on transcriptional activation. *Cancer Res* 1992, **52**, 6976–6978.
31. Robinson M, Barr L, Fisher C, *et al.* Treatment of extremity soft

tissue sarcomas with surgery and radiotherapy. *Radiother Oncol* 1990, **18**, 221–223.
32. Shapiro S, Venet W, Strax P, Venet L. *Periodic Screening for Breast Cancer. The Health Insurance Plan Project and its Sequelae*, 1963–86. Baltimore, John Hopkins University Press, 1988.
33. Moran RE, Black MM, Alpert I, Straus MJ. Correlation of cell-cycle kinetics, hormone receptors, histopathology and nodal status in human breast cancer. *Cancer* 1984, **54**, 1586–1590.
34. Chang EH, Pirollo KF, Zou Z-G, *et al.* Oncogenes from radioresistant, noncancerous skin fibroblasts from a cancer-prone family. *Science* 1987, **237**, 1306–1309.
35. Bischoff FZ, Yim SO, Pathak S, *et al.* Spontaneous abnormalities in normal fibroblasts from patients with Li–Fraumeni cancer syndrome: aneuploidy and immortalization. *Cancer Res* 1990, **50**, 7979–7984.
36. Bischoff FZ, Strong LC, Yim SO, *et al.* Tumorigenic transformation of spontaneously immortalised fibroblasts from patients with a familial cancer syndrome. *Oncogene* 1991, **6**, 183–186.
37. Lane DP. p53, guardian of the genome. *Nature* 1992, **358**, 15–16.
38. Cuzick J, Baum M. Tamoxifen and contralateral breast cancer. *Lancet* 1985, **i**, 282.
39. Easton DF, Bishop DT, Ford D, Crockford GP and the Breast Cancer Linkage Consortium. Genetic linkage analysis in familial breast and ovarian cancer—results from 214 families. *Am J Human Genetics* 1993, **52**, 678–701.
40. Kash KM, Holland JC, Halper MS, Miller DG. Psychological distress and surveillance behaviors of women with a family history of breast cancer. *JNCI* 1991, **1**, 24–30.
41. Birch JM. Germline mutations in the p53 tumour suppressor gene: scientific, clinical and ethical challenges. *Br J Cancer* 1992, **66**, 424–426.

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Uveal Melanoma

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UVEAL MALIGNANT melanoma is the commonest primary intraocular malignancy with an annual incidence averaging 7 per million [1]. Although congenital tumours have been reported, peak incidence is in late middle age: in one study there were 3 cases per million under 50 years and 21 per million per year over this age [2]. It is predominantly a tumour of fair-skinned Caucasians and is uncommon in Asians and Orientals and rare in Negroes. Sunlight exposure and other environmental stimuli are not known to be predisposing factors although iris melanomas are much more common inferiorly where this structure is not covered by the upper eyelid. A study from Denmark demonstrated no overall increase in the frequency of ocular melanomas during a period in which the incidence of its cutaneous counterpart had increased five or six times [3]. Host factors play a strong part in the development of this malignancy. Most choroidal melanomas are now thought to arise in pre-existing naevi. Naevi are present in up to 2% of eyes clinically and up to 6.5% at autopsy [4]. The chance of malignant change in a naevus has been estimated at less than 1 in 500 during a 10-year period [4]. Congenital ocular and oculodermal melanocytosis are strongly associated with uveal melanoma [5] and annual ophthalmoscopic screening is recommended. There have been reports of a familial incidence [6] and of bilateral uveal melanomas and some of these cases have been linked to the atypical mole syndrome (AMS) [7]. There is an increased incidence of uveal naevi in AMS [8] and unilateral and bilateral uveal melanomas have been seen to coexist with cutaneous melanomas in affected individuals [7]. AMS sufferers should be screened for ocular melanoma and *vice versa*.

There is no convincing evidence that local ocular treatments reduce the high mortality rate of uveal melanoma. Large tumour size is the single most important clinical indicator of a poor life prognosis [9]. Histology is also highly predictive and individuals with tumours containing epithelioid cells fare worse than those with pure spindle cell lesions [10, 11]. The clinical and histopathological features may not be independent predictors of outcome because it has been shown that large tumour volume is closely associated with epithelioid cell type [12]. Furthermore, although extrascleral extension is unfavourable, this too is closely associated with epithelioid cell tumours [13] and multivariate analysis does not demonstrate an independent adverse effect of extrascleral extension on survival rate [14]. Location within the uvea appears to have a prognostic significance which is independent of tumour size. Iris melanomas tend to have a good prognosis and, although this may be due in part to early detection because they are visible to the patient, a higher proportion of these lesions have a relatively benign spindle cell histology compared with their counterparts in the ciliary body and choroid [15]. Posterior choroidal melanomas may be detected when quite small because they disturb vision early during their development by encroachment on the macula. Furthermore, asymptomatic posterior melanomas are easy to see on routine ophthalmoscopy during a sight test. By contrast, ciliary body melanomas are difficult to visualise, tend not to disturb vision until late in their development when they produce a secondary retinal detachment and so are often very large when first detected. Although large size at diagnosis clearly contributes to the exceptionally poor prognosis associated with ciliary body melanomas, anterior location appears to have an adverse effect which is independent of size [11].

Diagnosis of large melanomas anywhere within the uveal tract poses few difficulties and, aided by non-invasive ancillary investigations and particularly by ultrasound, specialist ophthalmic oncologists can distinguish such tumours from simulating lesions with an accuracy approaching 98% [16, 17]. Cases of difficulty can usually be resolved by open biopsy of anterior tumours or fine needle aspiration biopsy of posterior lesions with relatively little risk of extrascleral spread or damage to the eye. Most typical melanomas are in excess of 3 millimetres in

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