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Genetic susceptibility to cancer

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Abstract—A Task Group of the ICRP Committee 1 (Radiation Effects) has reviewed relevant data with the objective of advising the Main Commission of the ICRP on the possible implications for radiological protection of emerging views on genetic susceptibility to cancer (Chapter 1).

Chapter 2 considers DNA damage and its processing/repair after ionising radiation and serves principally to demonstrate that a few rare cancer-prone, human recessive genetic disorders show DNA repair deficiency and profound increases in radiosensitivity. Less dramatic changes in radiosensitivity are also apparent in a wider range of such disorders. The cellular mechanisms that underly the association between DNA damage processing and tumorigenesis are discussed.

Chapter 3 reviews the mechanisms and genetics of solid tumours illustrating the ways in which mutations in proto-oncogenes, tumour suppressor genes together with those in DNA repair and cell cycle control genes can contribute to tumour development. Specific examples are given of how germ line mutation of such genes can predispose to familial cancer. It is judged that up to 5% of all solid tumours have a recognisable genetic component. Heritable organ-specific effects are most usual and cancers of the breast and colon tend to show the most obvious genetic components. Clearly discernible genetic effects are seen when rare dominant germ line mutations express strongly as familial cancer (high penetrance mutations), but the existence of perhaps less rare low penetrance mutations and gene–gene interactions are recognised but not well understood.

Chapter 4 considers the mechanisms and genetics of lympho-haemopoietic tumours. Specific chromosomal translocations and proto-oncogene activation events are much more frequent in human leukaemia/lymphoma than in solid tumours. Genetic predisposition to leukaemia/lymphoma is found in a number of non-familial recessive genetic disorders of DNA processing and/or chromosomal instability. Familial manifestation of susceptibility to these tumours is, however, extremely rare. The genetic component, although poorly defined, is judged to be less than that of solid tumours and expressed largely in childhood.

Chapter 5 reviews and discusses limited data that comment upon tumorigenic radiosensitivity in cancer-prone genetic conditions. From knowledge of the fundamental processes involved it is judged that in most, but not all, cases genetic susceptibility to spontaneous tumours will be accompanied by a greater-than-normal risk after radiation. A review of epidemiological, clinical and experimental data relevant to this issue suggests that although a wide range of different sensitivities may be involved, a factor of 10 increase in sensitivity broadly accords with the limited human data available. This interim judgement of a factor of 10 increase in radiation risk in such human genetic disorders is made for the purposes of illustrative modelling and calculation. In addition, specific attention is given to breast cancer risk in heterozygotes for the radiosensitive human disorder, ataxia-telangiectasia; this association, while in no way discounted, is judged to be less strong than that claimed by some.

Chapter 6 discusses and develops computational modelling procedures that aim to describe the impact of genetic factors on radiation-tumorigenesis in human populations. Estimates of the prevalence of known cancer-prone genetic disorders are made but breast cancer susceptibility is used to illustrate the application of the model developed. The most important message to emerge from this work is that, even at an assumed high level of radiation sensitivity, the prevalence of familial (high penetrance) genetic disorders in the population is too low ($< 1\%$) for there to be a significant impact on risk in typical human populations. In principle, however, there is the potential for such impact in atypical inbred sub-populations where these mutations can be more common. These modelling procedures are also used to illustrate how incomplete penetrance of these mutations will dilute any impact on population risk.

In conjunction with the Main Commission of the ICRP, in Chapter 7 the Task Group discusses the potential implications of the main report for radiological protection. Their principal conclusions are: (i) That current estimates of radiation cancer risk already include an unknown contribution from genetically radiosensitive sub-populations. (ii) Using the data cited,

the likely contribution to radiation risk from familial cancer disorders is too low to generate an unacceptable distortion of current estimates of cancer risk in the vast majority of human populations. (iii) There is insufficient knowledge to judge the contribution to risk from mutations of low penetrance that do not express as familial cancer. (iv) Because of the high risk of spontaneous cancer in familial disorders, low doses of radiation (say 100 mSv) are most unlikely to impact significantly on life-time cancer risk in an affected individual; at high doses, such as those experienced in radiotherapy, this relative risk may however become important. (v) Because organ-specific cancer risk is predicted in most familial disorders, the absolute increase overall in risk to an affected individual will be diluted, ie. comparing normal and affected individuals. (vi) The utility of genetic testing for cancer predisposition in the context of radiological protection is currently limited by technical factors and concerns on predictive power. In the future genetic testing may find selected use prior to certain medical exposures to radiation, but the value of such procedures, as applied to low-dose occupationally exposed individuals, is open to doubt; it would also be subject to major ethical scrutiny outside the remit of the ICRP.

The Task Group and the Main Commission of the ICRP stress that, because of the current lack of knowledge, the above judgements should be regarded as preliminary. The report serves principally to provide a framework on which to develop further views in this rapidly advancing area of human genetics.

1. INTRODUCTION

(1) The last two decades have witnessed a spectacular increase in our fundamental understanding of the cancer process. This has come about largely through the development of powerful molecular genetic techniques that have allowed cancer-associated genes to be identified, isolated, functionally characterised, and mapped to specific regions of the human genome. These same techniques, together with advances in epidemiology and clinical studies, have revolutionised human genetics and coupled with advances in cancer biology have allowed the characterisation of a significant number of heritable disorders associated with a familial predisposition to neoplasia.

(2) In addition to the benefits accruing to clinical and medical genetic judgements this advance in knowledge also has potentially important socio-economic implications. Included in this latter category is the question as to whether germ line gene mutations that predispose to spontaneously arising human cancer also impose an increased cancer risk after exposure to environmental genotoxic agents such as ionising radiation.

(3) Current ICRP recommendations on radiological protection provided in *Publication 60* (ICRP, 1991) are based essentially on the estimation of excess cancer risk after exposure of whole populations. Since however all such populations will be genetically heterogeneous, there is a strong expectation that, even taking account of all other exogenous factors, the excess cancer risk per unit of dose following radiation exposure will be non-uniform because of specific differences in genetic make-up between individuals.

(4) On the above basis, this report seeks to review fundamental, clinical, and epidemiological data relevant to a heritable cancer predisposition and from this to make interim judgements on tumorigenic radiosensitivity in the genetic disorders of this type and also on the likely contribution that genetic factors may make to radiation-induced cancer in the human population.

(5) It is however important to stress at the outset that, in the context of radiation effects, directly informative epidemiological data on a cancer predisposition are essentially lacking. For this reason emphasis is placed on the implications of data from experimental studies and clinical observations, on the development of computational genetic models of cancer risk and on possible research strategies for the future. Together, these are intended to provide a framework to facilitate future judgements by the Commission on this potentially important aspect of radiological protection.

(6) The Task Group met to review data and compile their report between 1993 and 1996. The report was essentially completed and adopted by the Commission during 1997 with minor revisions in early 1998.

2. DNA DAMAGE AND REPAIR

(7) Throughout this report reference will be made to specific cancer-prone human genetic disorders and the clinical, epidemiological, and cellular/molecular data that serve to define their phenotypes. Since however a prime objective of this report is to review tumorigenic mechanisms after radiation in relation to a cancer predisposition, it is important to briefly consider data relating to radiation-induced cellular damage, DNA repair mechanisms, and mutagenic processes.

(8) Assessment of the nature and extent of radiation-induced cellular damage has preoccupied radiation biology for many decades. Although enumeration of such induced lesions is undoubtedly appropriate, evaluation of the forms of cellular damage that are most relevant to neoplastic development is of principal importance for the purpose of this report. In order to achieve such meaningful evaluations, it is necessary to take full account of the nature of the cellular lesions that principally characterise the neoplastic state itself. In this way it becomes possible to correlate specific radiation-induced cellular events identified in model experimental systems with the in vivo induction of tumours. It is fortunate that, from the reviews of the mechanisms of neoplastic development provided in Sections 3 and 4 of this report, it is possible to identify gene mutations and chromosomal events including loss of heterozygosity (chromosomal and gene deletions) as critical events in tumorigenesis.

(9) This section considers the nature of radiation-induced genomic lesions in somatic cells and the cellular repair processes which correct these or lead to gene and chromosomal mutations; also described are the somatic cell mutants and human genetic disorders in which the processing of radiation-induced DNA damage is believed to be impaired. We have elected to avoid discussion of the specific effects of ultraviolet radiation (UVR) and chemical genotoxic agents except where they directly contribute to our broad understanding of the problem in hand.

2.1. The DNA damage spectrum after radiation

(10) The nature of the initial DNA damage produced by ionising radiations and the effects of radiation quality have been extensively reviewed (Ward, 1988, 1991; Price, 1993; Prise, 1994). There is a strong consensus that cellular DNA is the critical target for radiobiological effects and that differences in radiation track structure influence the nature of induced DNA lesions and their cellular consequences.

(11) The DNA molecules of cellular nuclei may not always represent the direct target for energy loss events from radiation and DNA damage may be mediated by the products of the radiolysis of water or perhaps other cellular constituents. These radiolysis products, i.e. reactive chemical radicals, which represent the primary stage of the damage induction process, are incapable of diffusing over significant cellular dimensions because of endogenous scavenging capacity. As a consequence, radiation action on DNA is essentially a local process.

(12) Radical induced damage as a consequence of a radiation track intersection of DNA may occur within all the chemical moieties of DNA and, since it is local in nature, LET-dependent clustering of DNA lesions is to be expected. Spontaneous chemical reversal of such DNA lesions may occur but, if not, the damaged site becomes the potential substrate for enzyme-mediated repair which either restores the molecular lesion to its original undamaged state or results in stable DNA damage, i.e. gene or chromosomal mutation.

(13) Radiation-induced DNA lesions recognisable at the biochemical level are largely defined by the nature of the assay technique employed and include damage to DNA nucleotide bases, single and double strand breaks in the sugar-phosphate DNA backbones, and DNA–DNA or DNA–protein cross links. Complex lesions involving local clustering of different forms of molecular damage are also believed to occur and may be critical for radiation quality effects (Ward, 1991; Prise, 1994).

2.2. Post-irradiation cellular DNA repair and mutagenesis

2.2.1. *Damage to DNA nucleotide bases*

(14) DNA base damage can arise from direct radiation interaction with the macromolecule or perhaps most frequently via the generation of reactive free radicals in the water shell surrounding DNA. Various classes of reactions are involved in the radical attack on DNA bases and in the case of DNA base dissociation, oxidation, and cross-linking the local biological function of DNA is frequently impaired or lost.

(15) Amongst the principal DNA base damage induced by ionising radiation are 8-hydroxydeoxyguanosine (8-OHdG) and thymine glycols although a broad range of other forms of oxidative damage has been characterised (Ward, 1988, 1991). 8-OHdG may be regarded as the form of induced DNA base damage of greatest potential biological significance. It is known to be removed by a DNA base repair mechanism (Tchou *et al.*, 1991) but, most importantly, since it is a mis-coding lesion it is potentially mutagenic (Woods *et al.*, 1990). The available data suggest however that such base damage will play only a minor role in radiation mutagenesis (Ward, 1995).

2.2.2. *Single strand breaks (ssb) in DNA*

(16) It appears that DNA ssb do not contribute greatly to the process of radiation mutagenesis. These lesions are repaired rapidly (Kohn *et al.*, 1981) and, since such repair utilises the base sequence on the undamaged complementary DNA strand, repair fidelity is expected to be high. The first step in the repair process is the action of an exonuclease (phosphodiesterase) that serves to remove ssDNA at the damaged site. The resulting single strand gap is then filled by the action of DNA polymerase β with a short repair patch of 1 or 2 nucleotides; DNA strand rejoining is subsequently achieved probably via the action of DNA ligase 1.

(17) Some mutant rodent cells hypersensitive to the inactivating effects of ionising radiation have impaired DNA ssb repair but data for one such mutant (*irs* 1SF) reveals it to be hypomutable (Zdzienicka and Jongmans, 1994). Thus, while DNA ssb may not be completely neutral in terms of biological effects, they are clearly not a major contributor to radiation mutagenesis (Fuller and Painter, 1988).

2.2.3. *Double strand lesions in DNA*

(18) DNA double strand lesions including frank breaks are believed to be the lesion type of greatest importance in the induction by radiation of cell lethal events, chromosomal abnormalities, and gene mutations (Goodhead, 1994). For the purposes of simplicity the use of the term DNA double strand break (dsb) will be used in the text to describe collectively all forms of DNA double strand lesion. Misrepair or non-repair of a proportion of coincident lesions in DNA is to be expected through DNA dislocation and/or the non-availability of an undamaged complementary sequence for polymerase action. Such misrepair may include the interaction of DNA dsb through some form of illegitimate recombination in order to form the chromosomal exchanges and interstitial deletions that

are characteristic of radiation and which are implicated in neoplastic development (Sections 3 and 4). The biological importance of DNA dsb in radiation response is most clearly evident from studies of mutant rodent cell lines that are defective in the repair of radiation induced damage (Table 2.1; Thacker, 1992, Collins, 1993, Jeggo, 1994; Zdzienicka, 1995).

(19) The *xrs* cells of Table 2.1 are radiosensitive and demonstrate both an enhanced induction of chromosomal damage either measured biochemically (Nagasawa *et al*., 1991) or by premature chromosome condensation techniques (Iliakis and Pantelias, 1990). Direct demonstration of the importance of DNA dsb in the production of chromosome aberrations is further provided by the correlation in the time course of the loss of DNA dsb and aberration yield. For mutation induction, a five fold hypermutable state is observed when mutations at the *hprt* or *tk* locus were assessed at equal radiation doses in the genetic complementation group 5 sensitive mutants in comparison with their controls. When, however, the response at equitoxic doses is compared these cells showed a similar or reduced induced mutant frequency in comparison with the parental cell line. These results imply that the excess of DNA dsb detected at a given level of dose in these radiation sensitive cells is processed via a repair pathway which is essentially error prone; the result at equitoxic doses further suggests that there is a close relationship between the repair of potentially mutagenic and lethal lesions involving DNA dsb. This accords with early data relating to the lethal and mutagenic effects of low LET radiation in human and rodent cells (Thacker and Cox, 1975; Thacker, 1992).

2.2.4. *DNA repair and recombinational processes*

(20) The central role of DNA dsb repair has been revealed by the genetic analysis of rodent cell DNA repair mutants (Table 2.1). In combination with DNA ssb defective mutants, this compilation shows that there are at least seven human genes involved in the repair of DNA damage induced by ionising radiation, i.e. *XRCC1–XRCC7*, of which five are involved in DNA dsb repair. A further genetic approach which exploits the evolutionary conservation of DNA repair genes has made it possible to clone a further four human homologues of the yeast *rad* 6, 51, 52, and 54 genes; these latter three genes are also involved in DNA dsb repair (Table 2.2). The first direct evidence that recombination was important in the repair of DNA dsb induced by ionising radiation derived from studies on the radiation response of a severe combined immune deficiency (*scid*) mutant of the laboratory mouse (Biedermann *et al*., 1991; Hendrickson *et al*., 1991). In this autosomal recessive disorder whole animal radiosensitivity is reflected at the cellular level and is accompanied by defects in the repair of DNA dsb and impaired recombination of *V(D)J* immune sequences which in turn generates the immunodeficiency phenotype of these animals. *V(D)J* recombination requires several DNA breakage and rejoining steps, and is directed towards conserved recombination signal sequences that flank each of the germline V, D, and J genomic segments (see also Section 4). This recombination process utilises an array of cellular components and the evidence available suggests that the DNA dsb repair machinery which is used routinely to handle DNA damage is recruited to participate in the DNA dsb rejoining steps of *V(D)J* recombination. The relationship between competent *V(D)J* recombination and dsb repair is further evidenced by the observation that those radiosensitive mutants which are competent to carry out DNA dsb repair have normal *V(D)J* rejoining (Pergola *et al*., 1993; Taccioli *et al*., 1993; Thacker *et al*., 1994).

Table 2.1. Radiosensitivity and repair deficiency in rodent somatic cell mutants

Group	Mutant designation	Cell line	Phenotype	Human chromosome location	Human gene
1	EM9	CHO AA8	Defective ssb repair,	18q13.2–13.3	*XRCC1*
	EM-C11	CHO	hypermutable; elevated SCE		
2	irs1	V79	Reduced fidelity of dsb rejoining	7q33.1	*XRCC2*
3	irs1SF	CHO-AA8	Defective ssb repair hypomutable	14q32.2	*XRCC3*
4	XR1	CHOK1gly	Defective dsb repair	5q13–14	*XRCC4*
	M10	L5178Y	Defective *V(D)J* recombination		
5	*xrs5,6*	CHOK1	Defective dsb repair	2q.35	*XRCC5*
	XR-V15B	V79	Defective *V(D)J* recombination		(*Ku80*–DNA end binding)
6	sxi-2,3	V79-4	Defective dsb repair and *V(D)J* recombination	22q13	*XRCC6*
	sxi-1	V79-4			
7	V-3	AA8	Defective dsb repair	8q11	*XRCC7*(DNA–PK sub unit)
	scid	C.B-17 (mouse)	Defective *V(D)J* recombination		
	irs-20	CHO			
8	V-C4	V79	Radioresistant DNA synthesis (an AT like defect)	Mouse 9	*XRCC8*
	V-C5		ssb and dsb repair normal		
	V-G8				
	irs-2	V79			

Based on the reviews of Collins (1993) and Zdzienicka (1995).

Table 2.2. Human homologues of yeast genes involved in radiation response

Yeast mutant	Defect	Human gene location	Human gene
rad51	dsb repair	15q51	*hHR51*
rad52	dsb repair	12p13.3	*hHR52*
rad54	dsb repair	1p32	*hHR54*
rad6	Ubiquitination	Xq25–25	*hHR6A*
	processes	5q23–31	*hHR6B*

(21) The DNA dsb repair defective *xrs* mutants are defective in the DNA end-binding activity of a heterodimeric protein known as Ku (de Vries *et al*., 1989; Rathmell and Chu, 1994) and a gene encoding the Ku 80 kD subunit and the *XRCC5* gene map to the same location at human chromosome 2q33–35 (Hafezparast et al, 1993). *Ku80* cDNA corrects both the *V(D)J* rejoining defect and the radiation sensitivity of *xrs* cells and, since both phenotypes co-segregate in human/hamster hybrids containing segments of human chromosome 2, it was judged that the 80 Ku subunit was almost certainly the product of the *XRCC5* gene (Taccioli *et al*., 1994). A direct demonstration of this relationship was available when the defect in DNA dsb repair was corrected by introducing the *Ku80* gene into *xrs-6* cells (Ross *et al*., 1995). In addition to the 80 kD subunit, Ku is composed of a second tightly bound 70 kD component, and the binding of these peptides to DNA termini activates a third 350 kD subunit with protein kinase activity. This complex is known as DNA–PK and the kinase activity of this is totally dependent upon the binding of Ku to the broken DNA ends. The large 350 kD subunit of Ku has been shown to be the product of the *XRCC7* gene on chromosome 8q11 (Blunt *et al*., 1995) and is clearly also the product of the gene that determines murine *scid* phenotype. Genetic and biochemical studies on the human disorder ataxia-telangiectasia (A-T) provide additional important information on the role of DNA repair and recombinational processes in radiation response. The recent identification of the first human cells that are clearly defective in dsb repair (Badie *et al*., 1995) may assist in further mechanistic studies.

2.2.5. *Human ataxia-telangiectasia*

(22) The importance of DNA dsb repair has been revealed by the study of the human autosomal recessive cancer prone syndrome ataxia-telangiectasia (A-T) (Sedgwick and Boder, 1991; Taylor, 1992; Bundey, 1994). The syndrome is characterised by cerebellar ataxia as a consequence of progressive neurodegeneration together with permanently dilated blood vessels, known as telangiectases, particularly in the eye but which may occur on the ears or nose. Its frequency in the population is around one in 300,000 (Woods *et al*., 1990). Both cellular and humoral immune deficiencies are common and there is a considerably elevated incidence of tumours. Approximately 10% of A-T patients develop cancer in the first two decades of life, the majority of these tumours are of haemopoietic origin (see Section 4) and some may well be associated with the appearance of spontaneous chromosome translocations between chromosomes 7 and 14 which can expand to form large clones in the circulating T-cell populations of some patients (Taylor, 1982). The high frequency of chromosome aberrations in blood derived cells has led to this syndrome being defined as a ‘chromosome breakage or chromosome instability syndrome’. The syndrome became the focus of radiobiological interest following the demonstration that a dramatic clinical radiosensitivity (Cunliffe *et al*., 1975) was reflected

at the cellular level by hypersensitivity to both the lethal and chromosome breakage action of ionising radiation (Taylor *et al.*, 1975; Taylor, 1982). Hypersensitivity to the lethal effects of radiation has been seen in all cell types tested to date (Cole *et al.*, 1988) and there is some evidence implicating the misrepair of DNA dsb in the disorder (Cox *et al.*, 1986; Thacker, 1992), a feature that may be consistent with the immunological defects in the disorder. Formal investigations on the repair of dsb in A-T cells have generated conflicting results. The most recent of these (see Foray *et al.*, 1995) included low dose-rate irradiation and extended repair times and are consistent in showing higher levels of residual dsb than in normal cells. In addition there is evidence that, during 6 h post-irradiation, repair in A-T is faster than normal (Foray *et al.*, 1995). However, the search for a biochemically defined defect in the repair of ionising radiation damage in A-T has proved difficult. Several laboratories have produced convincing evidence for an abnormal cellular response of DNA synthesis following treatment with ionising radiation which may relate to the faster early repair in A-T cells. Thus, in normal cells there is a marked inhibition of DNA synthesis following radiation which is not observed in A-T cells (Houldsworth and Lavin, 1980; Painter and Young, 1980). This cellular phenotype, termed radioresistant DNA synthesis, has been used to carry out genetic complementation tests which suggested that A-T could be subdivided into four complementation groups (Jaspers *et al.*, 1988, but see paragraph 23). The relevance of a defect giving radioresistant DNA synthesis is not however obvious. In the 'A-T like' radiation-sensitive hamster cell mutants (Table 2.1) transfection with a human gene from chromosome 4q corrects the DNA synthesis defect but does not ameliorate the lethal or clastogenic effects of radiation (Verhaegh *et al.*, 1995). In addition to the abnormality in radiation resistant DNA synthesis A-T cells also show other perturbations in progression through the cell cycle (Thacker, 1994). Since A-T cells remain hypersensitive under conditions where they are not proliferating and where, by definition, the cell cycle abnormalities cannot be operating, there must be additional mechanisms of defective DNA repair (Thacker, 1994).

(23) Subsequent genetic linkage studies showed first that the A-T gene(s) mapped to chromosome 11q22.23 (Gatti *et al.*, 1988) and that three of the complementation groups mapped to the same region. Finally, Savitsky *et al.* (1995) succeeded in cloning a cDNA covering a significant part of the gene including the important functional domains. The site of the mutation in the *ATM* gene was next identified in a number of A-T patients, some proved to be homozygotes, others compound heterozygotes. These results laid to rest the concept of a series of complementation groups in A-T because an identical homozygous mutation was found to be present in two individuals previously assigned to distinct complementation groups. The majority of the mutations identified to date are frame shifts resulting in a total loss of function of the gene product, a result which might be considered surprising in the light of the multi-system nature of the disease. Approximately 20% of A-T patients from within the United Kingdom are recognised as having intermediate radiation sensitivity and to exhibit milder clinical symptoms (Taylor *et al.*, 1996). They also show a common haplotype and are compound heterozygotes characterised by a large 138bp insertion at position 5764/5 in one allele which leads to a frameshift and termination of the message. The second mutation in this group varies from patient to patient and it is probably this second, less severe, mutation which determines the phenotype. Unpublished information on the distribution of mutations, again from within the United Kingdom, suggests that they are almost all compound heterozygotes and that there is no common mutation.

(24) The most important consequences which flow from these studies concern the predicted nature and mode of action of the gene product. The A-T gene product is a protein with considerable homology with a family of signal transduction molecules generated by a gene family known as the phosphatidylinositol (PI) 3-kinase family. The signal transduction molecules include the TOR1 and TOR2 proteins from yeast (Helliwell *et al*., 1994), the mTOR and RAFT protein in rat and the FRAP protein from humans (Sabers *et al*., 1995). However, the most interesting comparison is with the *rad*3 gene of *Schizosaccharomyces pombe* and its homologue FSRI (MEC1) of *Saccharomyces cerevisiae*. These genes are involved in the checkpoint response to DNA damage giving delayed progression through the cell cycle which, it is believed, maximises the opportunity for cellular repair. In *S. pombe* the mutated gene confers hypersensitivity to the lethal effects of both UV-C and ionising radiation (Al-Khodairy and Carr, 1992). These mutants have been subjected to extensive genetic analysis and are involved in both the DNA-damage checkpoint system and recovery and repair processes which, as yet, have not been defined (Lehmann and Carr, 1995). This dual role of the *rad3* protein is very reminiscent of the responses of A-T cells to radiation and suggests that there exists a family of genes, common to all eukaryotes, which is involved in both repair and monitoring of DNA damage. Through studies on the localisation/function of *ATM* protein and the phenotype of *ATM null* mice it is becoming more clear that *ATM* plays a complex and interactive role in DNA damage recognition, DNA rearrangement and cell cycle arrest. These data which are summarised by Hoekstra (1997) imply interaction between *ATM, ATR* (*RAD*3-related), *p53* and *RAD51* proteins in DNA damage response. It is also speculated that there may be similar interactions with *BRCA* proteins (see Section 2.3).

(25) Overall, the cancer-proneness of A-T patients seems likely to be related to the chromosome instability characteristic of the syndrome with misrepair of DNA dsb, disrupted signal transduction and impaired cell cycle checkpoint functions as causative mechanisms.

2.2.6. *Radiation mutagenesis and its relationship to DNA repair*

(26) The accuracy and competence of relevant DNA repair processes are further revealed in studies of mutation in human cell lines of variable radiosensitivity. Kent *et al*. (1994) demonstrated hypomutability at the *hprt* locus in a radiosensitive human bladder cell line UI-S40b when it was compared with the parental line (MGH-U1) and it was speculated that the hypomutability was a reflection of the poor recovery of multilocus lesions involving the target *hprt* gene and closely linked essential sequences. Support for this interpretation is provided by investigation of the response of two human lymphoblastoid lines, WI-L2-NS and TK6, isolated from the same original donor isolate. Mutability was examined at two structurally different genetic loci, *hprt* and *tk* (Amundson *et al*., 1993). At the hemizygous *hprt* locus the response to x-ray induced mutation is very similar in the two cell lines which differ in their radiosensitivity, TK6 proving to be significantly more radiosensitive. The kinetics of the mutation induction curves were linear for TK6 and linear quadratic for WI-L2-NS. WI-L2-NS cells proved however to be 50-fold more mutable at the heterozygous *tk* locus than TK6 cells. Previous studies by the group showed that in TK6 cells one allele ($tk^{+/-}$) was up to 10-fold more mutable by x rays than the alternative allele ($tk^{-/-}$) and that this was explained by the lack of recovery of *tk* mutants with associated loss of heterozygosity (LOH), i.e large deletions. All the mutants analysed from WI-L2-NS derivatives were shown to have lost heterozygosity

at the *tk* locus but had nonetheless retained two copies of the gene implying that mutation induction involved DNA recombinational processes.

(27) Further support for the involvement of recombination processes has been gained from molecular analysis of mutations at the *hprt* locus. Despite the hemizygous state of *hprt*, the availability of pseudogenes in principle allows for *hprt* participation in recombination events. The fact that *V(D)J* mediated deletions are responsible for the majority class of *hprt* mutations recovered from foetal T-lymphocytes of human origin (Fuscoe *et al*., 1991) is also relevant to this argument. Non-homologous recombination-like events have been recorded amongst radiation-induced *hprt* deletion mutants from human fibroblasts (Morris and Thacker, 1993) and from a human myeloid leukaemia line (Monnat *et al*., 1992a, b). In essence, non-homologous recombination represents an error-prone pathway for repair and may account for the induction of a significant proportion of radiation-induced chromosomal mutations.

(28) In contrast to these results, the Chinese hamster *irs* lines have moderate radiation hypersensitivity combined with near normal DNA dsb repair capacity and are proficient in *V(D)J* rejoining (Thacker and Wilkinson, 1995). These observations suggest that radiation hypersensitivity in respect of cell lethality and gene mutation in some mutant cell lines may have origins in mechanisms other than overt defects in DNA dsb repair and recombination.

(29) Further support for the potential radiobiological importance of DNA dsb repair and recombinational processes comes from additional investigations of the *scid* phenotype. Roth *et al*. (1992) showed that the *V(D)J* coding intermediates in *scid* thymocytes are manifest as hairpin structures and the generation of such structures is the normal pathway for rearrangement while failure to resolve these may represent the *scid* phenotype. Thus, both the *scid* phenotype and the associated DNA dsb repair deficiency probably derive from recombinational errors in DNA processing which may involve higher order DNA structures. Similar arguments have been made in respect of the involvement of higher-order DNA structures in the formation of neoplasia-related chromosomal events (see for example, Bouffler *et al*., 1993).

(30) The importance of DNA dsb for radiation mutagenesis is also believed to be reflected in the predominance of a DNA deletion mechanism in many of the somatic genes of repair proficient cells analysed in vitro (Thacker, 1986, 1992; Sankaranarayanan, 1991). Although radiation-induced DNA base pair mutations have been identified in some somatic mutation systems, in the absence of genomic constraints imposed by, for example, the proximity of essential flanking genes, radiation mutagenesis may principally proceed via DNA deletion through misrepair or misrecombination at DNA dsb.

2.2.7. *Chromosomal structure and radiation mutagenesis*

(31) The importance of chromosomal structure for radiation-induced gene mutation is evident from a number of studies (see Thacker, 1992). Of principal importance is the relationship between the DNA deletion mechanism that predominates after radiation and the tolerance of genomic regions to DNA loss events. In addition to the data cited in Section 2.2.6 this feature is clearly illustrated by studies on mutation induction at the *aprt* locus (see Meuth and Bhattacharyya, 1994).

(32) The *aprt* gene is autosomally encoded and, thus, normally present as two copies in the genome. In order to study induced mutagenesis it is therefore necessary to use cell lines having one allele carrying a point mutation (heterozygotes) or deleted (hemizygotes) as part of a larger chromosome loss event. In this way the remaining wild type copy of

aprt is available as a target for radiation. Using such cell lines it has been shown that *aprt* hemizygous cells have greatly reduced mutability compared with heterozygotes and that unlike heterozygotes few large DNA deletions are associated with *aprt* mutation. Although yet to be fully confirmed at the molecular level these data are wholly consistent with the presence of an essential gene in the chromosomal segment encoding *aprt*. On this basis homozygous loss of the essential gene is not compatible with cell viability, thus largely restricting the spectrum of mutational damage observed in *aprt* hemizygotes to intragenic events with a commensurate decrease in the apparent mutagenic efficiency of radiation.

(33) It may be concluded therefore that the mutagenic radiosensitivity of functionally similar genes should be expected to be non-uniform and will be highly dependent upon the function and activity of flanking sequences which in turn is dependent upon chromosomal location. It follows from this that since the criticality of gene function will tend to differ between cell types in a given species, organ-specific effects on the tolerance of radiation-induced genomic losses should be anticipated. Given the known differences in genomic organisation, such tolerance to loss is also expected to differ between mammalian species. The possible implications of these mutagenesis factors for tumour suppressor gene loss in radiation carcinogenesis are discussed in Section 5.

(34) Effects of chromatin structure on the repair of UVR damage are well established. DNA photoproducts present in the transcribed DNA strand are repaired preferentially (e.g. Van Zeeland et al, 1994) and *hprt* mutation induction correspondingly proceeds principally via mutation of the non-transcribed strand (Vreiling *et al*., 1992). For UVR induced DNA damage there is also a difference in repair between active and inactive genes in the genome which probably reflects chromatin-dependent genome domain effects on repair. Together with the known role of UVR DNA repair proteins in gene transcription, the existence of such differential repair may explain some of the complex and unexpected clinical manifestations of DNA photoproduct repair-deficient human genetic disorders (Arlett and Lehman, 1995).

(35) Much less information is available on the genomic distribution of repair of ionising radiation-induced damage. Leadon and Cooper (1993) have shown that preferential repair of radiation damage occurs within the transcribed strand of DNA of human cells of normal radiosensitivity while in a radiosensitive cell line of Cockayne syndrome (group B) there was no DNA strand-selective repair in spite of a normal overall rate of repair. The cytological investigations of Pandita and Hittleman (1994) suggest that cells of A-T patients and A-T heterozygotes have an altered chromatin structure and that this may be associated in increased radiosensitivity. Atm, the protein mutated in A-T, has been shown to localise to synapsed chromosome axes during meiosis, further implicating the gene in chromosome structure and function (Keegan *et al*., 1996).

(36) Differences in the state of condensation of nucleoprotein complexes in chromatin may be expected to influence radical scavenging and post-irradiation DNA repair rates (e.g. Oleinick and Chiu, 1994) but as yet it is not possible to relate such effects to mutagenesis.

2.2.8. *Genomic instability*

(37) The current consensus is that the development of neoplasia is a multistage process (Fearon and Vogelstein, 1990; see also Section 3). The assumption of independence for five or more steps in this process, each with a spontaneous rate of say 10^{-7} per cell generation is not biologically feasible and several lines of evidence point towards the acquisition of

inherent genomic instability at a relatively early stage of neoplastic development in order to accelerate the neoplastic process (see Section 3).

(38) Of possible relevance to this is the induction of genomic instability by ionising radiation. Earlier studies on the kinetics of the induction of malignant transformation of mouse 10T1/2 cells (Kennedy *et al*., 1980, 1984) suggested that radiation induces a type of genomic instability in a large fraction of the cell population leading to the occurrence of the actual transforming event amongst the progeny of the original irradiated cells after many generations of cell replication. Chang and Little (1992, 1994) examined the frequency of mutations in clonal populations derived from single cells surviving radiation exposure. They showed that the rate of mutation at the *hprt* locus of a cultured hamster cell line was persistently elevated for up to 50 population doublings following radiation exposure. The spectrum of DNA structural changes for these late-arising mutations differed significantly from that of direct radiation-induced mutants in that they were primarily the result of small scale events (point mutations) rather than deletions (Little, 1994). A persistently enhanced mutation rate following irradiation has also been described by Simons and Niericker (1994) in a mouse lymphoma cell line by use of a fluctuation analysis of *hprt* mutagenesis. Li *et al*. (1994) reported finding elevated frequency of microsatellite mutations in human lymphoblastoid cell clones selected for induced mutation at the *TK* locus. Together these data on multiple mutational events imply that in some cellular systems there is induction of some form of trans-acting mutagenic process, but the nature of this process and its genetic control remain unresolved.

(39) At the chromosomal level, persistent alpha-particle induced genomic instability has been reported in some but not all bone marrow samples from mouse strains and human donors (Kadhim *et al*., 1994) and Marder and Morgan (1993) have reported on the induction of delayed chromosomal instability in hamster cells irradiated in vitro. Pampfer and Streffer (1989) irradiated mouse embryos in the single-cell zygote stage, and showed that cells derived from skin biopsies of the mature foetus exhibited an increased frequency of chromosomal aberrations and micronuclei as compared with animals receiving no irradiation at this early stage in development. The mechanisms underlying these chromosomal instability effects after radiation are however poorly understood and their relevance to neoplastic development remains to be established.

(40) Genomic instability may also be modulated by epigenetic factors acting in vivo. Paquette and Little (1994) isolated several x-ray transformed Type III foci from mouse C3H 10T1/2 cells, and split each of them into two groups. One group was injected into syngeneic, non-immunosuppressed C3H mice, while the other was cultivated in vitro for 25 passages (about 6 months). After 3–6 months, DNA was extracted from the sub-clones grown either in vivo or in vitro, and analysed by a DNA fingerprinting assay for instability in mouse minisatellite sequences. A high frequency of genomic rearrangements (50–100%) was found in sub-clones derived from the tumours that arose in vivo, whereas the frequency was very low ($< 10\%$) in sub-clones cultivated in vitro for a roughly similar number of population doublings.

(41) Of perhaps more relevance to tumorigenesis is the genomic instability that may be associated with the $(TTAGGG)_n$ telomeric repeat sequence arrays that serve principally to cap and thereby stabilise the termini of mammalian chromosomes (Blackburn, 1991, 1994). Telomere length shortens by around 50–150 base pairs per cell division and is believed to limit the proliferative capacity of normal human diploid cells. It has been proposed that when telomeric length shortens to a critical level, suppression of cell senescence is relaxed such that cells progressively lose proliferative capacity; such telomeric shortening is also

believed to destabilise chromosomes such that they are more prone to participate spontaneously in breakage–fusion cycles resulting in the increase in chromosomal abnormalities that characterises the senescence process. Escape from such senescence and subsequent cell immortalisation via the re-stabilisation of telomeres is now believed to involve the reactivation of the enzyme telomerase which is normally silent in human somatic cells (Kim *et al.*, 1994). Thus telomerase reactivation may be viewed as a crucial step in malignant development and the genes that control telomere sequence processing may well act as targets for neoplastic change (Kipling, 1995).

(42) In the context of ionising radiation there is evidence that induced life-span extension of human diploid fibroblasts may involve the expansion of clones carrying unstable telomeric rearrangements (Martins *et al.*, 1993). There is also evidence that telomeric sequence arrays play a role in DNA damage response, chromosome break healing (Blackburn, 1991, 1994) and when located at interstitial sites may represent preferential locations for the expression of radiation-induced chromosomal rearrangements (Bouffler *et al.*, 1993). The role of these repeat sequence arrays in some of the chromosomal events associated with haemopoietic neoplasia is discussed in Section 4.

2.3. Human genetic disorders affecting DNA repair and genomic instability

(43) The principal human genetic disorders associated with DNA repair deficiency, hypersensitivity to ionising radiation and/or inherent genomic instability, have already been noted. For the sake of completeness Table 2.3 provides a summary of the claims that have been made in respect of radiosensitivity in a range of human genetic disorders. It is important to stress however that many of these claims are contentious. A major problem in all such analyses is the extent to which technical factors cloud judgements on intrinsic cellular radiosensitivity. In this context the data of Table 2.4 illustrate the range of cellular radiosensitivity that has been ascribed to different sets of ostensibly normal human donors. There can be little doubt that a significant element in the apparent differences in normal human cellular radiosensitivity suggested by these data is of technical and not genetic origin. In addition, since in vitro cellular radiosensitivity is a gross phenotype and single mutant gene effects are being sought in heterogenous genetic backgrounds, only the most highly penetrant mutations will be clearly distinguished. Given these problems, on the basis of the data available on radiation-induced cell inactivation (Tables 2.3 and 2.4), it is judged that it is only the genetic disorders ataxia-telangiectasia and Nijmegen breakage syndrome (NBS) that show unambiguous evidence of radiation hypersensitivity to the lethal effects of ionising radiation.

(44) Cell inactivation tends however to be a rather crude measure of cellular radiosensitivity and there are data showing that a number of cancer-prone genetic conditions are associated with chromosomal radiosensitivity. Table 2.5 lists those disorders in which chromosomal radiosensitivity is judged to be increased, albeit moderately in most cases. In addition to A-T and NBS which show marked increases, certain disorders associated with tumour suppressor gene mutation are also somewhat increased in sensitivity, e.g. Li–Fraumeni syndrome, retinoblastoma and nevoid basal cell carcinoma syndrome. Although still uncertain it may be that, in these disorders, the consequences of mutation of certain tumour suppressor genes for cell cycle control may provide an explanation for the effect (see Section 3).

(45) New cell cycle-based assays of cellular radiosensitivity in human lymphocytes hold promise of a greater power of discrimination than that provided by conventional methods. The data of Sanford and Parshad (1990) which imply a degree of chromosomal

Table 2.3. Claims and counter-claims for cellular radiosensitivity in 23 human genetic disorders

Human disorder	Assay type[a]	Cellular response to radiation: Radio-sensitive	Heterogeneous response among patients	Normal response	Reference
Alzheimer's disease	1	+			Robbins *et al.* (1985)
	1		+		Chen *et al.* (1991)
Ataxia-telangiectasia	2	+			Taylor *et al.* (1975)
Ataxia-telangiectasia, heterozygotes			+		Kidson *et al.* (1982)
	3		+		Hannan *et al.* (1982)
Bloom syndrome	2			+	Arlett and Harcourt (1980)
	7	+			Weichselbaum *et al.* (1980)
	8	+			Parshad *et al.* (1983)
					Higurashi and Conen (1973)
					Kuhn (1980)
					Aumas *et al.* (1985)
				+	Evans *et al.* (1978)
Cockayne syndrome	2			+	Arlett and Harcourt (1980)
	2			+	Proops *et al.* (1981)
	4	+			Deschavanne *et al.* (1984)
Down syndrome	5		+		Lavin *et al.* (1989)
Duchenne's muscular dystrophy	2			+	Nove *et al.* (1987)
Fanconi anaemia	2	+			Arlett (1979)
	4	+			Arlett and Priestley (1984)
	2			+	Duckworth-Rysiecki and Taylor (1985)
	2			+	Burnett *et al.* (1994)
Friedreich ataxia	2	+			Lewis *et al.* (1979)
	4	+			Chamberlain and Lewis (1982)
Gardner syndrome	2	+			Little *et al.* (1980)
Hodgkin lymphoma	3		+		Hannan *et al.* (1989)
Huntington disease	2		+		Arlett (1980)
	2		+		Moshell *et al.* (1980)
	1		+		Chen *et al.* (1981)
	6			+	McGovern and Webb (1982)
	2	+			Baverstock and Simmons (1982)
	2		+		Nove *et al.* (1987)
Multiple sclerosis	1		+		Gipps and Kidson (1981)
Nevoid basal cell carcinoma syndrome	2			+	Taylor *et al.* (1975)
	2			+	Arlett and Harcourt (1980)
	4	+			Arlett and Priestley (1984)
	4			+	Featherstone *et al.* (1983)
	2	+			Chan and Little (1983)
	2			+	Little *et al.* (1989)
Nijmegen breakage syndrome	2	+			Taalman *et al.* (1983)
Parkinson disease	1			+	Robbins *et al.* (1985)
Porokeratosis	2	+			Olsuka *et al.* (1989)
Progenia	2			+	Arlett and Harcourt (1980)
					Weichselbaum *et al.* (1980)
	2	+			Little and Nove (1990)
					Saito and Moses (1991)

(continued on next page)

Table 2.3 (*continued*)

Human disorder	Assay type[a]	Cellular response to radiation: Radio-sensitive	Heterogeneous response among patients	Normal response	Reference
Retinoblastoma	2	+			Arlett and Harcourt (1980)
	2	+			Weichselbaum *et al.* (1980a)
	2			+	Woods and Byrn (1986)
	2		+		Little *et al.* (1989)
Rothmund–Thomson syndrome	2		+		Smith and Paterson (1982)
Tuberous sclerosis	1,2	+			Scudieno *et al.* (1981)
Usher syndrome	2	+			Nove *et al.* (1987)
Wilms' tumour	7	+			Sandford *et al.* (1989)
	2			+	Weichselbaum *et al.* (1980)
	2			+	Imray *et al.* (1984)
Xeroderma pigmentosum	2		+		Arlett *et al.* (1980)
	2			+	Arlett and Harcourt (1980)
					Weichselbaum *et al.* (1980)
	7	+			Parshad *et al.* (1983)

[a]Assay types: 1, cloning in lymphoblastoid cells; 2, cloning in fibroblasts; 3, cloning in fibroblasts with chronic irradiation of plateau-phase cells; 4, cloning in fibroblasts with repair of potentially lethal damage; 5, clastogenicity in lymphoblastoid cells; 6, vital staining in lymphocytes; 7, clastogenicity in fibroblasts; 8, clastogenicity in lymphocytes.Data compiled from the reviews of Arlett (1992) and Murnane and Kapp (1993) and references given therein.

radiosensitivity in a broad range of cancer-predisposing genetic disorders remain somewhat contentious but the recent work of Scott *et al.* (1994) and Jones *et al.* (1995) is judged to be more convincing and not only has achieved the discrimination of radiosensitivity in A-T heterozygotes but also raises the possibility that a significant fraction (~40%) of unselected

Table 2.4. Radiosensitivity of fibroblasts and lymphocytes from sets of normal human donors[a]

Number of donors	Range of D10 (cGy)	Cell type[b]	References
42	230–380	F	Cox and Masson (1980); Thacker (1989)
15	145–180	L	Kutlaca *et al.* (1982)[c]
10	350–450	F	Nagasawa and Little (1988)
6	300–360	F	Arlett *et al.* (1988)
24	196–372	F	Little *et al.* (1988)
21	213–448	F	Paterson *et al.* (1989)
56	210–370	F	Ban *et al.* (1990)[d]
31	180–420	F	Little and Nove (1990)
22	253–404	F + L	Kurshiro *et al.* (1990)[d]
33	220–390	F + L	Green *et al.* (1991)
32	320–410	L	Nakamura *et al.* (1991)[d]
8	498–295	L	Geara *et al.* (1992)
6	446–264	F	
32	353–253	F	Begg *et al.* (1993)[d]
5	305–242	F	Wann *et al.* (1994)

[a]Based on the review of Arlett (1992) and references given therein.
[b]F, fibroblast; L, lymphocyte.
[c]Cloning in agar.
[d]Some of these studies included cancer patients among the donors. Papers with data based largely on such donors have been excluded from this tabulation. Nakamura *et al.* (1991) refers to studies on a further 150 donors with similar results. The D10 values are either as quoted by the authors or determined by inspection of the survival curves.

breast cancer patients are also characterised by increased chromosomal radiosensitivity (Fig. 2.1, see also Section 3). While the inheritance of this phenotype in breast cancer patients has yet to be established, it is of clear importance that the application of these techniques is extended in order to gain a more complete picture of human chromosomal radiosensitivity and its possible relationship with a cancer predisposition.

(46) Rapidly developing evidence on the function of the breast cancer susceptibility genes *BRCA1* and *BRCA2* suggest that the associated genetic disorders may also be included in the DNA repair category. This evidence, summarised and discussed by Brugarolas and Jacks (1997), implies that in combination *BRCA1* and *BRCA2* proteins interact with *Rad51* to regulate the repair of DNA dsb. Another strand to this argument is that developmental arrest in *BRCA1* and *BRCA2* null mice is partially rescued by mutation of *p53*. The implication of this latter result is that in the absence of *p53* protein, unrepaired DNA damage in the *null* mice fails to fully activate cellular growth arrest pathways.

(47) Viewed overall, the mechanistic links between heritable cellular radiosensitivity, human predisposition to neoplasia and other health effects are less well established than those for UVR. In the case of UVR sensitivity the nature of the DNA repair deficiencies in xeroderma pigmentosum, Cockayne syndrome and trichothiodystrophy are becoming increasingly well understood and many of the responsible genes are cloned and characterised (see Arlett and Lehman, 1995). Of particular note is the clear indication that sensitivity to the in vitro cell lethal effects of UVR is not always reflected in proneness to neoplasia in vivo and that, with or without excess cancer, UVR repair deficiency may be manifest by developmental abnormalities probably as a consequence of repair-related perturbation of gene expression.

(48) It seems likely that a similar explanation applies to disease manifestation in radiosensitive ataxia-telangiectasia and it is anticipated that further molecular cloning and characterisation of genes relevant to human radiosensitivity will clarify the position in respect of the complex relationships between deficiency in these genes, cancer proneness and other human health effects.

(49) In the meantime, a strong message that emerges from UVR and ionising radiation studies is that in vitro human cellular radiosensitivity is not a reliable predictor of in vivo cancer proneness nor, by implication, tumorigenic radiosensitivity. It is therefore wholly

Table 2.5. Human genetic disorders showing evidence of chromosomal radiosensitivity

Disorder	References
Ataxia-telangiectasia homozygotes	Higurashi and Conen (1973); Taylor *et al.* (1976)
Ataxia-telangiectasia heterozygotes	Waghray *et al.* (1990); Scott *et al.* (1994); Jones *et al.* (1995)
Basal cell naevus syndrome	Featherstone *et al.* (1983)
Bloom's syndrome	Higurashi and Conen (1973); Kuhn (1980)
Common variable immune disorder	Vorechovsky *et al.* (1993)
Down's syndrome	Sasaki *et al.* (1970); Countryman *et al.* (1977)
Dyskeratosis congenita	DeBauche *et al.* (1990)
Fanconi's anaemia	Heddle *et al.* (1978); Duckworth-Rysiecki *et al.* (1985); Gibbons *et al.* (1995)
Klinefelter syndrome	Sasaki *et al.* (1970)
Li–Fraumeni syndrome	Parshad *et al.* (1993)
Nijmegen breakage syndrome	Taalman *et al.* (1983, 1989); Jaspers *et al.* (1988)
Rothmund–Thomson syndrome	Kerr *et al.* (1996)
Trisomy-18	Sasaki *et al.* (1970)
Porokeratosis of mibelli	Takeshita *et al.* (1994)
Retinoblastoma	Morten *et al.* (1991); Sanford *et al.* (1996)
Xeroderma pigmentosum	Price *et al.* 1991

inappropriate to make judgements upon the distribution of radiation-induced cancer in human populations on the basis of data surveys of the type presented in Tables 2.3 and 2.4.

(50) Finally, it is now well recognised that other human germ line defects in DNA repair and genomic maintenance show associations with cancer susceptibility. The most compelling examples of these are DNA mismatch repair deficiencies in hereditary non-polyposis colon cancer (see Section 3) and *p53* tumour suppressor gene mutation in the Li–Fraumeni syndrome (see Sections 3–5). In addition, heritable genomic instability is associated with the less well understood cancer-prone disorders Fanconi anaemia and Bloom syndrome (see Section 4); contentious claims of such associations have also been made for other disorders. A summary of the current position regarding heritable cancer, DNA repair deficiency and genomic instability is provided in Table 2.6. It is judged that it is only in the cases of ataxia-telangiectasia and Nijmegen breakage syndrome that there is a clear link between in vitro radiosensitivity and heritable cancer susceptibility and even for these disorders much remains to be learned of the molecular mechanisms involved.

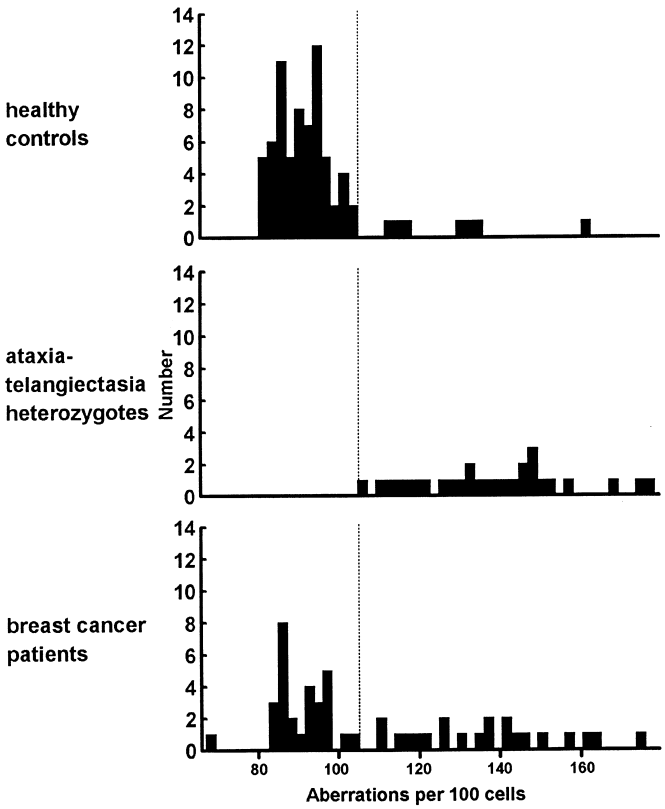

Fig. 2.1. Chromosome damage in human lymphocytes exposed to 0.5 Gy x rays in G2 phase of the cell cycle (data from Scott *et al.*, 1994). Top panel: healthy controls, 74 donors (111 samples), 39 male and 35 female (no sex effect), aged 5–68 (no age effect), mean = 93.8 (SD 13.6) (coefficient of variation 14%), difference between donors $p < 0.001$. Middle panel: obligate A-T heterozygotes, 28 donors (30 samples), aged 27–67 (no age effect), mean = 145 (40.4) (28%). Bottom panel: breast cancer patients, 50 donors, aged 35–73 (no age effect), mean = 109 (26.8) (25%). Vertical line gives maximum discrimination between controls and A-T heterozygotes. Both A-T heterozygote and breast cancer groups are significantly more sensitive than controls (Mann–Whitney test, $p < 0.001$).

Table 2.6. Human disorders affecting DNA repair and genomic maintenance

Disorder	Major clinical features	Cancer	Specific cellular hypersensitivity	Proposed DNA repair defect	No. of genes	Data source
Xeroderma pigmentosum	Photosensitivity and cancer of UVR-exposed skin	SCC, BCC and melanoma	UVR, some chemicals	Excision repair or daughter strand repair	8	This Section
Cockayne Syndrome	Photosensitivity dwarfism	No excess	UVR, ionising radiation?	Preferential repair of transcribed strands	2	This Section
Trichothiodystrophy	Photosensitivity abnormal, sulphur-deficient hair	No excess	UVR	Excision repair	2	This Section
Bloom syndrome	Photosensitivity dwarfism	Various	UVR some chemicals	DNA helicase	1	This Section, Section 4
Ataxia-telangiectasia	Neurological defects, immunodeficiency	Lymphoma	Ionising radiation, radiomimetic chemicals	Kinase activity	1	This Section, Section 4
Nijmegen breakage syndrome	Microencephaly, immunodeficiency	Lymphoma	Ionising radiation, radiomimetic chemicals	?	2	This Section, Section 4
Fanconi anaemia	Bone marrow deficiency, skeletal abnormalities	Leukaemia	Bi-functional alkylating agents ionising radiation?	Repair of intrastrand cross links	4	This Section, Section 4
Werner syndrome	Accelerated ageing	Various	?	DNA helicase	1	Section 4
Nevoid basal cell carcinoma syndrome	Sskeletal abnormalities, cancer	Basal cell nevus and medulloblastoma	Ultra-violet? Ionising radiation?	Defect in repair of potentially lethal ionising radiation damage?	1	This Section, Section 3
Retinoblastoma	Retinal tumours	Retinoblastoma and others	Ionising radiation?	Via cell cycle control?	1	This Section, Section 3
Down syndrome	Mongoloid features	Leukaemia	Ionising radiation?	None known	1	This Section, Section 4
Hereditary non-polyposis colorectal cancer	Colon and other cancers	Colon and other cancers	None known	Defective DNA mismatch repair	4	This Section, Section 3
Li–Fraumeni syndrome	Cancer excess	Leukaemia, sarcoma, breast cancer	Spontaneous genomic instability ionising radiation (chromosomal)	Via cell cycle control	>1	This Section, Sections 3 and 4

(51) In spite of reservations on the degree to which heritable cellular radiosensitivity may correlate with human cancer predisposition, a significant cancer contribution from low penetrance repair-related mutations of the type suggested by the work of Scott *et al*. (1994) should not be discounted and it is expected that further studies will clarify the potential role of *BRCA* mutations in cellular radiation response.

2.4. Summary and conclusions

(52)

1. Cellular DNA damage after ionising radiation is mediated directly or indirectly by induced chemical radicals. Radical induction local to DNA is judged to be most important.
2. DNA double-strand lesions appear to be radiobiologically far more important than DNA single-strand lesions or base damage. High LET radiation-induced DNA damage may be more complex than that produced by low LET and, as a consequence, appears to be subject to less effective repair.
3. A total of 13 human genes involved in the repair of radiation damage have been cloned and/or mapped; currently the most important of these is the gene (*ATM*) that determines the radiosensitive and cancer prone human disorder ataxia-telangiectasia.
4. DNA double-strand lesion repair by DNA recombination and the associated activity of DNA end-binding proteins and kinases are important for cellular radiobiological response.
5. The study of in vitro cellular inactivation and chromosomal radiosensitivity show ataxia-telangiectasia and Nijmegen breakage syndrome disorders to have unambiguously elevated radiosensitivity. Less dramatic increases in cellular or chromosomal radiosensitivity are apparent for a range of human genetic disorders and conditions including some known to be associated with tumour suppressor gene mutation such as Li–Fraumeni syndrome and retinoblastoma.
6. Genomic instability without dramatic manifestation of enhanced radiosensitivity in respect of cellular inactivation is evident in the disorders Li–Fraumeni syndrome, Fanconi anaemia, Bloom syndrome and ataxia-telangiectasia heterozygotes. The cancer prone genetic disorder, hereditary non-polyposis colon cancer, has its basis in deficiency in DNA mismatch repair; this is manifested by a specific form of DNA sequence instability in tumour cells. The relevance of radiation-induced persistent genomic instability to neoplastic development remains to be established.
7. Evidence is developing that heritable breast cancer associated with *BRCA1* and *BRCA2* mutation may also be associated with DNA repair deficiency.

3. MECHANISMS AND GENETICS OF SOLID TUMOURS

(53) The molecular genetic era of cancer research is now firmly established and it is clear that, in most instances, cancers arise via a monoclonal multi-step process, involving multiple genetic alterations (e.g. Fearon and Vogelstein, 1990). The identification of the determinants of multi-step neoplastic development has led to the realisation that at least three major classes of genes are involved: oncogenes, whose protein products serve to stimulate cell growth and survival; tumour suppressor genes, whose products appear to negatively regulate growth, promote apoptosis, and maintain in vivo homeostatic growth and differentiation potential; and DNA repair genes, defects in which lead to increased genomic instability (Stanbridge, 1990; Bishop, 1991; Loeb, 1994). As will be seen, mutations in a number of these genes represent the germ line determinants of familial predisposition to solid cancers of various tissues. This section provides a general description of the genes involved in cancer initiation and progression and seeks to identify genetic features that may be relevant to radiation carcinogenesis. Also included is an outline of the principal genetic disorders associated with a predisposition to solid tumours and information relevant to judgements on the contribution of genetic factors to human cancer risk after radiation which will be provided later in the document.

3.1. Oncogenes

(54) The plethora of functions associated with oncogenes is, at first glance, bewildering (Table 3.1). However, recent studies have indicated that many oncogene products interact with each other in signal transduction cascades that result in mitogenic signals that drive the cells into a proliferative state and also have pleiotropic effects on cell mobility and cytoskeletal architecture (Hunter, 1991; Khosravi-Far and Der, 1994). One of the most intensively studied group of oncogenes that illustrates this signal transduction well is the *RAS* family. Initially, it was thought that the Ras protein signals through a single pathway, i.e. Ras $\rightarrow$ Raf $\rightarrow$ MEK $\rightarrow$ MAP kinase. It is now known that multiple pathways of transduction occur, with both independent and interacting components (Karin, 1995) Fig. 3.1 depicts these signal transduction cascades, indicating the pleiotropic effects on membrane ruffling and cytoskeletal architecture, and gene transcription leading

Table 3.1. Functions of oncogene products

Class 1	Growth Factors e.g. PDGF-β chain (sis) and FGF-related growth factor (hst)
Class 2	Receptor and non-receptor protein tyrosine kinases (RPTK and NRPTK) e.g. src (NRPTK) and erbB (RPTK)
Class 3	Receptors lacking protein kinase activity e.g. Angiotensin receptor (mas)
Class 4	Membrane-associated G proteins e.g. Ras family
Class 5	Cytoplasmic protein–serine kinases e.g. raf-1 and mos
Class 6	Cytoplasmic regulators e.g. SH2/SH3 protein (crk)
Class 7	Nuclear transcription factors e.g. myc, myb, jun, fos
Class 8	Cell survival factors e.g. bcl-2
Class 9	Cell cycle genes e.g. PRAD1 (cyclin D1)

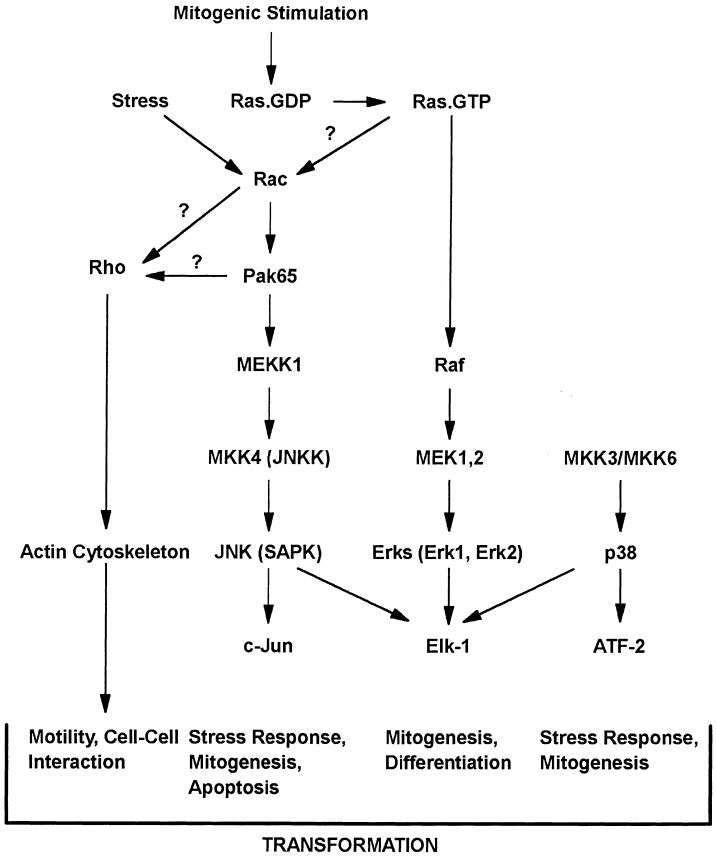

Fig. 3.1. Schematic representation of Ras-mediated signal transduction pathways.

to mitogenic signals. It should be appreciated that constitutive activation of these pathways results in constant mitogenic signalling and that activation of any member of the cascade, as a consequence of mutation, will result in activation of downstream members accompanied by further enhancement of mitogenesis. Thus, mutational activation of any one member of the cascade is sufficient for constitutive mitogenic signalling, thereby creating the potential for proliferative advantage.

(55) Other oncogenes play critical roles in cell survival (e.g. *BCL2*) and control of the cell cycle (e.g. *PRAD1* or cyclin D1), functions which again play pivotal roles in tumour development (Hockenberry *et al.*, 1993; Hunter and Pines, 1994). In considering the role of oncogenes in neoplasia, it is important to note that point mutations are only one mechanism whereby activation of oncogenes occurs. One may also encounter amplification, over-expression, inappropriate expression (temporally, developmentally, or tissue-specific), and gene translocation resulting in activation of gene expression and/or the generation of fusion products. The key role that proto-oncogenes play in differentiation can also be seen from the fact that oncogene over-expression may result in neoplastic transformation or induction of differentiation and/or apoptosis (Bar-Sagi and Feramisco, 1985; Garrido *et al.*, 1993; Han *et al.*, 1996).

(56) Numerous proto-oncogenes have been shown to be activated in human tumours. Activation by point mutation is seen in the *RAS* family, which has been detected in a

variety of human cancers. Point mutations in critical codons, i.e. codons 12, 13, or 61, can activate these genes. In some tumours mutation of the *RAS* gene can be found even in premalignant stages of cancer development, for instance in the adenomatous stages in the development of colorectal cancer, and myelodysplastic syndrome in leukaemia development (Fearon and Vogelstein, 1990).

(57) Gene amplification is often seen in the *MYC* family. Sometimes, the amplified DNA segments containing the oncogene can be identified as a homogeneously staining region (HSR) in Giemsa-banded chromosomes or extra-chromosomal double minutes (DM). Gene over-expression in the absence of gene amplification also occurs in the development of many human tumours (Bishop, 1991). The amplification of oncogenes is usually associated with the late stages of tumour progression. However, amplified *HER2/neu* has been observed in early clinical stages of breast cancer (Slamon, 1990).

(58) Germ line mutations of a proto-oncogene have been reported in the *RET* gene, which predispose to multiple endocrine neoplasia, namely MEN2A and MEN2B (Carlson *et al*., 1994). Mutations of RET are also found in sporadic medullary thyroid carcinoma. This is one of the few examples of an inherited proto-oncogene mutation in contrast to tumour suppressor genes, where many of those so far identified are found as germline mutations in familial cancer syndromes, as discussed below.

(59) There is some suggestive evidence indicating that certain rare alleles of proto-oncogenes are associated with the susceptibility to some types of cancers (Krontiris, *et al*., 1993). High frequencies of specific alleles of *HRAS* have been reported in patients with breast cancer (Lindereau *et al*., 1986; Saglio *et al*., 1988), lung cancer (Heighway *et al*., 1986), melanoma (Radice *et al*., 1987), colorectal cancer (Wyllie *et al*., 1988) and Wilms' tumour (Hayward *et al*., 1988). The issue of *HRAS1* allelic variation is discussed in Section 3.4.8. A rare allele of the *MOS* gene has also been shown to be linked to the development of breast cancer (Lindereau *et al*., 1985). A specific allele of L*MYC* appears in high frequency in bone and soft-tissue sarcomas (Kato *et al*., 1990) and has been associated with a tendency to develop metastasis in lung and kidney cancers (Kawashima *et al*., 1988; Kakehi and Yoshida, 1989). The true significance of these observations is unclear at this time.

(60) A relationship between oncogene expression and radiation response has been claimed by various investigators (e.g. Cassoni, 1994). There is relatively consistent evidence that expression of *RAS* oncogenes increase cellular radioresistance in some rodent cell lines but not in normal human cells. Radioresistance conferred by other activated oncogenes, e.g. *MYC* and *RAF*, is relatively tenuous and yet others, e.g. *MYB*, *FOS*, and *FES* have no measurable effect. The field of inquiry remains somewhat confused and direct clear links to radiation oncogenesis have yet to be established. In spite of this, the pivotal role of some oncogenes in mitogenic signalling and hence cell cycle control (Figs 3.1 and 3.3) provides a link with DNA damage response and repair (Hartwell and Kastan, 1994).

3.2. Tumour suppressor genes

(61) Whereas the protein products of many oncogenes are thought to function as promoters of cell proliferation and cell survival, tumour suppressor gene products appear to be involved in negative regulation of cell growth, induction of differentiation and programmed cell death.

(62) Loss of function of tumour suppressor (TS) genes is the hallmark event in the genesis of many cancers. Loss of function via deletion, rearrangement or point mutation, of both alleles of a given autosomal TS gene is a common occurrence in tumour cells.

Table 3.2. Some known or candidate tumour suppressor genes

Gene symbol	Chromosome map location	Cancer types	Product location	Mode of action	Hereditary symptoms
APC	5q21	Colon carcinoma	Cytoplasm	Transcriptional control	Familial adenomatous polyposis
DCC	18q21	Colon carcinoma	Membrane	Cell adhesion molecule	–
NF1	17q11	Neurofibromas	Cytoplasm	GTPase-activator	Neurofibromatosis type 1
NF2	22q12	Schwannomas and meningiomas	Inner membrane?	Links membrane to cytoskeleton?	Neurofibromatosis type 2
p53	17p13	Multiple	Nucleus	Transcription factor	Li–Fraumeni syndrome
RB1	13q14	Multiple	Nucleus	Transcription factor	Retinoblastoma
VHL	3p25	Kidney carcinoma	Membrane?	Transcription	von Hippel–Lindau disease
WT-1	11p13	Nephroblastoma	Nucleus	Transcription factor	Wilms tumour
p16	9p21	Multiple	Nucleus	CDK inhibitor	Familial melanoma
BRCA1	17q21	Breast and ovarian carcinoma	Nucleus?	Transcription factor? DNA metabolism?	Familial breast and ovarian cancer
TSC2	16p13	Multiple	?	?	Tuberous sclerosis
BRCA2	13q	Breast and ovarian carcinoma	Nucleus?	DNA metabolism?	Familial breast and ovarian cancer
FHIT	3p14.2	Multiple	?	?	–
DPC4	18q21.1	Pancreatic carcinoma	?	?	–
PTC	9q22.3	Basal cell carcinoma	Transmembrane	?	Basal cell nevus syndrome
ATM	11q23	Lymphoma	PI3 kinase	Cell cycle checkpoint control (others?)	Ataxia-telangiectasia

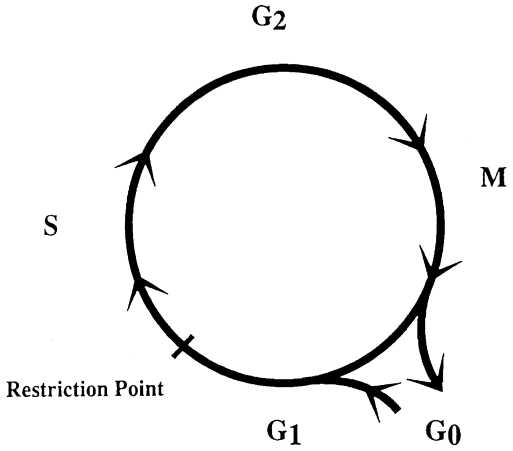

Fig. 3.2. The cell cycle. Non-dividing cells enter the cell cycle from the quiescent G_0 phase into the G_1 (gap) phase. Late in G_1, a restriction point exists at which the decision is made to progress through the rest of the cell cycle. Passage through the restriction is an absolute commitment to synthesise DNA in S (synthesis) phase. After S phase, the cell enters the G_2 phase in which the mass of the cell is increased and necessary enzymes are manufactured to allow the cell to divide into two daughter cells in M (mitosis) phase. In M phase, the duplicated chromosomes that were synthesised in S phase are separated and cytokinesis results in two daughter cells. At the end of M phase, the cell may either re-enter the cell cycle at G_1 or go into G_0.

Thus, these TS genes would seem to be recessive, although there are exceptions to this notion, e.g. the *NF1* gene (Li *et al.*, 1992). Loss of function of certain TS genes seems to be relatively cell lineage-specific, for example, the *NF2* gene in meningioma, whereas defects in other TS genes are encountered in a wider array of malignancies (Stanbridge, 1990).

(63) It should be appreciated that whereas more than 100 oncogenes have been identified, only a relatively small number of known or candidate TS genes have been reported (see Table 3.2). This arises principally because there are no good functional screening assays for TS genes and the majority of those identified have been isolated by the arduous process of positional cloning, i.e. via the screening of DNA clones from the known chromosomal position of the gene of interest. Interestingly, the deduced functions of TS genes in many instances mimic those of oncogenes. These include transcription factors, cell cycle regulators, surface adhesion molecules, and G protein regulators. However, these functions are antagonistic to oncogene functions. To illustrate this yin-yang effect, two examples are provided, namely, cell cycle regulation and DNA damage checkpoint control.

3.2.1. *Tumour suppressor genes in the cell cycle*

(64) The characteristic proliferative pattern of cell growth and division has been defined as the cell cycle (Fig. 3.2). The cell cycle is divided into four unequal parts or phases for dividing cells, termed G1, S, G2, and M, and one phase for non-dividing cells, termed G0. G1 is the first gap in the cell cycle during which the cell prepares for DNA synthesis. The G1 checkpoint has been delineated by a point in late G1 that occurs two to four hours prior to the G1/S boundary. This point has been defined as the restriction point after which the cell is committed to proceed into S phase and synthesise DNA. Chromosome duplication in the form of DNA synthesis occurs during S (synthesis) phase. G2 is defined

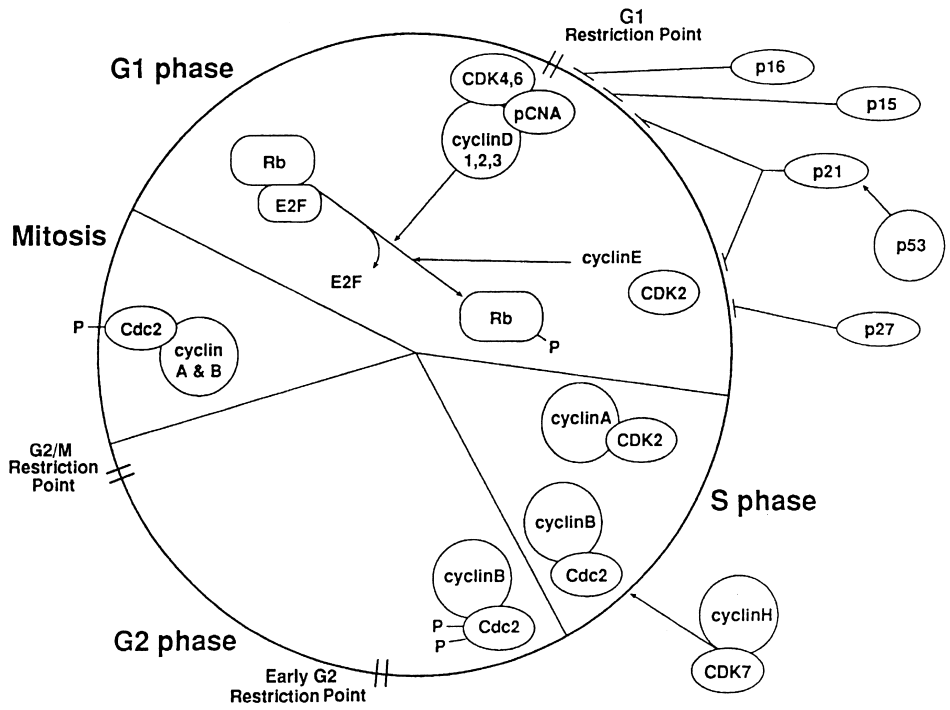

Fig. 3.3. Schematic representation of factors regulating the cell cycle; p15, p16, p21, and p27 are negative regulators.

as the gap between S and M phases during which the cell prepares the necessary enzymes and metabolic machinery to enter mitosis. Mitosis occurs in M phase during which the duplicated chromosomes are separated via the spindle apparatus, followed by cytokinesis and the generation of two daughter cells. At the end of M phase, the two daughter cells may enter either G1 or G0. The G0 phase is defined as a state of quiescence in which the cell exits the cell cycle and stops dividing. G0 cells may be restimulated by growth factors or mitogens to enter the cell cycle at the G1 phase. In the case of post-mitotic stem cells the fate of the two daughters is usually different. Whereas one daughter may proceed through further division cycles in order to provide a clonal population of differentiating cells, the other will re-enter a quiescent phase. In this way stem cell equilibrium is maintained. The cellular factors that govern this equilibrium are, for most tissues, poorly understood.

(65) The cyclic regulation of the cell cycle is determined by the accumulation and degradation of proteins during the specific phases of the cell cycle. These cyclically expressed and degraded proteins have been termed cyclins. Cyclins A and B were first discovered in molluscs based on the observed accumulation and degradation of these proteins during the cell cycle. This observation was later tied into the observation that in yeast there is an absolute requirement for the presence of S phase and M phase serine/threonine kinase activity for the cell to proceed to the next phase of the cell cycle. The kinase was identified as cdc2 (cell division cycle), which encodes the catalytic subunit. In yeast, cdc2 performs both of these regulatory functions by complexing with different cyclins, which comprise the regulatory subunits, during different phases of the cell cycle. In

mammals, there is a complex family of these serine/threonine kinases that regulate the different phases of the cell cycle. These serine/threonine kinases have been termed cell cycle dependent kinases (cdk), with cdk1 being equivalent to the cdc2 homologue from yeast.

(66) The free cdk catalytic subunit is inactive, due to phosphorylation, until a cyclin molecule complexes with it which leads to a dephosphorylation of the cdk and hence activation. Free cdks are present during the entire cell cycle. At a specific point in the cell cycle a specific cyclin is expressed and forms a heterodimer with a cdk molecule. This specific cyclin:cdk dimer is then activated and phosphory–phosphorylates the required substrates to allow the cell to progress through a given point of the cell cycle. The cdk/cyclin complex is then inactivated by the programmed degradation of the cyclin (Morgan, 1995).

(67) Malignant cells lose the ability to correctly regulate the cell cycle; this loss of control of the cell cycle can come in the general form of two signals. First, the increase of positive growth signals from mutant proto-oncogenes may override the negative regulatory control on the cell cycle. Second, the genes encoding the negative growth regulatory proteins (tumour suppressor genes) may be inactivated by mutation, loss, or in the case of Rb and *p53* oncoproteins, by complexing with the transforming proteins of DNA tumour viruses. The summation of these two processes profoundly compromises control of the cell cycle resulting in abnormal patterns of cell proliferation.

(68) The complex nature of these positive and negative cell cycle regulatory elements is illustrated in Fig. 3.3. A number of the proteins involved in cell cycle control have recently been shown to be oncogene products or TS gene products. One of the most intensively studied proteins is the Rb protein, which is the product of the *RB1* TS gene. Its identification and positional cloning represents the classic methodology for isolation of candidate TS genes.

(69) Cytogenetic and restriction fragment length polymorphism (RFLP) analyses mapped the putative retinoblastoma gene to chromosome 13q14. RFLP analysis further showed the loss of heterozygosity (LOH) by DNA deletion in this region in tumour tissue DNA as compared to normal constitutional tissue DNA (Cavenee *et al.*, 1983). This now classic LOH allelotyping has been used to map multiple candidate TS gene loci (Stanbridge, 1990). The *RB1* gene was finally cloned in 1986 (Friend *et al.*, 1986) and exhibited loss of function of both alleles in tumour DNA, as predicted by the Knudson two hit theory (Knudson, 1971; see also Section 5).

(70) Functional analyses of the Rb protein have shown that it plays an important role in progression of the cell cycle and functions as a gatekeeper. Rb is a phosphoprotein that localises to the nucleus. The phosphorylated state of the protein is the critical determinant of its activity and phosphorylation is dependent on cyclin-dependent kinase (cdk) activity. As cells transit G1, they reach a restriction point where the metabolic decision to proceed to S phase or not is made. The oscillation of Rb from the hypo- to the hyperphosphorylated state provides or relieves this block, respectively, i.e. the hypophosphorylated state is the active form of Rb. In this active state it acts as a negative regulator of the cell cycle and inhibits progression through G1 to S; the cells may then enter the quiescent G0 state that may herald induction of differentiation or apoptosis.

(71) Key to the regulation of G1 progression in mammalian cells are D-type cyclins (D1, D2, and D3) which interact with the cyclin dependent kinases (cdks) cdk4 and cdk6, and cyclin E which interacts later in G1 with cdk2. The critical role of cyclins D and E is illustrated by the fact that over-expression of these cyclins results in a shortening of the G1 period and a reduced dependency of the cell for growth factors (Sherr, 1994). In the

continued presence of mitogens, the D-type cyclins persist and they exhibit only moderate oscillations during the cell cycle with peak levels near G1/S. However, when the mitogens are withdrawn, the D-type cyclins are rapidly degraded. If this degradation occurs in G1, the cells fail to enter the S phase.

(72) Unlike the D-type cyclins, cyclin E is expressed periodically with a maximum level at G1/S. As the cells enter S phase, cyclin E is rapidly degraded and the freed cdk2 then complexes with cyclin A. The cyclin A–cdk2 complex correlates with initiation of S phase, and cyclin A is degraded late in G2. Cyclins B1/B2 are also periodic in their expression. They are expressed during late G2 phase and degraded during late M phase (Morgan, 1995).

(73) There is a distinct functional association between D-type cyclins and Rb protein. Cyclin D binds directly to Rb and the cyclin D–cdk4/cdk6 complex phosphorylates Rb, thereby inactivating it and relieving Rb-mediated G1 inhibition. The growth suppressive effects of Rb are mediated in part by the ability of hypophosphorylated Rb to bind to E2F and negatively regulating its transcriptional activation of genes required for DNA replication (Nevins, 1992). Hyperphosphorylation of Rb releases E2F and frees it to activate the requisite genes (see Hunter and Pines, 1994).

(74) The connection between D-type cyclins or Rb and tumorigenesis is compelling. Amplification and over-expression of cyclin D1 are seen in many malignancies (Hunter and Pines, 1994). Loss of function (via mutation or deletion) or loss of expression of Rb are noted not only in retinoblastomas and osteosarcomas but also in a variety of haematopoietic and solid tumours. Furthermore, Rb protein binds to DNA tumour virus transforming proteins, e.g. SV40 T antigen, HPVE7 and adenovirus E1A (Nevins, 1992). The Rb protein is inactivated as a consequence of binding to the viral oncoproteins.

(75) Yet other levels of control of the cell cycle have been recently discovered that have significance for tumour suppression. Both cyclin E-bound cdk2 and cyclin D-bound cdk4 undergo phosphorylation on a single threonine residue, a modification needed for their activation. The enzyme responsible for this activation is a cdk-activating kinase (CAK) which is itself a multi-subunit enzyme composed of a catalytic subunit (MO15 or cdk7) and a novel cyclin (cyclin H).

(76) Active binary complexes of cyclins–cdks are catalytically active. However, other proteins co-purify with these binary complexes in extracts from normal cells (Xiong *et al.*, 1993). Among these is a 21 kDa protein (p21Cip1) which is a universal cdk inhibitor and is capable of inhibiting the activity of cyclin–cdk holoenzymes that have undergone CAK-mediated phosphorylation. *p21* expression is regulated by the *p53* tumour suppressor protein in response to DNA damage (see below). As illustrated in Fig. 3.4 *p21* is found in a quaternary complex that includes not only cyclin and cdk but also the proliferating cell

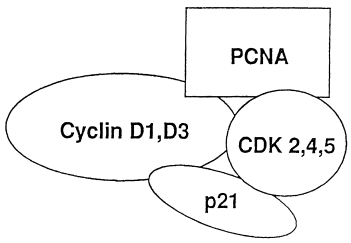

Fig. 3.4. Postulated quaternary complex between D-type cyclins, CDKs, p21, and PCNA (after Xiong *et al.*, 1992).

nuclear antigen (PCNA), which functions in both DNA repair and replication as a subunit of DNA polymerase delta (Xiong *et al.*, 1992).

(77) Another regulator of G1 progression is the protein *p27*Kip1 which also inhibits the activity of all cdks (Polyak *et al.*, 1994). It is the relative levels of cyclin D–cdk complexes and *p27* that determine inhibition or progression through G1 (Xiong *et al.*, 1993).

(78) One notable G1 cyclin–cdk inhibitor (CDI) is *p16*INK4. The *p16* protein apparently inhibits cdk4 and cdk6 by binding in competition with cyclin D. There is a reciprocity between the levels of Rb and *p16* expression in tumour cells. Those cells that express wild type Rb protein have low levels of *p16* and cells lacking functional Rb expression express high levels of *p16*. This suggests that Rb may suppress *p16* expression, the reciprocal of Rb-stimulated cyclin D expression.

(79) The *p16* gene maps to chromosome 9*p21* in humans. Another gene, *p15*, is in close proximity. The *p15* protein is also an inhibitor of cdk4 and cdk6 and shares strong sequence identity with *p16* (Hannon and Beach, 1994). The *p16* locus is deleted, rearranged, or mutated in the majority of tumour cell lines (Kamb *et al.*, 1994a). This finding led to the suggestion that *p16* may be the TS gene (*MTS1*) that maps to this region of chromosome 9 and whose inactivation has been implicated in many malignancies, including sporadic carcinomas and familial melanomas (Hussussian *et al.*, 1994). Confirmation of this hypothesis has been controversial, possibly due to the haste in which the analyses were performed. For example, the linkage to familial predisposition to melanoma has both its proponents (Hussussian *et al.*, 1994) and opponents (Kamb *et al.*, 1994b). Resolution of this controversy should shortly be forthcoming and may have implications for DNA damage repair (see Section 3.4.6). It is also possible, of course, that *p15* may represent a critical TS gene.

(80) In summation, it is clear that regulation of the cell cycle is critical for the development and maintenance of normal cell behaviour. Dysregulation serves as a powerful driving force for abnormal proliferation. Several genes that govern control of the G1/S progression are known to be, or are candidate, oncogenes (*PRAD1*/cyclin D1) or tumour suppressor genes (*p15*, *p16*, *p21*, *p27*, *RB*, and *p53*). Presumably, any of the critical cell cycle regulatory genes are candidates and their aberrant functioning in cancers is being actively investigated.

(81) Relatively little is known about checkpoint controls in S, G2, and M phases of the cell cycle. An early G2 checkpoint response in normal human cells has been associated with inhibition of $p34^{CDC2}$/cyclin B kinase activity (Paules *et al.*, 1995). There is also evidence for a later G2 checkpoint closer to the G2/M boundary.

(82) Despite the small amount of knowledge available, there are some intriguing findings. For example, cyclin E is thought to be important in the initiation of DNA replication. Cyclin E is over-expressed in many human tumour types, including breast cancers (Hunter and Pines, 1994). Experimental evidence for an oncogenic activity for cyclin E is, however, not compelling. The cyclin A gene (*CCNA*) is the unique insertion site of a hepatitis B virus (HBV) in clonal hepatoma. The integration produced a chimeric fusion protein that lacked the so-called cyclin destruction box (Wang *et al.*, 1992), thus the chimeric cyclin A protein was not degraded in mitosis. Since cyclin A is required for progression through S phase and G2/M, its constitutive expression may contribute to aberrant cell cycle control (Hunter and Pines, 1994).

(83) Knowledge on cell cycle control continues to develop rapidly and a series of recent reviews provide further insights into this critical area of cellular physiology (see Nasmyth, 1996).

3.2.2. *The* p53 *gene in DNA damage checkpoint control*

(84) Damage to DNA can elicit two early cellular responses: cell cycle arrest followed by repair, or apoptosis (programmed cell death). Loss of either response can lead to accumulation of mutations and genomic instability, both of which may contribute to neoplastic development.

(85) A key regulator of the G1/S checkpoint control in the cell cycle is the protein product of the *p53* TS gene. *p53* is the most commonly mutated TS gene found in human cancers. The *p53* protein was first identified through its ability to form a stable complex with SV40 large T antigen. The SV40 T antigen was subsequently found to block the activity of wild type *p53*, an important component of the transforming activity of SV40 T Antigen. *p53* protein has been shown to bind to DNA and function as a transcriptional activator (Lane, 1994). It also represses the transcription of certain genes (e.g. the multi-drug resistance gene or *MDR*) via protein:protein interactions.

(86) The *p53* protein has a very short half-life (approximately 15 minutes) in normal cells. Upon exposure of cells to DNA-damaging agents, e.g. UV light, ionising radiation and DNA intercalation agents, the half-life of *p53* protein increases significantly (to several hours) and the cells arrest in G1 or undergo apoptosis, depending on cell type. The mechanisms by which *p53* induces G1/S checkpoint control are unknown. However, it has been shown that *p53* upregulates the expression of *p21*CIP1/WAF1, the cdk inhibitor that forms a quaternary complex with cyclin D, cdk and PCNA (Fig. 3.4). This quaternary complex seems to be required for efficient cell cycle control (Xiong *et al.*, 1993). It is currently thought that the induction of the G1/S checkpoint control allows the cell to either repair the DNA damage—in which case the G1/S block is temporary—or, if the damage is irreparable, the cell is permanently arrested or diverted into the programmed cell death or apoptotic pathways. The sensitivity of the DNA damage sensing by wild type *p53* is remarkable; Wahl and colleagues have suggested that the existence of a single DNA double strand break in a cell might be sufficient to activate the *p53*-mediated G1/S arrest (Huang *et al.*, 1996).

(87) Loss of function of the *p53* gene is a critical event in neoplastic progression. This may occur as a consequence of deletion of the *p53* gene or, more commonly, due to missense or nonsense point mutations. The majority of point mutations are missense, thereby preserving the open reading frame of the gene. The gene is approximately 20 kb in length, transcribing a 2.8 kb mRNA which is translated into a 393 amino acid protein. The gene has 11 exons. The functional domains of the *p53* protein are illustrated in Fig. 3.5. There are five evolutionarily highly conserved domains which correspond closely

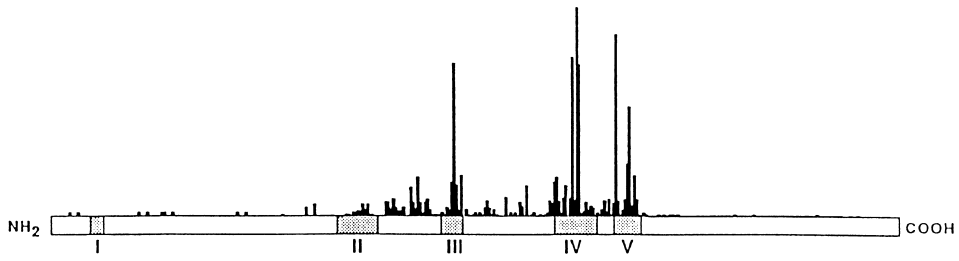

Fig. 3.5. Mutational spectrum within conserved domains of p53 protein. Conserved domains I–V are hatched; bars represent relative frequency of mutation, the highest being mutational hot-spots.

to the regions most frequently mutated in sporadic cancers. Domains II–V correspond to the region of the molecule responsible for binding to sequence-specific DNA regions in *p53*-responsive promoters. The carboxy terminal domain includes the nuclear localising signal and the oligomerisation domain. The crystal structure of *p53* predicts a tetrameric complex binding to DNA (Cho *et al*., 1994, Jeffrey *et al*., 1995). Missense mutations in *p53* result in loss of binding to *p53* consensus sites in DNA and/or loss of transcriptional activation (Prives, 1994).

(88) Cells that lack wild type *p53* function fail to arrest at the G1/S checkpoint thereby increasing the likelihood of development of genetic lesions created by replication errors and their subsequent fixation in the genome. Consequential DNA damage may include point mutations, DNA deletions, insertions and rearrangements, and gene amplification. In addition to its G1/S phase roles, in some cell types *p53* protein may also play a role in checkpoint repair in G2/M (Bouffler *et al*., 1995). The importance of this for cellular radiation response and tumorigenesis is discussed Section 5. There are also indications that *p53* may play a role in correct maintenance of centrosome replication. The centrosome plays a critical role in mitotic stability, ensuring the establishment of bipolar spindles and balanced chromosome segregation. The centrosome duplicates only once during each cell cycle. Fukasawa and colleagues (1996) report that in mouse embryonic fibroblasts lacking the *p53* tumour suppressor protein, multiple copies of functionally competent centrosomes are generated during a single cell cycle. The presence of multiple copies of the centrosome lead to unequal chromosome segregation and consequent chromosomal aneuploidy.

(89) Evidence that loss of function of the *p53* gene is a critical event in neoplastic development has come from several sources. One of the most compelling is that a germ-line mutation in *p53* is the heritable trait in the Li–Fraumeni syndrome, a rare autosomal dominant condition that is characterised by the tendency to develop multiple malignancies—particularly soft tissue sarcomas and breast carcinomas—at an early age (Malkin *et al*., 1990). It has now been shown that tumours arising in these individuals have suffered deletions or point mutations in the remaining wild type *p53* allele.

(90) Transgenic mice have been produced that are either heterozygous or null for *p53*. The $p53^{+/-}$ heterozygous mice develop normally and a significant fraction of the animals develop tumours after approximately one year. Interestingly, the majority of mice that are *p53* null also develop normally—indicating that *p53* is not essential for normal development—but the vast majority succumb to malignancy within six months of birth (Donehower *et al*., 1992). This is in contrast to *RB1* nullizygosity which in mice is an embryonic lethal condition (Lee *et al*., 1992). The tumorigenic and cytogenetic responses of *p53*-deficient mice to radiation are discussed in Section 5.

(91) It should be noted that *p53* inactivating mutations and dysfunction of the *p53* protein are the most common alterations observed in human cancers. They have been associated with some fraction of virtually every sporadically occurring type of malignancy (Greenblatt *et al*., 1994). For example, in sporadic colon carcinoma—the first malignancy where *p53* loss of function was implicated—loss of heterozygosity and mutation of *p53* is seen in more than 70% of cases.

(92) There is an increasing appreciation that tumours that retain wild type *p53* alleles may also have compromised *p53* protein function by indirect means. For example, Howley and colleagues showed that the HPV16 or HPV18 E6 transforming protein complexes with *p53* protein and mediates its rapid degradation via a ubiquination pathway (Scheffner *et al*., 1990). They found that cervical carcinomas that are HPV-positive have wild type *p53* whereas those that are HPV-negative have mutant or null *p53* alleles. The obvious

inference is that the expression of the E6 protein renders the cell functionally null for *p53* activity. Similarly, Levine and colleagues (Momand *et al.*, 1992) have found that the mdm2 protein, often over-expressed in breast carcinomas and other malignancies, complexes with *p53* protein and inhibits its transcription activation function.

3.2.3. *Somatic mutations of* p53 *in human tumours*

(93) The *p53* tumour suppressor gene has recently received much attention because of its frequent mutation in diverse types of human cancers. Frequently, the initial mutation in one allele is accompanied by a loss of the second allele. A data base, consisting of published *p53* mutations, has been reported that provides a mutation spectrum for somatic mutations in the *p53* gene (Greenblatt *et al.*, 1994). All classes of mutations occur: single base substitutions predominate and constitute more than 80% of all mutations. Among those, the most common mutations are G:C → A:T and A:T → G:C transitions and G:C → T:A transversions, and more than 60% of base substitutions are found at G:C base pairs (Fig. 3.6). However, the mutation spectra differ depending on tumour type, indicating that the origins of mutations are distinct among different tumour types.

(94) Mutations scatter over the entire region of evolutionally conserved domains (codons 97–292). While mutational hot spots are not evident for deletion/insertion mutations and nonsense base substitutions, the missense base substitutions tend to cluster at high frequency in codons 175, 245, 248, 249, 273, and 282. Codons 175, 245, 248, 273, and 282 contain CpG dinucleotides. The prevalence of G:C → A:T transitions in these sites suggests the spontaneous deamination of 5-methylcytosine (^{5mt}C) converting into uracil or thymine. Aliphatic alkylation of guanine at the O6 position is also expected to generate G:C → A:T transitions by mispairing with thymine during DNA replication. The bulky adducts produce uninstructive lesions which, along with abasic sites, tend to be replicated by the addition of adenine opposite the lesions ('A rule'). This 'A rule' accounts for the G to T and A to T transversions.

(95) While the types and sites of mutations are the driving force of mutation and selection they also provide etiologic clues in some tumours. Predominance of the G:C → A:T transition with frequent involvement of CpG sites is evident for colon, brain, and stomach cancers, basal cell carcinoma of skin and leukaemia/lymphoma. In contrast, the involvement of CpG sites in C to T transitions is comparatively less frequent for lung

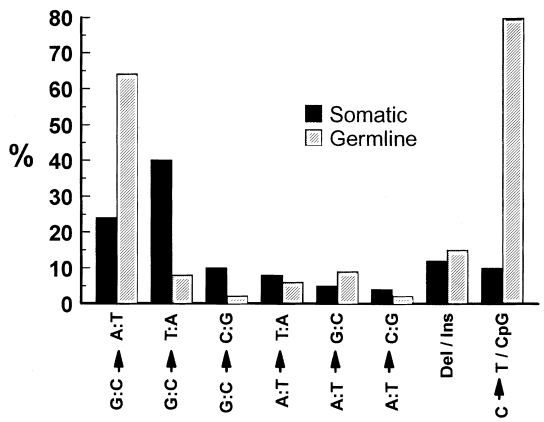

Fig. 3.6. Somatic and germ-line mutation of the human *p53* gene (from Greenblatt *et al.*, 1994).

and liver cancers, where G:C → T:A transversions predominate. In these organs, tobacco smoking and intake of mutagens from diet may be the origin of mutation, forming uninstructive bases by mutagen action. In breast cancer, while G:C → A:T transitions constitute the majority of mutations, with a high frequency at CpG sites, G:C → T:A transversions are frequent in Europe and America but very rare in Japan, indicating different etiological mechanisms among these countries. In skin cancer, particularly squamous cell carcinoma of the skin, mutations in the *p53* gene are highly UV specific, i.e. CC to TT or C to T changes at dipyrimidine sites (Brash *et al.*, 1991; Ziegler *et al.*, 1993; Sato *et al.*, 1993). The thymine moiety included in TT, TC, and CT dimers is expected to pair with adenine, resulting in restoration of the original base sequence. In liver cancer, AGG to AGT change (or G:C → T:A transversion at the 3rd position) in codon 249 has been specifically correlated with the exposure to aflatoxin B1. These lines of evidence, together with base-directed mutagenesis, show that the *p53* mutations are highly tissue specific and agent specific, coming from either spontaneous deamination of ^{5mt}C or mutagen-induced base damage.

(96) It is noteworthy that the UV-related CC to TT mutations in *p53* gene have been also found at a high frequency (74%) in normal skin samples from sun-exposed areas of the skin cancer patients (Nakazawa *et al.*, 1994). Similarly, AGG to AGT mutations at codon 249 are also found in non-malignant samples of liver from patients in Qidong, China, and Western countries, and are correlated with exposure to aflatoxin B1 (Aguilar *et al.*, 1994). In bladder cancer most of the mutations are G:C to A:T transitions. The involvement of the CpG doublet is relatively high, but in smokers 4/16 (25%) of mutant *p53* genes showed tandem double mutations (Spruck *et al.*, 1993). Such multiple mutations in one allele are also found in mutagen-induced cancers in rats (Makino *et al.*, 1992). Multiple mutations in the *p53* gene also have been found in skin tumours that arose in patients with xeroderma pigmentosum, a genetic disease lacking excision repair of UV-induced DNA damage (Sato *et al.*, 1993).

(97) Data are now available for *p53* mutations in some radiation-associated cancers (Fig. 3.7). Vähäkangas *et al.* (1992) studied mutations in *p53* genes in 19 cases of lung cancer that developed in uranium miners and identified nine mutations, including two deletions, in seven of the patients. Among six base substitution mutations, three were G:C → T:A transitions. More recently, Taylor *et al.* (1994b) reported that 16 of 52 lung

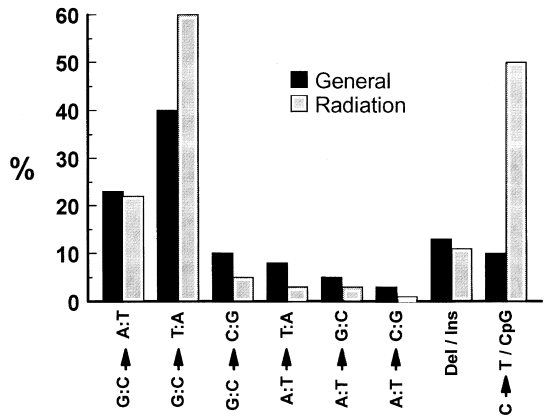

Fig. 3.7. *p53* mutations in human lung cancer. Data on lung cancer in the general population taken from Greenblatt *et al.* (1994). See text for references on radiation-associated lung cancer.

cancers from uranium miners contained AGG to ATG transversions at codon 249. This site-specific mutation, particularly G to T transversion at the 2nd position of codon 249, is a rare event in *p53* mutations that are found in conventional lung cancers, and they reported that such mutations might be a potential marker for radiation-induced lung cancer. This is, however, a controversial finding. Takeshima *et al.* (1993) studied *p53* mutations in lung cancers that arose in nine low-dose atomic bomb survivors who were nonsmokers and identified four mutations. The frequencies and types of mutations were not different from those in lung cancers of nonsmoker controls, where G:C → A:T transitions predominated, in contrast to the predominance of G:C → T:A transversions in smokers. Recently, Ohtsuyama *et al.* (1994) studied *p53* mutations in mouse skin tumours and osteosarcomas, which appeared after β-irradiation, and found that the majority of mutations were at CpG sites. Thus, no clear evidence is emerging for radiation-specific mutations in radiation-associated tumours, although data remain rather sparse.

(98) In summary, tumour suppressor genes may be seen to occupy a central position in the negative regulation of a range of cellular functions that are relevant to neoplastic development. Of principal importance to normal cell physiology are the tumour suppressor gene functions in signal transduction, cellular interactions, cell cycle regulation and the maintenance of genomic stability. Loss or mutation of such genes can act to compromise these critical biochemical and biophysical pathways, often in a fashion that implies a degree of cell type, and thereby tissue specificity. Most tumour suppressor genes are recessive in function and loss/mutation of both autosomally encoded copies will be necessary for full phenotypic expression during neoplastic development. In some cases, however, single-copy gene mutation is sufficient for the generation of partial phenotypic effects. Mutant tumour suppressor genes and proto-oncogenes appear to operate in a synergistic and often gene-specific fashion during multistage oncogenesis. The consistency of somatic tumour suppressor gene mutations in many tumour types, their critical involvement in familial tumours, and in vitro experimental evidence, tend to support the view that they are the principal rate-limiting genetic component of human cancer.

3.3. DNA repair and replication genes in solid tumours

(99) Inherited syndromes that are associated with DNA repair deficiencies, radiation sensitivity, and increased risk of malignancies have been known for many years. An illustration of such genes and the disorders they are associated with are given in Table 3.3.

Table 3.3. Cloned DNA replication and repair genes implicated in human carcinogenesis

Gene symbol	Chromosomal location	Syndrome	Mode of Action
XPAC	9q34.1	Xeroderma pigmentosum (XP) A	Nucleotide excision repair
XPBC	2q21	Xeroderma pigmentosum XPB	Nucleotide excision repair
XPCC	3p25	Xeroderma pigmentosum XPC	Nucleotide excision repair
XPDC	19q13.2	Xeroderma pigmentosum XPD	Nucleotide excision repair
XPGC	13q32-q33	Xeroderma pigmentosum XPG	Nucleotide excision repair
hMSH2	2p16	Hereditary nonpolyposis Colorectal cancer (HNPCC)	Mismatch repair
hMLH1	3p21	HNPCC	Mismatch repair
hpMS1	2q31-q33	HNPCC	Mismatch repair
hpMS2	7p22	HNPCC	Mismatch repair
BS	15q26	Bloom's syndrome	Helicase (DNA ligation)

These conditions, and their relevance to lymphohaemopoietic neoplasia are discussed in Sections 2 and 4 of this document.

(100) A specific set of DNA repair genes involved in DNA mismatch correction have been recently implicated in the genesis of solid tumours. A variety of tumours have been found to harbour alterations in microsatellite sequences distributed throughout their genome (Ionov *et al*., 1993; Aaltonen *et al*., 1993). This microsatellite instability indicates malfunctions(s) in the replication or repair of DNA which persist throughout the life span of the tumour, constantly providing a source of genetic instability. The molecular determinant of microsatellite instability was resolved when it was found that germ line mutations in a DNA mismatch repair gene, *hMSH2*, the homologue of the prokaryotic *mutS* mismatch repair gene, characterise hereditary nonpolyposis colorectal cancer (HNPCC) patients (Fishel *et al*., 1993; Parsons *et al*., 1993). HNPCC (or Lynch syndrome) is one of the most common cancer-predisposing syndromes (see Section 6). Colonic tumours derived from *hMSH2* HNPCC individuals contain the germline mutation in *hMSH2* plus mutation or deletion of the remaining wild type allele. Recent experiments have confirmed the recessive nature of this gene (Casares *et al*., 1995).

(101) Not all HNPCC patients have a *hMSH2* germ line mutation. It has now been shown that germ line mutations in three other DNA mismatch repair genes, namely *hMLH1* (a homologue of the bacterial *mutL* gene), *hPMS1*, and *hPMS2* (two other homologues of the prokaryotic *mutL* gene) are mutated in a subset of HNPCC cases (Table 3.4) (Bronner *et al*., 1994; Nicolaides *et al*., 1994). In these cases, somatic mutations in the respective remaining wild type allele are also found in tumours obtained from these patients.

(102) Based on the high frequencies of chromosomal abnormalities and mutations that are found in human cancers, Loeb hypothesised that cancer is manifested by a mutator phenotype (Loeb, 1994). Although attractive, this hypothesis lacked experimental support until the discovery of defects in DNA repair genes. The replication error (RER) phenotype that characterises microsatellite instability is also associated with an increased in vitro somatic mutation rate (Eschelman *et al*., 1995). Loeb has developed a hypothetical scenario (Fig. 3.8) that predicts that mutations in genomic stability genes (including DNA repair and replication) are early events in carcinogenesis. Defects in DNA repair, replication, and chromosomal segregation are likely to increase the probability of damage to, or loss of, oncogenes and tumour suppressor genes that are critical for the final manifestation of the malignant phenotype. Experimental evidence is now accumulating to support this contention. It is noteworthy that mismatch repair gene defects are found in sporadic colorectal cancers (and other cancers) with microsatellite instability. Furthermore, a significant number of cancer cells that exhibit the RER phenotype do not show defects in the four known mismatch repair genes (Liu *et al*., 1995). This is not a surprising finding since it is known that in the bacterium *Escherichia coli*, approximately 10 gene products are involved in mismatch repair. Thus, the RER phenotype in these cases may be due to defects in other genes responsible for DNA repair or replication.

(103) In summary, mutation of DNA mismatch repair genes appears to be an important component of sporadic and familial solid tumours with mutant gene effects being most pronounced in the intestinal tract. Evidence that has accumulated in this new area of study has further highlighted the critical importance of the early acquisition of genomic instability for neoplastic development. Two further advances in our understanding of how defective genes associated with DNA replication/repair play a role in neoplastic progression came with the cloning and characterisation of the *ATM* and Bloom's

Table 3.4. Genetic determinants of solid tumours in different organs

Organ system	Disorder	Genes	Principal neoplasms	Other features or comments (see text)
Mammary/ovarian	Heritable breast/ovarian cancer	*BRCA1* *BRCA2* (others?)	Breast/ovarian cancer Breast cancer (also male)	2–5% of all cancers., ~40% of early onset disease, moderate-high penetrance
	Ataxia-telangiectasia heterozygotes	*ATM*	Breast cancer	See Section 5
Gastro-intestinal	Familial adenomatous polyposis	*APC*	Colorectal cancer (multiple polyps)	~80% penetrance, <1% of all cancer
	Gardner and Turcot syndromes	*APC*	Colorectal cancer	
	Hereditary non-polyposis colon cancer	*MLH1* *MLH2* *PMS1* *PMS2*	Colon cancer (proximal) Endometrial cancer	Up to 5% of total cancer, early onset (<45 years), high but variable penetrance
Renal	von Hippel Lindau	*VHL*	Renal cancer	Adult early onset disease, variable penetrance Nephroblastoma <15 years, high penetrance, complex genetics
	Denys Drash syndrome	*WT1*	Nephroblastoma	
Nervous tissues	Neurofibromatosis type 1	*NF-1*	Neurofibroma	High penetrance but low mortality rates, high de novo contribution in *NF-1*
	Neurofibromatosis type 2	*NF-2*	Schwannoma Meningioma Neurofibroma	
	Nevoid basal cell carcinoma syndrome	*PTC*	Medulloblastoma Meningioma	See Section 5
Prostate	Familial prostate cancer	*HPC1*	Early onset prostate cancer	5–10% of all cancers, ~40% of early onset disease
Skin	Familial melanoma	*MLM1* *MLM2*	Melanoma	5–10% of all cancers is familial, 50% is associated with *MLM1*; variable penetrance, see Section 5
	Nevoid basal cell carcinoma (others, e.g. skin pigmentation, genotypes, xeroderma pigmentosum)	*PTC*	Basal cell skin cancer	Variable penetrance, see Section 5 Not considered in this document
	Familial cylindromatosis	*16q*	Cylindromas	
Endocrine	Multiple endocrine neoplasia type 2A	*RET*	Medullary thyroid cancer, Parathyroid hyperplsia	Correlations between *RET* mutation type and disease expression
	Multiple endocrine neoplasia type 2B	*RET*	Medullary thyroid cancer	
	Familial medullary thyroid cancer	*RET*	Medullary thyroid cancer	
Multiple organs	Li–Fraumeni syndrome	*p53* (others?)	Soft tissue/bone sarcoma. Breast, brain and adrenal cancer (myeloid/lymphoid leukaemia)	Rare disorder but high penetrance 50% by 30 years, 90% by 70 years
	Tuberous sclerosis	*TSC1* *TCS2*	Hartomas of skin, nervous tissue, heart and kidneys	Vaiable expression, high de novo contribution
	Retinoblastoma	*RB1*	Retinal tumours, bone/soft tissue sarcoma, brain cancer and melanoma	High penetrance for retinoblastoma <15 years see also Section 5

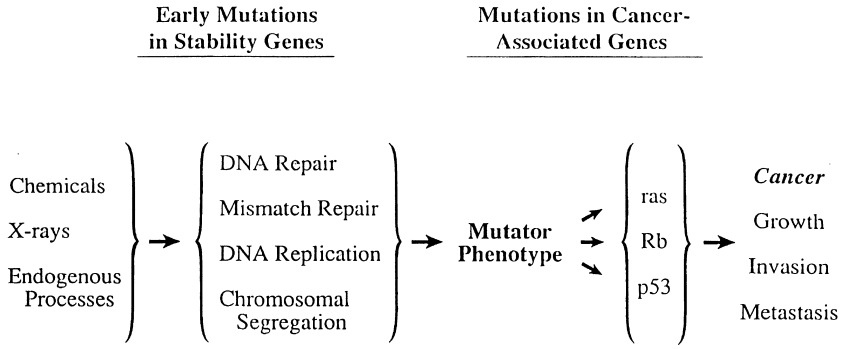

Fig. 3.8. Sources of multiple mutations during cancer development (see Loeb, 1994).

syndrome genes. The *ATM* gene is mutated in patients with the cancer-prone genetic disorder ataxia-telangiectasia (Savitsky *et al.*, 1995). It encodes a cell cycle checkpoint protein related to the yeast *Rad3* gene, a member of the phosphatidyl-3′ subgroup of kinases (Walworth and Bernards, 1996). Other functions of this large gene (transcript size of 12 kb) are currently under investigation.

(104) The Bloom's syndrome gene, *BLM*, plays an important role in the maintenance of genomic stability in somatic cells. The *BLM* gene has recently been cloned and sequence comparison shows homology with RecQ helicases (Ellis *et al.*, 1995).

(105) The relevance of these gene defects, as well as those responsible for xeroderma pigmentosum, Cockayne's syndrome, and Werner's syndrome, with respect to radiosensitivity and predisposition to cancer, are addressed in Section 2 and also noted in Section 5.

3.4. Genetic susceptibility to solid tumours

(106) Having established the general role of oncogenes, tumour suppressor and DNA repair genes in the genesis of human solid tumours, and having provided illustrative examples of the cellular/molecular mechanisms involved, it becomes possible to consider the specific germ line mutations that predispose to cancer. Such predisposition has been the subject of a number of reviews (Knudson, 1993; Eng and Ponder, 1993; Lanfrancone *et al.*, 1994; Karp and Broder, 1995; Eeles *et al.*, 1995) and here we provide an outline of this rapidly developing field on the basis of the expression of disease in different organ systems.

(107) Although individuals with cancer-predisposing germ line mutations carry a mutant gene in each and all of their somatic cells, the subsequent age-related development of overt cancer is usually limited to a few of these cells and often presents as cancer in a subset of tissues (see also Section 5). Thus the mutant gene in question, although not assuring neoplastic transformation, must reduce or bypass a given rate limiting step in carcinogenic development. It also follows that the relative importance of such a mutation to physiological control differs between cell types.

(108) These features may also underlie apparent differences in the penetrance and expressivity of the germ line tumour genes of interest. In the context of tumour predisposition, the term penetrance principally applies to the probability that a given gene carrier will express the mutant genotype as neoplastic disease. Expressivity is less well defined but essentially applies to the nature of disease in carriers, e.g. the age of onset

(which is usually at an earlier age than seen in the normal population), the manifestation of multiple tumours at the same or different sites, and the relative numbers of benign and malignant neoplasms. Thus, penetrance and expressivity are the principal biological determinants of cancer morbidity and mortality in tumour-predisposed individuals.

(109) The factors that influence penetrance and expressivity of germ line tumour gene mutations are poorly understood. However, they seem to include the specific nature of the mutation in question, the presence or absence of other genomic determinants (so-called modifier genes), epigenetic factors, such as parent-of-origin effects on gene expression, and perhaps, what is most important, environmental factors such as dietary intakes and exposure to genotoxic agents. Some of these issues are discussed in this and other sections of the document.

3.4.1. *Heritable breast and ovarian cancer*

(110) Genetic linkage studies have established that a gene predisposing to breast and ovarian cancer (*BRCA1*) accounts for the inherited predisposition to cancers in a high proportion of families with multiple breast cancer and ovarian cancers, and approximately half of all early-onset breast cancer families without ovarian cancer (Ford *et al*., 1994). It was estimated that the penetrance of the gene is 87% by age 70 (Easton *et al*., 1992). Significant excesses for colon cancer and prostate cancer were also observed in some families (Ford *et al*., 1994).

(111) The *BRCA1* gene is situated on chromosome 17q21 and has been cloned (Miki *et al*., 1994). It is expressed in numerous tissues, including breast and ovary, and encodes a predicted protein of 1863 amino acids. The *BRCA1* gene is large, comprising 22 coding exons distributed over approximately 100 kb of genomic DNA.

(112) Follow-up studies have confirmed the original finding of germ line *BRCA1* mutations in a significant proportion of breast/ovarian cancer families. These have both deletion and chain terminating mutations as the principal mutational events (Friedman *et al*., 1994; Simard *et al*., 1994; Castilla *et al*., 1994; Collins, 1996). Such protein-inactivating mutations are broadly consistent with a tumour-suppressor role for *BRCA1*. In spite of this, somatic *BRCA1* mutation appears not to be a principal feature of sporadic breast cancer (Futreal *et al*., 1994), which might indicate a specific developmental role for the gene. The size and complexity of *BRCA1* may, however, be a confounding factor in these analyses; an alternative explanation is that the *BRCA1* protein may be inappropriately expressed in tumours (see Boyd, 1995). Efforts are currently underway to clarify these possibilities (e.g. Kinzler and Vogelstein, 1996; Steeg, 1996).

(113) Early linkage studies suggested that the chromosome 17-linked locus now identified as *BRCA1* could account for only approximately half of the strongly expressing heritable breast cancers. In accord with this, a second gene *BRCA2* has been mapped to chromosome 13q 12–13 (Wooster *et al*., 1994) and has recently been cloned (Wooster *et al*., 1995). Mutations in the *BRCA2* gene account for approximately one-third of high-risk families. Functionally, the two known *BRCA* genes may be different since, while mutation to either gene gives a similarly high risk of breast cancer, *BRCA1* defects are associated with a much higher risk of ovarian disease and *BRCA2*, unlike *BRCA1*, can express as breast cancer in males. Friend (1996) has summarised recent findings in *BRCA2* kindred which confirm the relatively high risk of male breast cancer and that of very early onset disease in some female carriers. These studies do provide, however, evidence of the variable penetrance of a single *BRCA2* mutation in Icelandic populations highlighting the importance of genetic–genetic and genetic–environmental factors in the expression of

heritable cancer. The linkage study of Wooster *et al.* (1994) also shows that mutation of *BRCA1* or *BRCA2* cannot account for all cases of heritable breast cancer. Together they may explain no more than 5% of total breast cancer at all ages in Western populations but amongst the remainder there appear to be familial cases that may involve genes of lower penetrance. In the case of *BRCA1* sufficient breast cancer cases are now defined at the molecular level to allow comment upon the range of mutations in the population and their likely contribution to population risk (see Collins, 1996 and Section 6). An important finding is that a single mutation (185 delAG) propagated by common descent (founder effect) contributes to the high *BRCA1* contribution of around 38% of cases <30 years in Jewish women of Ashkenazi origins. By contrast, the total *BRCA1* contribution to breast cancer at <30 years in non-Jewish women was around 7%. Of equal importance was the finding of *BRCA1* germ line mutations in cases without striking family histories of breast or other cancers.

(114) There is growing evidence that there can be variation of risk of breast and ovarian cancer with different germ line mutations of *BRCA1* and *BRCA2* but that alleles of *BRCA1* that are common in the population do not have obvious implications for risk (Gayther *et al.*, 1997; Dunning *et al.*, 1997).

(115) With regard to the specific functions of breast cancer-predisposing genes evidence is now emerging that *BRCA1* and *BRCA2* proteins may play a role in DNA metabolism and damage response. In the case of *BRCA1* protein, binding to asynapsed regions of meiotic chromosomes has been demonstrated (Scully *et al.*, 1997) and interaction with *RAD51* seems likely. Equally, studies with genetically manipulated mice (Sharan *et al.*, 1997) suggest that *BRCA2* protein may be a co-factor in *RAD51*-dependent repair of DNA double strand breaks thereby acting as a co-determinant of cellular radiosensitivity.

(116) In Section 5 the contention that heterozygous carriers of the mutations associated with the 11q-linked radiosensitive disorder, ataxia-telangiectasia (A-T) have an increased risk of spontaneous breast cancer is discussed (see Easton, 1994). As shown in Section 6, such A-T heterozygotes may be relatively common in the population, having an estimated frequency of around 1 in 200.

(117) Recent data regarding breast cancer risk in A-T heterozygotes, i.e $ATM^{+/-}$ appear to be more equivocal than the earlier epidemiological studies reviewed by Easton (see Bishop and Hopper, 1997; also Section 5). While in a study of A-T heterozygous females ascertained by molecular methods, Athma *et al.* (1996) estimated that the relative risk of breast cancer was 3.8, Fitzgerald *et al.* (1997) found no evidence of such increased risk when comparing germ line *ATM* mutation incidence in 402 breast cancer cases diagnosed before age 40 years with that of 202 controls. As noted by Bishop and Hopper, these two data sets are not incompatible and further large-scale studies are required to resolve the issue.

(118) Finally, a relatively high frequency of breast cancer genes of low penetrance may account for the observations of Scott *et al.* (1994). These data show that around 40% of unselected breast cancer patients exhibit significantly elevated chromosomal radiosensitivity in lymphocytes (Section 2). A genetic basis for this phenotypic association has yet to be established but it is already evident that such radiosensitive phenotypes are present in the population at a frequency that is too high to be explained by a combination of *BRCA1*, *BRCA2*, and $ATM^{+/-}$ genotypes. Although there is no direct information on the nature of low penetrance breast cancer genes, Skolnick *et al.* (1990) have shown that genetic determinants of neoplasia-associated proliferative breast disease may be relatively common in the population. It was suggested that these underlie a greater proportion of

breast cancer at all ages than the more penetrant genes now identified as *BRCA1* and *BRCA2*.

3.4.2. *Heritable colon cancer*

(119) Clinical, genetic, and laboratory studies in respect of human susceptibility to colon cancer have been reviewed (Rustgi, 1994; Bodmer *et al*., 1994).

(120) Familial adenomatous polyposis (FAP) was the first genetic disorder to be associated with predisposition to intestinal tumours. The FAP disorder, which has a prevalence of between 1 in 5000 and 1 in 10,000, is principally characterised by the development of up to thousands of neoplastic adenomatous polyps throughout the large intestine. Approximately 80% of FAP patients develop early onset colorectal cancer but neoplasms at other gastrointestinal sites are not uncommon (Rustgi, 1994).

(121) A single locus responsible for FAP was mapped to chromosome 5q21 and subsequently cloned and characterised as the *APC* gene (Kinzler *et al*., 1991; Nishisho *et al*., 1991). *APC* shows the primary characteristics of a tumour suppressor gene and its function had been linked with those of catenin proteins with a putative role in cellular cytoskeleton function and cell adhesion (Su *et al*., 1993). More recently *APC* protein function has been linked with β-catenin degradation and transcriptional control of cellular proliferation (see Nakamura 1997).

(122) Gardner's and Turcot's syndromes share some of the clinical features of FAP but have been regarded as being clinically distinct. There is, however, evidence that these disorders are rare genetic variants of FAP. The rare conditions, Peutz–Jegher's syndrome, juvenile polyposis, Cowdens disease and Muir–Torre syndrome, are poorly understood genetic disorders associated with some degree of predisposition to intestinal neoplasia (Rustgi, 1994).

(123) Hereditary non-polyposis colon cancer (HNPCC) was originally suggested to have a frequency of around 1 in 200 to 1 in 500 in Western populations and was claimed to account for up to 15% of colon cancers (Lynch *et al*., 1993). As such, it is by far the strongest genetic determinant of such neoplasms (Lynch *et al*., 1993). Such estimates remain, however, highly controversial and depend critically on the criteria used for clinical and familial ascertainment. The prevailing view is that HNPCC is substantially less common than originally believed (Fishel and Kolodner, 1995; Liu *et al*., 1995). On the basis of the assumption that HNPCC contributes only around 2% of colon cancer and has a penetrance of around 60%, using a lifetime cumulative colon cancer rate of 3% for a USA population (Parkin *et al*., 1992) it may be provisionally estimated that around 1 in 2000 individuals carry the genetic determinants of HNPCC.

(124) HNPCC, unlike the colon cancer predisposing disorders determined by *APC* mutation, does not involve the widespread development of multiple colonic polyps. HNPCC polyps are few and largely confined to the proximal colon, within which early onset and poorly differentiated colon cancers subsequently develop. Some HNPCC patients also develop neoplasia at other sites, principally the endometrium, stomach and pancreas, the penetrance of mutations in HNPCC kindred is lower than that of FAP and tends to be somewhat variable (Lynch *et al*., 1993; Rustgi, 1994). Like heritable breast cancer, HNPCC has been subject to intense molecular genetic investigation over the last few years and evidence for the specific involvement of mutant DNA mismatch repair genes in this disorder and the ensuing genomic instability of colonic tumours has already been discussed (Section 3.3).

3.4.3. *Heritable renal cancer*

(125) The 3p26 gene, *VHL*, of the von Hippel–Lindau disorder is principally responsible for early-onset familial kidney cancer and, less frequently, hemangioblastoma and phaeochromocytoma (see Gnarra *et al.*, 1994). Characterisation of the germ line mutations involved, and of loss of the normal allele in the renal cancers of *VHL* patients, is strongly suggestive of tumour suppressor functions. This is also consistent with the finding that around 65% of sporadic renal tumours harbour somatic *VHL* mutations (Gnarra *et al.*, 1994). Recent studies point towards a role for the *VHL* gene in the regulation of transcriptional elongation (Duan *et al.*, 1995).

(126) While *VHL* is clearly a critical gene for renal carcinogenesis, a second locus responsible for hereditary papilliary renal cell carcinoma has also been identified (Zbar *et al.*, 1994; Schmidt *et al.*, 1997). In addition to *VHL*-determined adult kidney cancer, an embryonal kidney tumour (Wilms' tumour) can arise, often bilaterally, in a clinically complex chromosome 11p disorder termed Wilms' aniridia, genitourinary malformation and mental retardation (WAGR) (Housman *et al.*, 1986). While an 11p13 tumour suppressor gene *WT1* encoding a transcriptional regulator is occasionally inactivated in Wilms' tumour cells, this gene is not the sole determinant of WAGR. Mutation of *WT1* is seen in the Denys–Drash syndrome which is associated with urogenital abnormalities and kidney tumour susceptibility (Hastie, 1994). The complexity of the genetics of Wilms' tumour is further evidenced by the finding that a distinct genetic entity, the Beckwith–Wiedemann syndrome (BWS), that maps to 11p15, predisposes, amongst others, to Wilms' tumour. Also epigenetic, parent-of-origin effects influence both the expression of BWS and loss of heterozygosity for *WT1* in tumours (e.g. Steenman *et al.*, 1994; Bartolomei, 1994; Skuse and Ludlow, 1995). Although of great academic interest both WAGR and BWS are rare genetic disorders and do not impact strongly on childhood kidney cancer incidence.

3.4.4. *Heritable tumours of the nervous system*

(127) The two principal genetic disorders predisposing to nervous system tumours are neurofibromatosis type 1 (NF1) and type 2 (NF2).

(128) NF1 or von Recklinghausen disease is a relatively common (~1 in 3000) genetic disorder determined by mutation of a 17q11 gene with tumour suppressor functions (Skuse *et al.*, 1989). The *NF1* gene product, neurofibromin, is believed to be a GTPase-activating protein which may function in cellular signal transduction pathways. The principal neurological system tumours in *NF1* patients are neurofibromas (Riccardi and Eichner, 1986) but as noted in Section 4 there is also evidence for predisposition to juvenile myeloid leukaemia.

(129) NF2 is less common than NF1 and predisposes to tumours of the vestibular branch of the 8th cranial nerve (schwannomas), meningiomas and peripheral neurofibromas (Wertelecki *et al.*, 1988). The *NF2* gene maps to 22q12 and shows the primary features of a tumour suppressor gene. Its gene product, termed merlin, has been proposed to act to link elements of the cell membrane with those of the cytoskeleton (Trofatter *et al.*, 1993).

(130) The third genetic entity to be briefly considered here is Nevoid basal cell carcinoma syndrome (NBCCS). The predisposing gene (*PTC*) has recently been cloned (see Section 3.4.6). While multiple basal cell carcinoma is the principal neoplasm in NBCCS a significant number of patients develop medulloblastoma (Bale *et al.*, 1994). Predisposition to central nervous system tumours, albeit not as the predominant neoplastic

manifestation, has also been associated with the Li–Fraumeni syndrome, tuberous sclerosis, familial polyposis and von Hippel–Lindau disease (see Bondy *et al.*, 1993).

3.4.5. *Heritable prostate and testicular cancer*

(131) Prostate cancer is increasingly recognised as a common neoplasm in Western populations. Current estimates suggest that 5–10% of all prostate cancer, and around 40% of the early onset variety in males < 55 years, is inherited in a Mendelian fashion (Carter *et al.*, 1993). An association between familial prostate and breast cancer is supported by the finding of excess early prostate cancer in the male relatives of women with *BRCA1* mutations. It is clear, however, that *BRCA1* is not the principal genetic determinant of prostate cancer. Other candidate genes for prostate cancer predisposition have been discussed by Cannon-Albright and Eeles (1995) and, more recently through the analysis of high-risk families, a major dominantly expressing susceptibility locus, *HPC1*, has been mapped to chromosome 1 (1q24–25). Specific candidate genes from this region have yet to be reported on but it has been estimated that *HPC1* may account for a significant fraction of hereditary prostate cancer and that mutant alleles are carried by up to 1 in 500 individuals in the USA (Smith *et al.*, 1996). A heritable component to testicular cancer is also supported by twin and family studies (Forman *et al.*, 1992) but, as yet, the underlying genetics remains obscure.

3.4.6. *Heritable skin cancer*

(132) The clinical and genetic aspects of heritable skin tumours have recently been reviewed by Hauck and Manders (1994) and Epstein (1996). An ICRP Task Group has reported in *Publication 59* upon some of the implications for radiological protection (ICRP, 1991).

(133) Two of the most important genetic determinants of heritable skin cancer, namely skin pigmentation genotypes (e.g. albinism) and DNA photoproduct repair disorders (e.g. xeroderma pigmentosum), while having clear implications for photo-carcinogenesis, are of less direct relevance to ionising radiation exposures. Hauck and Manders (1994) and Epstein (1996) have also noted that excess skin tumours may be associated with a range of genetic disorders, including Cowden's disease, Gardner's syndrome, neurofibromatosis, tuberous sclerosis, and familial cylindromatosis. Here we focus on two other disorders, familial melanoma and nevoid basal cell carcinoma syndrome (NBCCS), the latter being of particular relevance to subsequent judgements on the degree of radiosensitivity that may accompany genetic predisposition to cancer (see Section 5).

(134) Familial melanoma is an autosomal dominant, but genetically heterozygous, disease which may account for 5–10% of all cases arising in the population (Wainwright, 1994). Penetrance of the disease is largely incomplete and the expression is highly variable. Two principal loci, encoded on chromosome 9p (*MLM1*) and 1p (*MLM2*), have been associated with familial melanoma and most attention has been given to the identification of the *MLM1* gene, which may account for 50% of familial cases. Although contentious, there is evidence (Wainwright, 1994; Ranade *et al.*, 1995) that the *MLM1* locus may be identical with the $p16^{INK4}$ gene (the *MTS1* tumour suppressor), the function of which has already been discussed (Section 3.2.2). Further support for this notion has come from the functional analysis of mutations in the *p16* gene that have been shown to segregate with familial predisposition to malignant melanoma (Parry and Peters, 1996).

(135) The second condition (considered further in Section 5), NBCCS, is also an autosomal dominant disorder with variable expression but, unlike familial melanoma, is

characterised by multiple basal cell carcinomas (BCC) of the skin, medulloblastoma, jaw cysts, and diverse developmental abnormalities (Bale *et al*., 1994; Gorlin, 1995). NBCCS is a rare disorder in which there appears to be a surprisingly high contribution from de novo mutations and evidence of unusual patterns of expression.

(136) Genetic linkage of NBCCS with a locus encoded in an ~2cM interval of chromosome 9q 22.3–31 has been established and the gene has recently been identified (Johnson *et al*., 1996). Mutations have been found in the gene in the germline of NBCCS patients and in cases of sporadic BCC. The gene shows sequence homology to the *Drosophila* (fruit fly) *patched* (*ptc*) gene. The *ptc* gene encodes a transmembrane protein that in *Drosophila* acts in opposition to the *Erinaceus* (hedgehog) signalling protein, controlling cell fates, patterning, and growth in numerous tissues (Johnson *et al*., 1996). Thus, the human *PTC* gene appears to be crucial for both embryonic development and control of cellular proliferation.

3.4.7. *Heritable cancers of the endocrine system*

(137) Heritable predisposition to C-cell medullary thyroid carcinomas (MTC) is the principal manifestation of the multiple endocrine neoplasia syndromes MEN2A and MEN2B; MTC is also characteristic of a third genetic entity, familial MTC (FMTC). However, whereas MEN2A and MEN2B share an additional predisposition to adrenal phaeochromocytoma, MEN2A, but not 2B, is associated with parathyroid hyperplasia, and in MEN2B there are accompanying developmental abnormalities. In contrast, MTC is the sole manifestation of disease in FMTC (Schimke, 1984). In spite of this clinical complexity MEN2A, MEN2B, and FMTC all map to a specific region of chromosome 10q11.2 (Gardner *et al*., 1993). Recent studies have cast light on the genetic basis of these diseases and show that a high proportion of MEN2A and 2B patients carry germ line *RET* proto-oncogene mutations and that there is a strong correlation between the phenotypic expression of disease and the nature and distribution of *RET* mutation. RET mutations have also been found in some cases of FMTC (Sasaki, 1996).

(138) *RET* mutations analysed in MEN2A and FMTC patients were found to be point mutations in one of five cysteines of the extracellular domain. Mutations at cysteine 634 are the most common and have been found in 87% of MEN2 cases. The de novo mutations show a strong bias towards the paternal allele. Deletion mutations of the whole gene cause Hirschsprung disease, an autosomal recessive disease of the enteric nervous system. Overall, germ line *RET* mutation provides an excellent example of how subtle differences in specific gene mutation can have profound effects on the tissue specificity of ensuing disease (see also Romeo and McKusick, 1994).

3.4.8. *Genes associated with cancer at multiple sites*

(139) The tumour susceptibility loci so far considered exhibit a substantial degree of tissue specificity suggestive of different rate-limiting tumorigenic processes in different organ types. There are, however, many exceptions to this tissue specificity, e.g. the excess of medulloblastoma in NBCCS, juvenile AML in *NF1* and prostate cancer in *BRCA1* kindred. There is an expectation, therefore, that in certain genetic disorders tumour predisposition will express as tumours at multiple sites in developmentally unrelated tissues. The most well characterised of these are the Li–Fraumeni syndrome and tuberous sclerosis, but accumulating data show that retinoblastoma also falls into this category.

(140) Li–Fraumeni syndrome (LFS), although genetically heterogenous, is, in a proportion of patients, associated with mutation to the well characterised and somatically

ubiquitous 17q13-encoded *p53* tumour suppressor gene (Malkin *et al.*, 1990; Birch *et al.*, 1994). A broad range of tumours are found in excess in LFS kindred. Neoplastic expression in LFS is, however, particularly evident as soft tissue and bone sarcomas, breast, brain, and adrenocortical carcinomas, together with lymphocytic and myeloid leukaemia. Cancer risk in LFS is high: ~50% by age 30 and ~90% by age 70 but, fortunately, it is a rare condition with an incidence that appears to be less than 1 in 50,000. The functional aspects of *p53* mutation relevant to carcinogenesis have already been discussed (Section 3.2.2 and 3.2.3) but the other genetic determinants of LFS remain obscure. Tumorigenic radiosensitivity in *p53*-deficient genotypes is discussed in Section 5.

(141) Tuberous sclerosis (TSC) is a genetic condition characterised by neoplastic lesions, termed hamartomas, principally of skin, nervous tissue, heart, and kidney, e.g. astrocytomas, renal angiomyolipomas, and cardiac rhabdomyomas (Gomez, 1991). In some respects TCS resembles NF1, but tends to be somewhat less tissue specific. The prevalence of the disease is estimated to be around 1 in 10,000 with a high proportion of cases representing de novo mutation. Heterogeneity in TCS is evident, not only in terms of neoplastic development but also in respect of neurological manifestations (Gomez, 1991). Genetic heterogeneity in TSC has also been revealed by the identification of two principal unlinked TSC loci, encoded on chromosome 9q34 (*TSC1*) and 16p13 (*TSC2*). Loss of heterozygosity for the 16p13 locus in the tumours of *TSC2* patients is strongly suggestive of a tumour suppressor role (Green *et al.*, 1994) but the specific function of the gene remains to be resolved. Radiation response in *Tsc2*-deficient rats is discussed in Section 5.

(142) Retinoblastoma is an ocular embryonic tumour of children, around 40% of which is bilateral or multifocal and represents the heritable form of the disease (Knudson, 1971). The incidence of heritable retinoblastoma (RB) is estimated to be 1 in 25,000 to 1 in 50,000 and only a minority of RB patients fail to develop retinal neoplasia.

(143) Increasing survival rates in RB patients have allowed long-term follow-up studies to be conducted and these show that at later ages the increased mortality rate in RB is associated with excess bone and soft tissue sarcomas, tumours of the brain and meninges and melanoma. Benign tumours are also seen in excess in these patients (see Eng *et al.*, 1993). In addition there is also information, albeit limited, in respect of radiotherapy related second tumours in RB; these will be discussed in Section 5 of the report. Disease manifestation in RB and the genetics of the disorder provided the basis of the two-hit hypothesis of Knudson (Knudson, 1971; see also Section 5). Predictably, the responsible gene, *RB1*, was the first tumour suppressor gene to be isolated and characterised.

(144) An important message that emerges from RB studies is that while cancer predisposition associated with a given germ line lesion might at first sight be regarded as having a high degree of tissue specificity, intensive study and long-term follow-up of patients may often reveal more widespread and age-related effects.

(145) Finally, a large case control study coupled with meta-analysis of 22 smaller investigations has provided some evidence that there is an association of uncommon germ line *HRAS1* minisatellite alleles with cancer at multiple sites (Krontiris *et al.*, 1993). In the Caucasian population 94% carry combinations of four common alleles while the remainder (6%) have rare derivative alleles. The data presented suggest that the relative risk of cancer in carriers of one uncommon allele was 1.85 while that for homozygotes for these alleles was 4.62. Although these relative risks are modest and would not reveal as familial cancer, from the prevalence of uncommon alleles it was predicted that perhaps 1 in 11 cancers of the breast, colon, and bladder might be attributable to *HRAS1* allelic variation. Krontiris *et al.* (1993) discuss a possible mechanism of transcriptional

dysregulation involving the *HRAS1* minisatellite which would imply that the sequence might act as a low penetrance modifier of risk. Although some support for this notion has come from studies of *HRAS1* alleles and ovarian cancer risk in *BRCA1* carriers (Phelan *et al.*, 1996) the true impact regarding cancer in the whole population remains somewhat controversial.

3.5. The origin of mutations in tumour-associated genes

(146) There is a rapidly expanding literature on the frequency of somatic and/or germline mutations in sporadic and/or familial cancers. Analyses of somatic mutations have revealed that predicted environmental exposures result in the corresponding types of mutation, underscoring the criticality of environmental influences. Furthermore, parental origin of the mutations in certain malignancies is suggestive of epigenetic effects due to some form of genomic imprinting. Finally, mutational mosaicism may play an important role in the development of certain malignancies. These issues are briefly discussed below.

3.5.1. *Germline mutations of tumour-suppressor genes*

(147) Table 3.5 illustrates representative mutations of a number of germ line tumour suppressor genes. Except for the *p53* and *RET* genes, frameshift mutations by deletion or insertion, or nonsense mutations resulting in premature termination of translation, predominate. In contrast, mutations in *p53* and *RET* genes are mainly of the missense type, resulting in the substitution of amino acids. These differences in the mutation spectra probably reflect phenotypic selection of those mutations that provide for gene-specific proliferative advantage in respect of neoplastic development and/or those that are most compatible with cell viability and embryonic/neonatal development.

(148) In the germinal mutations of the base substitution type, G:C → A:T transition predominates. Moreover, more than 50% of G:C → A:T transitions are found at CpG sites, indicating spontaneous deamination of 5-methyl cytosine residues (^{5mt}C), resulting in

Table 3.5. Germline mutations in some tumour-suppressor genes

		Base substitution							
		G:C→			A:T→				
Tumour suppressor genes	Total	A:T	T:A	C:G	T:4	G:C	C:G	Del or Ins	CpG→ TpG, CpA No. (%)
p53 (Li–Fraumeni syndrome)	86	55	5	1	4	7	2	12	44(80.0)
RB1 (retinoblastoma)	150	73	9	7	4	6	5	46	53(72.6)
APC (familial adenomatous polyposis	92	29	7	3	4	1	3	45	12(41.4)
NF1 (neurofibromatosis type I)	6	1	0	0	0	1	0	4	–
NF2 (neurofibromatosis type II)	11	6	0	0	0	2	0	3	6(100)
WT1 (Denys–Drash syndrome)	25	21	0	0	0	1	0	3	14(66.7)
RET (MEN2A, MEN2B)	34	4	2	5	1	19	3	0	0
VHL (von Hippel–Lindau disease)	109	30	8	5	2	15	9	40	21(70.0)
BRCA1 (breast/ovarian carcinoma)	112	14	7	5	1	0	11	74	9(64.3)
BRCA2 (breast carcinoma)	43	1	3	1	1	1	2	33	–
PTC (basal cell neuvus syndrome)	8	2	0	0	0	0	0	6	–
$p16^{INK4A}$ (familial melanoma)	38	10	6	6	4	7	0	5	6(60.0)
MSH2/*MLH1*/*PMS* (HNPCC)	45	14	0	3	1	3	1	23	6(42.9)
Total	759	260	47	36	22	63	36	295	171(67.9)

The C to T transition at CpG site is shown by percentage of all G:C → A:T transitions.

thymine. This is surprising given that less than 1% of the bases of human DNA are ^{5mt}C (Bird, 1978), and that cells have a short patch repair process for G:T mismatches (Loeb, 1994). Only 3–5% of the cytosine residues are methylated and more than 90% of the ^{5mt}C are found in the ^{5mt}CpG dinucleotides. This implies a high spontaneous mutation rate at ^{5mt}CpG sites resulting from cytosine deamination.

(149) The frequency of loss-of-function mutations by deletions and insertions is relatively high compared to that seen in somatic mutations. Microdeletions of one or a few base pairs predominantly occur in runs of single base-pair repeats. Most of the deletions of longer stretches of base pairs are characterised by the presence of direct short repeats at the junctions (Canning and Dryja, 1989), and slippage or misalignment during DNA replication is a likely mechanism for their generation. These data imply that the majority of germ line mutations in tumour-associated genes arise via endogenous metabolic processes.

3.5.2. *Preferential involvement of parental alleles in tumour gene mutations*

(150) There is a growing list of the parental origin for human de novo mutations including chromosome structural rearrangements (Olson and Magenis, 1988). These are summarised in Table 3.6. As seen in the table, a strong bias exists towards a paternal origin for de novo structural rearrangements and germinal mutations, including loss-of-function mutations of tumour suppressor genes. The strong bias toward paternal origin of new germ line mutations of tumour suppressor genes might indicate differential expression of the gene of interest, depending on the parental origin of the chromosome on which it is encoded. Such bias might result from the operation of a poorly understood process termed genomic imprinting, whereby a subset of genes in the genome is subject to germ line mediated non-mutational (epigenetic) modification of expression which is dependent upon whether the genes in question are inherited from the mother or the father (e.g. Bartolomei, 1994). Tissue-specific imprinting of certain genes has been demonstrated, particularly in the mouse. DNA methylation changes resulting in the parentally dependent modification of gene transcription represents one mechanism for the imposition of these gene expression changes.

(151) In spite of early indications of parent-of-origin effects on the genesis of retinoblastoma, there is no evidence for the transcriptional imprinting of the *RB1* gene itself. In familial retinoblastoma the expressivity is not different whether the mutated *RB1* gene is transmitted from father or mother. For the *WT1* gene, maternally and paternally derived alleles are equally expressed in the foetal kidney (Little *et al.*, 1992). The parental origin of mutation in these cases has been determined by LOH, which suggests that the retained chromosome may harbour the initial germ line mutation. The preferential retention of the parental allele of interest could be due to chromosomal domain effects, e.g. a reflection of the loss of flanking chromosome regions which are transcriptionally imprinted (Blanquet *et al.*, 1991; Zhang *et al.*, 1993). Another possibility is that parent-of-origin effects are a reflection of the difference in the number of cell divisions involved in the production of germ cells between males and females (Dryja *et al.*, 1989). This hypothesis would predict that older males might have more chance to father de novo bilateral retinoblastoma patients. However, the mean paternal age at the time of birth of such bilateral retinoblastoma patients does not differ from those cases of sporadic unilateral retinoblastoma and those in the general population (Matsunaga *et al.*, 1990). The lack of paternal age effect suggests that the post-meiotic stages or the fertilised eggs

are the critical stage of mutagenesis, and differential allelic mutability by parent-of-origin effects remains as a possible explanation.

(152) The unilateral form of childhood cancer is assumed to arise from somatic mutations. There are some indications that the initial somatic mutations of certain tumour suppressor genes also preferentially occur on the paternally derived alleles in some tumours. In a study of sporadic unilateral Wilms' tumours, 11 out of 12 tumours retained the paternally derived copy of 11p13, suggesting imprinting of the paternally derived copy of the *WT1* gene. A similar situation also holds for rhabdomyosarcoma, and the *RB1* gene in sporadic osteosarcoma (see Feinberg, 1993).

(153) Observations on the involvement of the *RB1* parent of origin effects in sporadic unilateral retinoblastoma are controversial. Leach *et al.* (1990) showed a preferential involvement of the paternally derived *RB1* allele while others showed more equal involvement of paternal and maternal alleles in the initial loss-of-function mutations. However, these observations are based on LOH of chromosomal region 13q14. In the somatic mutation cases, the initial mutation can be large deletions, which by themselves give rise to LOH. Thus, the retained copy of *RB1* does not necessarily represent the chromosome segment which harbours the initial mutation. The somatic role of methylation-dependent effects in carcinogenesis has been discussed recently by Little and Wainwright (1995).

3.5.3. *Evidence for mutational mosaicism*

(154) Mutational mosaicism is another aspect of the nature of mutations which is relevant to human health effects, including the development of neoplasia (UNSCEAR, 1993). Somatic mosaicism has long been known for chromosome mutations (Hall, 1988). Firm evidence for the existence of somatic or germinal mosaicism has recently been presented for other genetic diseases such as osteogenesis imperfecta (Byers *et al.*, 1988; Cohn *et al.*, 1990; Edwards *et al.*, 1992), Duchenne muscular dystrophy (Darras *et al.*, 1988; Bakker *et al.*, 1989), Hemophilia A (Levinson *et al.*, 1990; Brücker-Vriends *et al.*, 1990), retinoblastoma (Greger *et al.*, 1990), Wilms' tumour (Chao *et al.*, 1993), Li–Fraumeni syndrome (Kovar *et al.*, 1992), and neurofibromatosis (type I) (Lázaro *et al.*, 1994). Mutational mosaicism is not always readily recognisable and the fraction of somatic cells carrying the mutation of interest varies between tissues and individuals. Accordingly, the extent of mutational mosaicism currently observed may be a substantial underestimate of this feature, which may make a significant but ill defined contribution to cancer risk.

(155) Mosaicism most likely arises by somatic mutation during early development and subsequent bi-clonal proliferation in developing tissues. The origin of germ cell-mediated mosaicism is, however, a matter of some debate. Delayed mutation of the Auerbach type is distinct since the hypothesised premutation would be expected to be progressively modified over one or more generations rather than being diluted by mosaicism. In this context, Carlson and Desnick (1979) have proposed that some sporadic retinoblastoma may be explained by delayed mutation, i.e. fixation of *RB1* premutation in the early stage of development giving rise to hereditary bilateral tumours and fixation in a later stage resulting in unilateral tumours with varying degrees of gonadal mosaicism.

(156) Suggestive evidence for gonadal mosaicism due to fixation of premutation has been reported by Greger *et al.* (1990). Two sibs with bilateral retinoblastoma had different intragenic *RB1* deletions which shared the same breakpoint. In explanation of this, the father might have carried a premutation and the two types of germ line deletion have emerged from this event, thus resulting in gonadal mosaicisms. In retinoblastoma patients,

about one-fourth of constitutional chromosome deletions of de novo origin involving the *RB1* gene are recognised as mosaics. While these are thought to arise from somatic mutations after fertilisation, yet again the deletions occur preferentially on paternally derived chromosomes (Sasaki and Shimizu, 1994).

(157) In summary, evidence is accumulating that distinct patterns of germ line mutation may distinguish different genes associated with human predisposition to cancer. In some cases these mutational variations will influence the degree to which such genes determine cancer development in different individuals and indeed different tissues. The origin, inheritance and expression of some cancer predisposing mutations may be subject to epigenetic processes which are termed parent-of-origin effects. In addition, some cancer-predisposed individuals may be mosaics, carrying a 'patchwork' of normal and tumour gene-mutated cells in their somatic and germinal tissues.

(158) The origin, mechanisms and impact of parent-of-origin effects and mosaicism in individuals have yet to be adequately described but there is reason to believe that, in some cases, they will be significant determinants of cancer risk. These processes, together with mutational variation and environmental factors, may serve to modify the penetrance and

Table 3.6. Parental origin of de novo mutations

Mutation	Parental origin			Significance
	Total	Pat	Mat	
Germline mutation				
Chromosome mutation				
Robertsonian translocation	26	7	19	$p = 0.0186$
Non-Robertsonian rearrangement				
Euploidy				
Reciprocal translocation	11	11	0	$p = 0.0009$
X/autosome (DMD)	7	7	0	$p = 0.0025$
Deletion:				
Retinoblastoma; del(13q)	11	10	1	$p = 0.0067$
Cri du chat syndrome; del(5p)	25	20	5	$p = 0.0027$
Miller-Dieker syndrome; del(17p)	7	6	1	$p = 0.0233$
WAGR syndrome; del(11p)	9	8	1	$p = 0.0077$
Wolf–Hirshhorn syndrome; del(4p)	7	7	0	$p = 0.0025$
Prader–Willi syndrome; del(15q)	35	32	3	$p < 0.0001$
Angelman syndrome; del(15q)	17	1	16	$p = 0.0003$
Other translocation (e.g. 15q+)	3	2	1	$p = 0.2482$
Aneuploidy (e.g. +15q-)	7	0	7	$p = 0.0025$
Submicroscopic or gene mutations				
Retinoblastoma (RB1)	17	16	1	$p = 0.0003$
WAGR syndrome (WT1)	9	8	1	$p = 0.0077$
Denys–Drash syndrome (WT1)	4	4	0	$p = 0.0124$
Duchene/Becker muscular dystrophy	7	5	2	$p = 0.1306$
von Recklinghausen disease (NF1)	24	22	2	$p = 0.0001$
Multiple endocrine neoplasia type 2B (RET)	26	25	1	$p < 0.0001$
Somatic mutation (alleles retained in tumours)				
Wilms tumour (WT1)	12	11	1	$p = 0.0039$
Retinoblastoma (RB1)	27	14	13	$p = 0.8474$
Osteosarcoma (RB1)	13	12	1	$p = 0.0023$
Osteosarcoma (*p53*)	14	7	7	$p = 1$
Rhabdomyosarcoma (11p)	6	6	0	$p = 0.0023$
Meningioma (chrom 22)	21	13	8	$p = 0.2752$

expressivity of a significant proportion of germ line determinants of neoplasia. Through such complex interactions normal Mendelian patterns of inheritance may be obscured.

3.6. The genetic component of solid tumours

(159) In spite of the fact that genetic influences on the development of most types of solid tumours have been identified it remains difficult to confidently judge an overall genetic contribution. The principal source of problems in making such a judgement is that familial ascertainment of heritable cancer is limited in its power and tends only to clearly identify those kindred carrying highly penetrant germ line mutations (Sorensen, 1995; Houlston and Peto, 1996). In illustration of this it has been calculated that for a dominant cancer predisposing gene with a frequency of 0.7 in the population the relative risk of all forms of cancer in a child of a carrier cannot exceed 1.02 (Peto, 1980). In order to resolve such cancer predisposition it is necessary to consider other clinical factors such as tissue at risk, histopathological type and age of onset. Thus, while it seems that genetic effects are most obvious for neoplasms of the breast and colon, where highly penetrant and specifically expressed mutations may account for around 2–5% of population risk, a larger overall contribution to risk at all sites from a range of less penetrant or less specifically expressed mutations cannot be discounted. This difficult issue, and its implications for judgements on genetic contributions to radiation risk, are discussed later in this report. With regard to familial cancer which by definition will largely involve germ line determinants of relatively high penetrance it is judged that prevalence in the population is likely to be less than 1% (see also Section 6).

3.7. Summary

(160)

1. Mutations of proto-oncogenes, DNA repair genes and tumour suppressor genes contribute to neoplastic development. Loss of function of tumour suppressor genes through mutation or deletion is the predominant event in the genesis of solid tumours.
2. Proto-oncogene and tumour suppressor gene products are central to regulation of cell–cell communication and differentiation, signal transduction and transcription. These processes contribute significantly to cell survival, cell death and control of the cell cycle.
3. Rare familial genetic determinants of neoplasia in most human tissues have been identified and in some cases isolated and characterised. Most of these determinants are mutations to tumour suppressor genes; some involve DNA repair genes but few involve proto-oncogenes. Many such mutations are expected to impact on the regulation of proliferation and on the quality of DNA replication and repair.
4. Mutant genes predisposing to breast and colon cancer have received greatest attention because they appear to be more common than other categories.
5. There is much information accumulating on the specific nature of germ line DNA sequence mutations in cancer predisposing genes; most are DNA base pair changes or intragenic deletions.
6. Predisposing germ line mutations expressing as familial cancer are judged to contribute no more than 5% of all solid cancers. There is insufficient evidence to judge the contribution from mutations of lower penetrance that do not express as familial cancer.
7. Poorly understood epigenetic processes can impact on the expression of tumour-associated mutations; there is also evidence that mosaicism for cancer-predisposing mutations can occur. The true extent of the influence of these processes on cancer in the population cannot however be judged.

4. MECHANISMS AND GENETICS OF LYMPHO-HAEMOPOIETIC NEOPLASIA

(161) From aetiological and clinical viewpoints, neoplasia of the lympho-haemopoietic system differs substantially from that of other organs of the body. For these reasons, the leukaemias and lymphomas that comprise this category of neoplasms have been traditionally regarded in clinical, epidemiological and laboratory studies as a particularly distinct group of malignancies.

(162) In this section of the report, the cellular and molecular mechanisms underlying the development of lympho-haemopoietic neoplasia are outlined following which the data relating to genetic susceptibility to these malignancies is addressed. In these respects, points of contrast are made between the mechanisms and genetics of lympho-haemopoietic neoplasia and those of solid tumours.

(163) In the concluding paragraphs, a general view of the likely extent of the genetic component of human lympho-haemopoietic neoplasia is provided in order to establish a basis for judgements on the contribution of genetic factors to the risk of leukaemia and lymphoma in irradiated human populations to be provided later in the document.

4.1. Mechanisms of induction of lympho-haemopoietic neoplasia

(164) A broad range of lympho-haemopoietic neoplasms (leukaemias and lymphomas) have been identified in man and experimental animals. These arise in blood forming and lymphatic tissues and have been the subject of intensive study particularly with regard to the involvement of specific chromosomal events in different neoplastic sub-types. The following paragraphs outline the structure and function of the lympho-haemopoietic system and summarise information available on cytogenetic and molecular mechanisms that underlie the development of neoplasia.

4.1.1. *Structure and function of the lympho-haemopoietic system*

(165) The lympho-haemopoietic (blood-forming) system is composed of a complex arrangement of cell lineage hierarchies with multiple options for cellular differentiation and clonal amplification in order to generate mature haemopoietic effector cells (Fig. 4.1). Thus the pluripotent stem cell compartment in bone marrow is able to supply upon demand an increased transit population of lineage-restricted precursor cells of the myeloid and lymphoid types. These precursors subsequently amplify through a lineage committed compartment and, on subsequent entry into a maturation compartment, convert to the mature effector cells of the blood and lymphatic system. This complex population of mature cells essentially comprises granulocytes and monocytes/macrophages which act to scavenge microbes, parasites and endogenous cellular debris; erythrocytes which transport oxygen; platelets for wound repair; plasma (B) cells for antibody secretion; suppressor (T) cells for cellular toxicity and helper (T) cells for immune regulation (Greaves, 1991; Henderson *et al.*, 1996).

(166) The generation of these mature cell lineages and the self-renewal of the pluripotent stem cells is subject to compartmentalised control, not only between bone marrow, lymph nodes, thymus, and spleen but also within these tissues. For example, stem cells are largely restricted to bone marrow where they are organised within a three-dimensional stromal matrix, while within lymph nodes and spleen there is some degree of topological constraint on T and B cell populations (Greaves, 1991; Henderson *et al.*, 1996).

(167) The control of lympho-haemopoietic cell proliferation and differentiation is a highly complex process (Metcalf, 1988; Robinson and Queensberry, 1990; Metcalf, 1991;

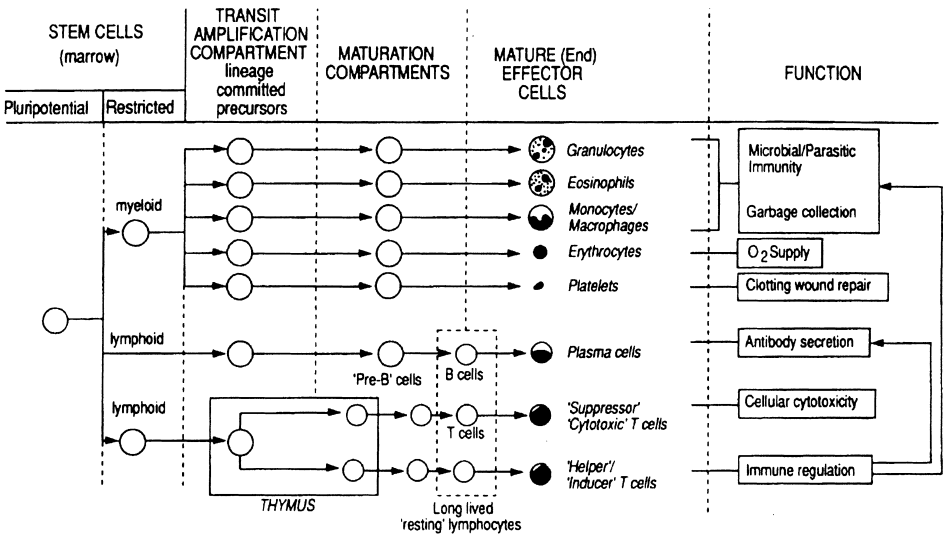

Fig. 4.1. Haemopoietic cell lineages and their functions (from Greaves, 1991).

Ness and Engel, 1994; Orkin, 1996), the detail of which is outside the scope of this review. In brief, in association with topological constraints the various cellular compartments of the system are subject to both long- and short-range control mediated by growth and maturation factors. In most instances these factors are specific to the lympho-haemopoietic system and their action is often mediated by or coupled to the interaction of the stem and committed progenitors with cells that make up the stromal elements. Such interactions would include those mediated by some of the cytokine proteins of the interleukin (IL) family, granulocyte–macrophage colony stimulation factor (GM-CSF), granulocyte colony stimulating factor (G-CSF), macrophage colony stimulating factor (M-CSF) and stem cell factor (SCF). Acting in concert and in association with appropriate membrane-bound receptors and intracellular transcription factors, these cytokines provide the fine control for lympho-haemopoietic development, maintenance, and function.

(168) Other long-range controlling factors circulate more freely in blood and lymphatic systems and either target cells directly or express their effects via the stromal elements. Such long-range factors include epidermal growth factor (EGF), platelet derived growth factor (PDGF), insulin, and various steroids.

(169) The control of lympho-haemopoietic development and maintenance should not however be viewed simply as a matter of regulating cellular proliferation and functional differentiation. The overall size of cell compartments and the individual clonal contributions within these are also regulated by protein factors interacting with other cytokines that either stimulate or block an active process of programmed cell death termed apoptosis (Cohen, 1991; Raff, 1992; Korsmeyer, 1992). Apoptosis is a crucial element in the control of normal lympho-haemopoiesis and may be viewed as a most important mechanism for the clonal restriction or elimination of degenerate or aberrant cells.

(170) In recent years much has been learned of the genetics and biochemistry of lympho-haemopoietic regulation, largely as a result of the molecular cloning of the genes involved and the in vitro analysis of their multifaceted functions (Metcalf, 1991; Metcalf, 1993; Ness and Engel, 1994; Orkin, 1996). In the main, differentiation of cell lineages is

dependent upon sequential activation or suppression of specific genes, a process that is mediated by an array of transcription regulators. Maturing T- and B-cell subtypes are however subject to what is currently understood to be a unique process of clonal DNA sequence-specific rearrangement in order to specify novel proteins that subsequently mediate immune reactions against non-self protein antigens (Tonegawa, 1983). In brief, T-cell antigen receptor (*TCR*) and B-cell immunoglobulin (*Ig*) genes in appropriate precursor cells are naturally rearranged at specific recombinogenic sites in these genes. These controlled rearrangements occur between specific gene segments and are catalysed by a complex system of recombination and hypermutation enzymes (Lieber, 1993; Willerford et al, 1996). The net outcome of these processes is the potential for clonal expression in lymphocyte subsets of a vast array of antigen-specific proteins which, in T-cells, function as membrane-bound recognition molecules for cell-mediated immune response and, in B-cells, as secreted antibodies for humoral immunity.

(171) In conclusion, although the cellular and molecular biology of lympho-haemopoietic development is probably better understood than that of any other mammalian tissue, much remains to be learned. Nevertheless, as will be seen, the current understanding is sufficiently good to allow specific comment on the role of specific genes in neoplastic processes.

4.1.2. *Lympho-haemopoietic neoplasia*

(172) Neoplasms of the lympho-haemopoietic system may be broadly divided into those of the myeloid and lymphoid cell lineages. These two major categories may be further subdivided into sub-types on the basis of clinical, haematological, and histopathological criteria and includes pre-leukaemic, chronic, and acute manifestations of neoplasia (Greaves, 1991, 1993; Henderson *et al*., 1996).

(173) Lympho-haemopoietic neoplasia accounts for around 5% of all cancer in Western populations but there are increases in this contribution that depend upon age, ethnicity, and demography (Magrath *et al*., 1984). Of particular note is the relatively high contribution (˜30%) that leukaemia and lymphoma makes to neoplasia at ages below 15 years (Stiller et al, 1991). Viruses, chemical carcinogens, and ionising radiation have been implicated in the appearance of lympho-haemopoietic neoplasia in a number of human groups and, in the context of this report, a most important data base is that concerning the excess neoplastic disease in irradiated human populations. These data are discussed in detail elsewhere (UNSCEAR, 1994).

(174) Lympho-haemopoietic neoplasia presents as the clonal dominance of aberrant cells expressing myeloid or lymphoid characteristics. Leukaemia is characterised by excess of white cells in blood which, in the acute form, may be accompanied by disturbances in bone marrow function, e.g. anaemia and infection; indolent (chronic and preleukaemic) forms have also been characterised. Lymphomas most usually present as the accumulation of neoplastic cells in lymphatic tissues; many of these conditions have an indolent phase. Indolent forms of lympho-haemopoietic neoplasia may, with time, convert to an acute form through the evolution of a more aggressive cellular phenotype and such transitions provide clinical evidence of the multi-stage nature of disease (Greaves, 1991; Henderson *et al*., 1996).

(175) Cytogenetic, molecular, and biochemical studies have been used to explore the cellular origin of leukaemia/lymphoma and with the possible exception of disease in some bone marrow transplant and acquired immunodeficiency disease (AIDS) patients, these

studies provide compelling evidence of the monoclonal (single cell) origin of neoplasia (Woodruff, 1988; Lieber, 1993; Greaves, 1993).

(176) There is also evidence that the target cell population for the vast majority of leukaemias and lymphomas is one that is relatively undifferentiated and that the subsequent maturation of potentially neoplastic cells is not synchronous with that of the respective normal cell lineage. Consistent with this, Greaves (1986, 1991, 1993) has described a model of leukaemogenesis where the critical cellular event results in changes to the differentiation programme such that the probability of self-renewal of leukaemia-initiated cells exceeds that of maturation and clonal extinction. In this way a clone of relatively immature cells that would normally have only a transitory presence in a given maturation compartment (see Fig. 4.1) may accumulate. According to the model, the degree of uncoupling of proliferation and maturation provides for the phenotypic heterogeneity that characterises many lympho-haemopoietic neoplasms. Thus, the monoclonal expansion of pre-leukaemic cells predicted by the model can account for indolent forms of disease and also allows for the accumulation of further mutational events within the maturation-arrested clone. These mutations will be subject to phenotypic selection and will result finally in the blast crisis that is the hallmark of the acute phase of disease.

(177) In general, latency periods for the development of haemopoietic neoplasia tend to be shorter than for solid tumours (UNSCEAR, 1994) illustrating the natural age-related process of oncogenesis and implying, perhaps, a requirement for rather fewer genomic changes for the development of malignancy. While still somewhat uncertain, this contention receives some support from the cytogenetic and molecular studies on leukaemia and lymphoma outlined in this section.

4.1.3. *Cytogenetic and molecular mechanisms of lympho-haemopoietic neoplasia*

(178) The most consistent feature of human leukaemias and lymphomas is the presence of primary, non-random clonal chromosomal rearrangements that are characteristic of leukaemia sub-types (Mitelman, 1991; Sandberg, 1993). In some cases a single chromosomal event is present within an essentially diploid chromosome complement, in others the karyotype is more complex with multiple changes, some of which cannot be cytogenetically defined. Although there is some knowledge of the principal chromosome specific translocations in leukaemia and lymphomas many such events remain obscure and there is also uncertainty on the chromosome deletions, numerical chromosomal changes, DNA amplifications, point mutations and genomic instabilities that characterise many of these neoplasms. These data have been extensively reviewed (Mitelman, 1991; Sawyers *et al.*, 1991; Sandberg, 1993; Cline, 1994; Young, 1994; Rabbitts, 1994) and here we seek only to summarise the current position.

Chromosome translocations

(179) Around 50 gene-specific chromosome translocations have been characterised at the molecular level in human lympho-haemopoietic neoplasms; these fall into two broad categories.

(180) *Gene deregulation through translocation*. Table 4.1 and 4.2 summarise the translocations that result in the deregulation of genes, the outcome of which is to change the proliferation and/or maturation of haemopoietic precursors. All of these events may be regarded as gain-of-function mutations and as such the target genes for deregulation can be classified as proto-oncogenes (Cline, 1994; Young, 1994; Rabbitts, 1994). Two sub-

Table 4.1. Proto-oncogenes juxtaposed to T-cell receptor genes in lympho-haemopoietic neoplasia

Disease	Translocation	Gene juxtaposition	Protein type
T-ALL	t(1;7)(p32;q34)	*TCRβ-TCL5*	TCL5 (TAL1/SCL), HLH domain
T-ALL	t(1;14)(p32;q11)	*TCRδ-TCL5*	TCL5 (TAL1/SCL), HLH domain
T-ALL	t(1;7)(p34;q34)	*TCRβ-LCK*	LCK, tyrosine kinase
T-ALL	t(7;9)(q34;q32)	*TCRβ-TAL2*	TAL2, HLH domain
T-ALL	t(7;9)(q34;q34)	*TCRβ-TAN1*	TAN1 (TCL3), *Notch* homologue
T-ALL	t(7;10)(q34;q24)	*TCRβ-HOX11*	HOX11 (TCL3), homeodomain
T-ALL	t(10;14)(q24;q11)	*TCRδ-HOX11*	HOX11 (TCL3), homeodomain
T-ALL	t(7;19)(q34;p13)	*TCRβ-LYL1*	LYL1, HLH domain
T-ALL	t(8;14)(q24;q11)	*TCRδ-MYC*	MYC, HLH domain
T-ALL	t(11;14)(p15;q11)	*TCRδ-RBNT1*	RBNT1 (Ttg1), LIM domain
T-ALL	t(11;14)(p13;q11)	*TCRδ-RBNT2*	RBNT2 (Ttg2), LIM domain
T-ALL	t(7;11)(q34;p13)	*TCRβ-RBNT2*	RBNT2 (Ttg2), LIM domain
T-ALL	t(14;14)(q11;q32)	*TCRα-TCL1*	TCL1, ?
PLL	t(14;x)(q11;q28)	*TCRα-C6.1B*	C6.1B, ?

T-ALL, T-cell acute lymphocytic leukaemia; PLL, prolymphocytic leukaemia; HLH, helix–loop–helix; LIM, cysteine rich.

classes of these events may be further defined on the basis of the cell-of-origin for proto-oncogene deregulation and the partner DNA sequence that acts to effect this.

(181) In T-cell neoplasia, gene deregulating translocation has to date been shown to depend upon the juxtaposition of enhancer or controlling sequences of the lineage-specific *TCR* genes with certain proto-oncogenes. *TCR*α,β and γ sequences are known to mediate such events and the known proto-oncogene targets are the genes *TCL1*, *TCL5*, *LCK*, *TAL2*, *TAN1*, *HOX11*, *LYL1 MYC*, *RBNT1*, and *RBNT2* (Table 4.1).

(182) In B-cell neoplasia gene deregulating translocation depends upon similar gene juxtaposition but, in these cases, involving controlling elements of different *Ig* genes, i.e *IgH*, *IgK*, and *Igl*. The known target proto-oncogenes for these B-cell translocations are *MYC*, *REL*, *IL3*, *BCL1*, *BCL2*, *BCL3*, and *BCL6* and, as in the case of T-cell neoplasms, there is some degree of specificity in terms of both gene partnership and neoplastic subtype (Table 4.2).

(183) It is important to recognise that proto-oncogene translocations in T- and B-cell neoplasms are not the result of completely random DNA exchange. As noted earlier, *TCR* and *Ig* sequences are uniquely subject to lineage-dependent recombination, a process that

Table 4.2. Proto-oncogenes juxtaposed to immunoglobulin loci in lympho-haemopoietic neoplasia

Disease	Translocation	Gene juxtaposition	Protein type
BL/B-ALL (L3)	t(8;14)(q24;q32)	*IgH-MYC*	MYC, HLH domain
BL/B-ALL (L3)	t(2;8)(p11;q24)	*Igk-MYC*	MYC, HLH domain
BL/B-ALL (L3)	t(8;22)(q24;q11)	*Igl-MYC*	MYC, HLH domain
B-CLL	t(2;14)(p13;q32)	*IgH-REL*	REL, NF-kB family
Pre-B ALL	t(5;14)(q31;q32)	*IgH-IL-3*	IL-3, Growth factor
B-CLL	t(11;14)(q13;q32)	*IgH-BCL1*	BCL1 (PRAD1), G1 cyclin
FL/B-CLL	t(14;18)(q32;q21)	*IgH-BCL2*	BCL2, inner mitochondrial membrane
B-CLL/NHL	t(2;18)(p12;q21)	*Igk-BCL2*	BCL2, inner mitochondrial membrane
B-CLL/NHL	t(18;22)(q21;q11)	*Igl-BCL2*	BCL2, inner mitochondrial membrane
B-CLL	t(14;19)(q32;q13)	*IgH-BCL3*	BCL3, IkB family LIM domain
NHL	t(3;14)(q27;q32)	*IgH-BCL6*	BCL6, zinc finger
BLym	t(10;14)(q24;q32)	*IgH-lyt10*	lyt10, ?

BL, Burkitts lymphoma; B-ALL, B cell acute lymphatic leukaemia; B-CLL, B cell chronic lymphatic leukaemia; FL, follicular lymphoma; Blym, B-cell lymphoma NHL, non Hodgkins lymphoma; HLD, helix-loop-helix; LIM, cysteine-rich.

is mediated by DNA processing machinery which recognises specific DNA motifs in the normal immune sequences (Lieber, 1993). Molecular studies have provided compelling evidence that it is loss of fidelity of this recombination system that drives *TCR*/*Ig*-proto-oncogene translocations by aberrantly catalysing inter-locus recombination between target sequences. Coupled to this, target sequences may also be subject to DNA sequence additions, losses, and duplications. This loss of fidelity of DNA processing implies a close relationship between leukaemogenesis and DNA damage repair/recombinational processes; this issue is discussed further in Section 2 of the present report.

(184) It may be seen, therefore, that a critical event in T- and B-cell neoplasia is the expression of lineage-directed genes that are placed inappropriately under the control of active *TCR* and *Ig* sequences that are specifically expressed in T- and B-cell precursors respectively. Although the specific functions of the active oncogenes created by these translocations may differ, they tend to share the general property of having the potential to interfere with the normal patterns of gene transcription that mediate cellular differentiation. In illustration of this *MYC*, *TCL5*, *LYL1*, and *HOX11* have either been directly implicated as nuclear transcription factors or have features of DNA sequence that are strongly suggestive of this, i.e helix–loop–helix, homeobox or LIM domains (Young, 1994; Rabbitts, 1994). Thus, inappropriate transactivation of the genes targeted by these transcription factors may be viewed as a means of escape from normal cellular constraints on growth and development.

(185) However, genes with somewhat different functions may also be subject to this class of leukaemogenic event. The data of Table 14 show that *BCL1*, a cell cycle control gene; *IL3*, a growth factor gene and *BCL2*, a gene specifying mitochondrial protein may be activated in some B-cell neoplasms. In the case of *BCL2* the outcome of such activation is

Table 4.3. Gene fusions in lympho-haemopoietic neoplasia

Disease	Translocation	Gene fusion	Protein type
Pre-B ALL	t(1;19)(q23;p13)	*E2A-PBX*	PBX, Homeodomain E2A, HLH domain
AML (M2/4)	t(6;9)(p23;q34)	*DEK-CAN*	CAN, cytoplasmic DEK, nuclear
AML (M2/4))	t(9;9)(q34;q34)	*SET-CAN*	CAN, cytoplasmic SET, ?
AML (M2)	t(8;21)(q22;q22)	*AML1-ETO*	ETO, G1 cyclin AMLI, Runt homolog
CML/B-ALL	t(9;22)(q34;q11)	*BCR-ABL*	ABL, tyrosine kinase BCR, GAP for $p21^{ras}$
APML (M3)	t(15;17)(q22;q11)	*PML-RARA*	PML, zinc finger RARA, zinc finger
APML (M3)	t(11;17)(q23;q21)	*PLZF-RARA*	PLZF, zinc finger RARA, zinc finger
Pre-B ALL	t(17;19)(q22;p13)	*E2A-HLF*	E2A, HLH domain HLF, leucine zipper
ALL	t(4;11)(q21;q23)	*HTRX-AF4*	HTRX, zinc finger AF-4, ?
ALL	t(11;19)(q32;p13)	*HTRX-ENL*	HTRX, zinc finger ENL, ?
NHL	t(2;5)(p23;q35)	*ALK-NPM*	ALK, kinase NPM, nucleophosmin
T or BLym	inv 14(q11;q32)	*TCRα-IgH*	TCR, α, receptor IgH, immunoglobulin
ALL/ANLL	t(9;11)(q21;q23)	*AF9-MLL1*	AF9, Ser-Pro rich *MLL1*, zinc finger
T or B-ALL/ANLL	t(11;19)(q23;p13)	*MLL1-ENL*	MLL1, zinc finger ENL, Ser-Pro rich
T-ALL	t(X;11)(q13;q23)	*AFX1-MLL1*	AFX1, Ser-Pro rich MLL1, zinc finger
ALL	t(1;11)(p32;q23)	*AF1P-MLL1*	AF1P, Eps-15 homologue MLL1, zinc finger
ALL	t(6;11)(q27;q23)	*AF6-MLL1*	AF6, myasin homologue MLL1, zinc finger
AML	t(11;17)(q23;q21)	*MLL1-AF17*	MLL1, zinc finger AF17, leucine zipper
CML	t(3;21)(q26;q22)	*EVI1-AML1*	EVI1, zinc finger AML1, DNA binding
MDS	t(3;21)(q26;q22)	*EAP-AML1*	EAP, Sn protein AML1, DNA binding
TLym	t(4;16)(q26;p13)	*IL2-BCM*	IL2, growth factor BCM, ?
CML	t(5;12)(q33;p13)	*PDGFβ-TEL*	PDGFβ, growth factor TEL, DNA binding

ANLL, acute non-lymphocytic leukaemia; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; APML, acute promyelocytic leukaemia; TLym, T-cell lymphoma; BLym, B-cell lymphoma.

to block a normal apoptotic mechanism mediated by oxidative metabolism, thereby facilitating clonal expansion (Rabbitts, 1994).

(186) *Gene fusion through translocation*. Whereas gene deregulation through *TCR* or *Ig* sequence translocation is restricted to T- and B-cells respectively, a gene fusion mechanism appears to have the potential to drive leukaemogenesis in both lymphoid and myeloid lineages (Cline, 1994; Young, 1994; Rabbitts, 1994).

(187) Gene fusion can be initiated when chromosome breakage occurs within the introns of two physically unlinked target genes. If reunion of these breaks occurs (i.e a translocation) such that the flanking exons of the two genes are in phase then a correctly spliced hybrid mRNA can be transcribed from the novel fusion gene. The protein synthesised from this message is a chimera of the two normal proteins and would be expected to have a mixed but defective function. In any such reciprocal translocation there is the expectation of two such fusion products. Thus, a carrier cell would be expected to express these together with the normal gene products from the two remaining normal (unfused) gene copies of the fusion partners.

(188) Table 4.3 summarises the chromosome translocations currently known to be associated with specific gene fusions. It is notable that, with the sole exception of *TCRα-IgH* fusion in chromosome 14 inversion, the genes involved all differ from those seen in gene-deregulating translocations of T- and B-cells (Tables 4.1 and 4.2).

(189) On the basis of current knowledge it appears that there is a high degree of specificity in the majority of the gene partnerships in fusion events and also neoplastic sub-type specificity in respect to the genes involved. This is certainly the case for the *BCR-ABL* fusion for which there is only one known substitute *ABL*-related kinase gene (Cline, 1994; Young, 1994; Rabbitts, 1994). The major exception to this is the *MLL*1 gene encoded in the 11q23 region of the genome which is capable of production fusion with at least six different partners and, albeit in different combinations, contributes to neoplasia in T-cell, B-cell and myeloid lineages. It seems likely therefore that whereas most fusion oncoproteins principally target lineage-restricted haemopoietic progenitors, *MLL*1 fusion productions can exert phenotypic effects in pluripotent or early multipotential cells (Rabbitts, 1994).

(190) *MLL1* involvement in haemopoietic neoplasia also shows strong age-dependence. Translocations involving 11q23 are by far the most common events in childhood acute leukaemia and *MLL1* fusion could underly up to 70% of all cases of infant AML and ALL (Thirman *et al*., 1993). Relevant to this are studies on the leukaemias of identical twins noted later in this section (4.2.2); these show that *MLL1* translocation may be favoured during in utero development of the haemopoietic system when, perhaps, pluripotent stem cell activity is maximal.

(191) In the absence of a requirement for the recombinogenic motifs seen in T- and B-cell neoplasms the greatest constraint on the formation of productive gene fusions is the need to encompass the critical domains from each of the partner genes. This is certainly evidenced in the close breakpoint clustering within the *HTRX* gene. In the case of *BCR-ABL* there is, however, considerable variation in *BCR* breakpoints and, although somewhat contentious, some indications that this may have phenotypic consequences (Cline, 1994; Young, 1994).

(192) The cellular dysfunctions induced by the synthesis of leukaemia-associated fusion proteins remain uncertain and it is not clear in all cases which of the two potential chimeric products has the dominant effect. Evidence is however emerging that these proteins may be involved in the nuclear signalling pathways that regulate the

differentiation and survival of haemopoietic cell lineages. The normal genes of many of the fusion partners encode helix–loop–helix and zinc-finger protein domains associated with transcriptional control (Table 4.3); in a chimeric form such domains may be expected to disturb the normal patterns of gene transcription (Cline, 1994; Young, 1994; Rabbitts, 1994).

(193) In support of this the *PML-RARA* fusion protein has been shown to interact in a complex fashion with the normal partner protein products and, through this, is able to block myeloid differentiation (Warrell *et al*., 1993). Complex biochemical interactions also characterise the *E2A-PBX* fusion protein which is seemingly able to dysregulate the proliferation and survival of lymphoid cells (Young, 1994; Rabbitts, 1994). Evidence suggests that the expression of the *BCR-ABL* fusion protein plays a role in the inhibition of apoptosis.

(194) Two possible mechanisms of transcriptional dysregulation by fusion oncoproteins may be suggested. First, the chimeric fusion product may have enhanced activity or altered specificity in respect of transactivation of genes; there is some evidence in support of this for both BCR-ABL and PML-RARA fusion proteins (Rabbitts, 1994). Second, by interference with critical protein–protein interactions, fusion products could interfere with normal transcription in a dominant negative fashion that parallels that of mutant *p53* (Lanfrancone *et al*., 1994); the pattern of *MLL1* gene truncation as a consequence of translocation (Rabbitts, 1994) could in principle provide for this.

(195) While there remain major uncertainties on the functional aspects of fusion oncoproteins in haemopoietic neoplasia it is becoming increasingly clear that overall they play an important role in the development of these diseases. Such translocations have also been identified in certain solid tumours (Rabbitts, 1994) and a recently published catalogue of chromosomal events in human neoplasia provides further examples of specific translocations in both lympho-haemopoietic and solid cancers (see Mitelman *et al*, 1997).

Chromosome deletions and numerical changes

(196) A variety of non-random chromosomal deletions and numerical changes have been observed in haemopoietic neoplasias but in the main these have not been resolved at the molecular level (Mitelman, 1991; Sandberg, 1993; Johansson *et al*., 1993; Dohner *et al*., 1993; Young, 1994; Mitelman *et al*., 1997). Of particular note however are the deletions and losses of chromosomes 5 and 7 in acute myeloid leukaemias (AML) and related myelodysplastic syndromes (MDS) (Van den Berghe *et al*., 1985); in some cases these neoplasms have been associated with occupational or medical exposure to DNA damaging agents including ionising radiation. Such loss events may signal the presence of tumour suppressor genes in critical deleted chromosome segments and in pursuit of this much attention has been focused on the 5q deletions in AML and MDS.

(197) Extensive cytogenetic analysis of these neoplasms has identified the 5q31 segment as the critical region for gene loss (LeBeau *et al*., 1993; Willman *et al*., 1993). Molecular analysis of this region has recently revealed the presence of two candidate tumour suppressor genes, *EGR1* and *IRF1*. Both these genes have been shown to be lost in many of the 5q neoplasms so far investigated, and either could be the critical gene. It remains however possible that there is a cluster of suppressor-type genes in this chromosome region and that other candidates may come to light.

(198) Attention has also been given to 13q14 deletions and translocations in B-cell chronic lymphocytic leukaemia and molecular studies have recently identified a putative suppressor locus, *DBM*, which is located at least 530kb telomeric to *RB1* (Brown *et al*.,

1993). Chromosome 11q23 deletions have also been observed in haemopoietic neoplasia (Rabbitts, 1994); these are probably associated with *MLL1* gene effects noted earlier.

(199) Many more DNA deletions have however been characterised at the cytogenetic level. These data which have been summarised and analysed by Mitelman *et al.* (1997) give the impression that DNA loss events are more frequent in neoplasms of myeloid origin than in other lympho-haemopoietic subtypes. Non-random chromosome deletions characterise certain haemopoietic neoplasms of experimental animals and in the case of radiation-induced murine AML there is some information on the molecular mechanisms involved (see Section 4.1.3.4).

(200) Overall, in spite of the numerous examples of non-random chromosome deletion events in human haemopoietic neoplasms, it is only recently that significant progress has been made in the elucidation of the tumour-suppressor-type gene losses that are predicted to be involved. This is an area where much remains to be learned of the leukaemogenic process.

Other proto-oncogene and tumour suppressor mutations

(201) In addition to the many disease-specific chromosomal mutations identified in haemopoietic neoplasms there is also some information on the involvement of proto-oncogene and tumour suppressor gene mutations. In contrast to chromosomal events it is evident that these gene mutations do not arise in a strict cell lineage dependent manner (Cline, 1994; Young, 1994).

(202) In brief *RAS* proto-oncogene mutation is common in chronic myelomonocytic leukaemia and acute myeloid leukaemia but rather less so in B-cell neoplasms. *MYC* proto-oncogene mutation has been recorded in B- and T-cell neoplasms and in some myeloid leukaemias and *FMS* mutation is not uncommon in myeloid leukaemia. Of the tumour suppressor genes, *p53* and *RB1* mutations have been characterised in a range of haemopoietic neoplasms, particularly those of an acute form, and there is also some evidence of *WT1* involvement in a proportion of acute myeloid leukaemias. Homozygous deletions of the α interferon gene has been recorded more consistently in a range of leukaemias (Cline, 1994; Young, 1994).

(203) Proto-oncogene and tumour suppressor gene mutations have also been identified in spontaneously arising and chemically and radiation-induced leukaemias and lymphomas in experimental animals (UNSCEAR, 1993). Of particular note are recent studies with mice heterozygous for *p53* showing that the remaining wild type *p53* allele can be a direct target for the initiation of lympho-haemopoietic neoplasia (Kemp *et al.*, 1994; see also Section 5).

(204) Chromosomal and gene amplifications have been recorded in a few lympho-haemopoietic neoplasms but the specific contribution of these to neoplastic development has yet to be determined (Mitelman, 1991; Sandberg, 1993); new molecular cytogenetic techniques (Pinkel, 1994) appear to have the power of resolution to address this problem.

(205) The overall impression that may be gained from these studies is that proto-oncogene, tumour suppressor, and gene amplification mutations exert their principal effects during the progression of haemopoietic neoplasia and are, in many instances, secondary to lineage-specific chromosomal events.

Genomic instability in haemopoietic neoplasia

(206) The principal link between genomic instability and lympho-haemopoietic neoplasia has been established by the finding that the human genetic disorders ataxia-telangiectasia

and Fanconi anaemia (see Section 4.2.4), associated with defects in DNA processing and attendant chromosomal instability, are also characterised by predisposition to certain lympho-haemopoietic neoplasms. This finding is broadly consistent with the view gained from cytogenetic and molecular studies that the principal mechanisms for the initiation of lympho-haemopoietic neoplasia centres on gene-specific chromosome translocation or segmental chromosome loss.

(207) In addition to this there has been much debate on the role of so called fragile chromosomal sites (*FRA*) in the development of leukaemia and lymphoma. Analyses of the relationships between common human *FRA* and neoplasia-specific chromosome breakpoints suggest that such associations are at best weak but there is evidence that rare heritable *FRA* may show a somewhat stronger relationship with the position of chromosomal breakpoints in some haemopoietic neoplasms (see Section 4.2.4). In the absence of firm molecular data on the structure and location of potentially informative human *FRA* the statistical analyses so far presented and discussed do not allow for any firm conclusions on their role in the development of haemopoietic neoplasia. Nevertheless, chromosome 5q breakpoints in the deletions characterising myelodysplasia are believed to be non-random (Mitelman *et al.*, 1986) but equally, do not clearly correspond to known *FRA* loci.

(208) Albeit restricted to a relatively small number of cases, genomic instability in some haemopoietic neoplasms has been more convincingly linked at the cytogenetic level with telomere-associated chromosomal rearrangement (Shippey *et al.*, 1990). In these cases neoplastic chromosome translocations involving the non-reciprocal exchange between an interstitial and terminal (telomeric) site appears to create a chromosomal reunion that is highly unstable and is able to undergo secondary exchange or loss events. Such observations lead to the hypothesis that some human *FRA* might be represented by telomere-like repeat (TLR) sequences (Hastie and Allshire, 1989). Some support for this and for the relationship between telomeric sequences and leukaemogenic chromosomal rearrangement has been provided by recent animal studies noted below.

(209) In certain mouse strains radiation-induced AML is strongly associated with interstitial deletion/rearrangement of chromosome 2 and there is evidence that such gross changes may represent initiating events for this neoplasm. While molecular data implicating specific chromosome 2 encoded genes in this process are not compelling, there are somewhat more convincing data relating to the involvement of specific recombinogenic TLR sequences at the leukaemia-associated chromosome 2 breakpoints (Bouffler *et al.*, 1991; Meijne *et al.*, 1996; Clark *et al.*, 1996). Whether these TLR loci represent murine *fra* and the possibility that murine *fra* act as genetic determinants of leukaemia susceptibility remains, however, speculative (see Section 4.2.4). In spite of this uncertainty, recent advances in telomere biology (Blackburn, 1991; Kim *et al.*, 1994; Zakian, 1995) do suggest that these repeat sequences play a crucial role in chromosome stabilisation and may be involved in the processes of cell senescence and neoplastic transformation (see Section 2).

4.2. Genetic susceptibility to lympho-haemopoietic neoplasia

(210) Evidence relating to the heritable component of leukaemia and lymphoma comes from a variety of sources. Such evidence that there is has been considered over a period of some 20 years (Zeulzer and Cox, 1969; Gunz, 1974; Linet, 1985; Taylor and Birch, 1996).

(211) The principal sources of information derive from studies on neoplasia in: families, monozygotic and dizygotic twins, cancer-family syndromes, chromosome instability syndromes, chromosomal and developmental abnormalities, and immunological

deficiencies. Here we provide a brief summary of the available data with an emphasis on the degree of uncertainty that surrounds any judgement on the genetic component of human lympho-haemopoietic neoplasia.

4.2.1. *Family studies*

(212) While familial leukaemia and lymphoma is uncommon there are a number of reports which support the contention that, in rare instances, a tendency towards haemopoietic neoplasia is heritable (Zeulzer and Cox, 1969; Gunz, 1974; Linet, 1985; Taylor and Birch, 1996). In most of these families a clear Mendelian pattern of inheritance has not been established but as noted by Lynch *et al.* (1985) the extent of pedigree analysis is critical for ascertainment of heritable predisposition to neoplasia; this is particularly the case for mutations of low penetrance.

(213) Accepting these uncertainties there is little doubt that in rare instances heritable factors underly multiple cases of leukaemia/lymphoma within family pedigrees (Gunz *et al.*, 1975; Linet *et al.*, 1989; Knudson, 1993). For example, in his review Gunz (1974) noted one family with 13 cases of leukaemia in 293 family members over three generations; Li *et al.* (1979) reported on eight males with leukaemia/pre-leukaemia over four generations suggestive of X-linked inheritance and Gunz (1975) in an extensive study noted a three-fold increase in risk of leukaemia among first-degree relatives of patients with all sub-types of haemopoietic neoplasia. Dominantly inherited familial predisposition to acute myeloid leukaemia has also been reported recently (He *et al.*, 1994). In their review Taylor and Birch (1996) provides further information on familial clustering of, not only leukaemia, but also of Hodgkins and non-Hodgkins lymphoma.

(214) A notable feature of the available data is the finding of increased risk and concordance for familial CLL but the absence of this for CML (Zeulzer and Cox, 1969; Gunz, 1974; Gunz *et al.*, 1975; Linet *et al.*, 1989). Such familial concordance for leukaemic sub-type adds additional credibility to a genetic basis for a small proportion of haemopoietic neoplasia. Some of the leukaemia families noted in the reviews cited here probably fall into the genetic categories discussed later in this section but in the main they have yet to provide information on the specific nature of the underlying genetic basis of haemopoietic disease.

(215) Genetic contributions to disease may also be judged in sub populations where there is a significant degree of inbreeding (consanguinity) (Edwards, 1969; Vogel and Motulsky, 1986). Early studies suggested that consanguinity among leukaemia cases was not frequent (Zeulzer and Cox, 1969; Steinberg, 1960) but it has been reported that 10/29 Japanese families with two or more cases were consanguinous (Kurita *et al.*, 1974). In addition, there are reports of increased frequency of leukaemia in inbred Jewish and Hutterite communities in the USA (Feldman *et al.*, 1976; Martin *et al.*, 1980) and consanguinity has been noted in leukaemia-prone ataxia-telangiectasia (Swift *et al.*, 1986) and Fanconi anaemia (Schroeder *et al.*, 1976) families (see also Section 4.2.4). Of particular relevance to childhood leukaemia, mortality rates, and the effects of inbreeding are the recent studies of Tuncbilek and Koch (1994) and Bittles and Neel (1994). As judged by these data, the contribution of consanguinity to leukaemia risk appears to be low.

4.2.2. *Studies with twins*

(216) In principle the genetic component of lympho-haemopoietic neoplasia may be judged from the increased degree to which there is concordance for disease in identical,

monozygotic (MZ) twins over that seen in the non-identical, dizygotic (DZ) type (Falconer, 1990). In brief, if neoplasia were to be due to a monogenic dominant mutation then concordance rates should exceed 50% in MZ and if due to recessive mutation this concordance should exceed 25% with the specific degree of concordance dependent perhaps on the role of non-genetic factors. Early reports (Keith *et al*., 1975; Kurita *et al*., 1974) suggestive of high concordance rates for leukaemia in MZ twins and, by implication, a significant genetic component to the disease need to be interpreted with great caution, not only because neoplasia overall has been shown to be discordant in such studies (Hrubec and Neel, 1982) but also because of a confounding mechanism that applies specifically to leukaemia.

(217) Following the original hypothesis by Clarkson and Boyse (1971) it has been unambiguously shown by cytogenetic and molecular studies (Chaganti *et al*., 1979; Ford *et al*., 1993) that in many instances the concordance of leukaemia in MZ twins is not due to genetic factors but rather to in utero transplantation of pre-malignant cells between MZ foetuses having shared blood circulation. Thus, leukaemia in MZ twins is most frequently monoclonal in origin with, in these cases, both twins having the same breakpoint in the *MLL1* gene of chromosome 11q23; different *MLL1* breakpoints are seen in leukaemia in DZ twins.

(218) Further sources of confounding for the estimation of genetic components of leukaemia from twin studies have been identified (Phillips, 1993; Côté and Gyftodimou, 1991; Taylor and Birch, 1996) and include fetal loss due to leukaemogenesis, non-genetic factors such as birthweight variability between twin pairs and the possible role of early mitotic events in the twinning process leading to genetic imbalance. An association between the processes involved in twinning and malformation has also been suggested (Garabedian and Fraser, 1994).

(219) There are isolated reports of adult MZ twins with leukaemias having different cytogenetic abnormalities and/or immune gene sequence rearrangements. These provide evidence of post-zygotic genomic changes leading to leukaemia but do not allow comment on whether the common genetic background or similar environmental features of early life are the causal elements (Hecht *et al*., 1988; Brok-Simoni *et al*., 1987).

(220) In spite of these uncertainties there is increasing evidence for the role of inherited factors in Hodgkins disease (HD), a monoclonal inflammatory condition associated with lymphomagenesis. Of particular importance is a recent study of concordance for the early adult form (< 50 years) of HD in a large study of MZ and DZ twins (Mack *et al*., 1995). Whereas none of the 187 pairs of DZ twins became concordant for HD, in 179 pairs of MZ twins there were 10 instances of concordance; the median age of diagnosis of HD was 26 years. The expected incidence of HD in each of these twin groups was 0.1, thus providing no evidence of excess risk in DZ twins but a greatly elevated risk in the MZ group (standardised incidence ratio of 99, CI 48–182). A genetic basis for concordance for HD in MZ twins was further supported by the finding of a consistent pattern of HD histology (nodular sclerosis sub-type) in most of the pairs. There was however no evidence of disparity between MZ and DZ twin pairs in respect of other cancers. These data are discussed by Lynch and Marcus (1995) with emphasis on the possible role of inherited immunological defects in the aetiology of HD.

4.2.3. *Cancer family syndromes*

(221) Lympho-haemopoietic neoplasia is not the principal disease in any of the 20 or so cancer family syndromes having mutant genes that are cloned or mapped on the human

genome (Knudson, 1993; Eng and Ponder, 1993). Thus, although there are a few reports of leukaemia in, for example, heritable retinoblastoma (DerKinderen *et al*., 1988), it seems that, in the main, the well characterised germ line tumour gene mutations predisposing to solid cancers (Section 3) do not impact significantly on leukaemia or lymphoma. The two exceptions to this relate to type 1 neurofibromatosis (NF1) and Li–Fraumeni syndrome (LFS).

(222) There are conflicting reports concerning leukaemia in NF1 patients. Whereas Seizinger (1993) suggests that there is no association of myelodysplasia (MDS) or acute myeloid leukaemia (AML) with NF1, other authors cite data indicative of a positive association (Eng and Ponder, 1993; Brodeur, 1994; Shannon *et al*., 1992; Stiller *et al*., 1994). In support of this association is the recent observation that mice deficient in the *NF1* gene are prone to acute myeloid leukaemia (Jacks *et al*., 1994).

(223) Perhaps more universally accepted is the association between LFS and acute leukaemia occurring before age 45 (Eng and Ponder, 1993; Malkin, 1993; Birch *et al*., 1994). In a study of 43 LFS families Malkin (1993) reported on 231 neoplasms of which 14 were leukaemias, and since germ line *p53* gene mutation is not a wholly consistent finding in LFS, it is important to consider the possibility that leukaemia predisposition is not homogeneous amongst LFS kindred.

(224) There are also studies in *p53*-deficient mice that allow comment upon the role of this gene in heritable susceptibility to lympho-haemopoietic neoplasia. Mice heterozygous for *p53* (analogous to LFS) have been shown to develop osteosarcoma, soft tissue sarcoma, and lymphoma at a high frequency (Donehower *et al*., 1992; Kemp *et al*., 1994) and also show increased tumorigenic radiosensitivity (Kemp *et al*., 1994). The mechanisms of this elevated sensitivity to haemopoietic neoplasia appear to centre on increases in chromosome replication errors and particularly in the case of radiation lymphomagenesis chromosome loss/gain events associated with *p53* deficiency (Bouffler *et al*., 1995; see also Section 5).

(225) Finally, there are preliminary indications that families carrying *WT1* (Wilms tumour) mutations may have an increased incidence of haemopoietic neoplasia (Hartley *et al*., 1994).

4.2.4. *Chromosome instability syndromes*

(226) The principal chromosome instability syndromes in man having associations with lympho-haemopoietic neoplasia are ataxia-telangiectasia (A-T), Bloom syndrome (BS), and Fanconi anaemia (FA) (German, 1983; Murnane and Kapp, 1993). These disorders are autosomal recessive and the cellular cytogenetic and molecular data that provide clues on associated DNA processing defects are outlined in Section 2. Here we focus on the pattern of neoplasia in these diseases.

Ataxia-telangiectasia

(227) Ataxia-telangiectasia, although described earlier, was more completely characterised by Boder and Sedgewick (1958) as having complex clinical features that include early onset cerebellar ataxia, telangiectasia, immunodeficiency, increased risk of sino-pulmonary infection and haemopoietic neoplasia (Boder, 1975; Bridges and Harnden, 1982; Bundy, 1994). The clinical features of A-T and their possible relationships with the genetics, cytogenetics, and molecular biology of the disorder are well described in a series of monographs and meeting reports covering the period 1982–94 (Bridges and Harnden, 1982; Gatti and Swift, 1985; Taylor *et al*., 1994b).

(228) The cancer risk in A-T with a lifespan that rarely exceeds 20 years has been estimated to be between 5 and 20% (Cohen and Levy, 1989; Bundy, 1994) with the risk of lympho-haemopoietic neoplasia being up to 250-fold greater than that in the general population; there is also an increased risk of stomach cancer in some A-T families (Spector *et al*., 1982). Of particular note is the relative absence of an excess of common solid tumours and myeloid diseases in A-T, contrasting with the clear excess of T- and to a lesser extent B-cell neoplasms. This predisposition to specific sub-types of haemopoietic neoplasia broadly accords with cytogenetic, molecular and immunological findings indicating that major sequelae of the putative DNA processing defect(s) in A-T centre on recombination errors in respect of immune gene sequences (see Section 4.1.3.1 and 2).

(229) Tissue radiosensitivity in A-T following radiotherapy is well documented (see Section 5) and accords with the findings from in vitro cellular studies (Section 2); there are however no data on which to judge leukaemogenic risk in A-T homozygotes after radiation. The contention that individuals heterozygous for A-T mutant genes show increased in vitro radiosensitivity and are also prone to neoplasia is discussed in Sections 2 and 5. Here it is sufficient to say that there is no evidence that these A-T gene carriers are prone to lympho-haemopoietic neoplasia.

(230) Although the genetics of A-T may be relatively straightforward (see Section 2), a variant form, the Nijmegen breakage syndrome (NBS) has been described (Weemaes *et al*., 1981; Weemaes *et al*., 1994). Eight lymphomas and a single glioma have been reported amongst 19 NBS patients and it is notable that, unlike 'classical' A-T, NBS has yet to be associated with T-cell leukaemia.

Bloom syndrome

(231) Initially described by Bloom (1954), Bloom syndrome (BS) is very rare and principally characterised by erythema, sun sensitivity, growth and mental retardation, and predisposition to neoplasia (German, 1969, 1983, 1993). A registry of BS patients (German, 1992) provides data on neoplasms in 132 cases and suggests that the tumour spectrum is more broad than in ataxia-telangiectasia and Fanconi anaemia and includes not only leukaemias and lymphomas but also a range of common solid tumours. Cancer risk in BS patients appears to exceed 20% but heterozygotes for the BS gene show no evidence of increased malignancy.

(232) The specific nature of the defect that may link BS with genomic instability and tumour predisposition has yet to be uncovered but following the recent cloning of the BS gene (*BLM*) some form of DNA helicase-deficiency seems likely (see Korn and Ramkisson, 1995).

Fanconi anaemia

(233) Following the original description of Fanconi anaemia (FA) as a disorder associated with a childhood defect in bone marrow development (Schroeder *et al*., 1976; Alter, 1987), the disease has also been shown to include skeletal, neurological, skin, and renal abnormalities (German, 1983). Various reports also highlight the chromosomal instability and abnormal DNA damage response of FA cells in vitro (Section 2) and recent studies have confirmed the genetic heterogeneity of the disorder (see Buchwald, 1995).

(234) Unlike A-T, myeloid rather than lymphocytic leukaemia dominates amongst haemopoietic neoplasms in FA and the rate of leukaemia in FA appears to be around 10% with an increased incidence of myeloid disease perhaps 15,000-fold greater than in the normal population (Alter, 1987; Auerbach and Allen, 1991). The question of

neoplastic risk in FA heterozygotes has been addressed and although an early report (Swift, 1971) was suggestive of increased risk, this has not been confirmed (Swift *et al.*, 1980; Potter *et al.*, 1983).

(235) Taylor and Birch (1996) have grouped FA along with other familial disorders showing, amongst other features, abnormalities of bone marrow development together with some degree of predisposition to leukaemia. This group of diseases include the WT, IVIC, Schwachman–Diamond and Kostmann syndromes, ataxia-pancytopaenia, familial monosomy 7, dyskeratosis congenita, amegakaryocytic thrombocytopaenia, and Blackfan–Diamond anaemia.

(236) These are all rare disorders and with the exception of Blackfan–Diamond anaemia (Iskandar *et al.*, 1980) little is known of their chromosomal responses to radiation. However even in the absence of chromosomal instability it may be reasonable to assume that the associated bone marrow defect may in some circumstances increase the risk of lympho-haemopoietic neoplasia as an indirect consequence of cellular proliferative stress placed upon that organ.

Heritable chromosomal fragile sites

(237) Since site-specific chromosomal rearrangement is an important somatic hallmark of lympho-haemopoietic neoplasia it is feasible that inherited predisposition to site-specific chromosomal fragility can be a genetic determinant of these malignancies. In excess of 100 rare and common chromosomal fragile sites have been identified and although inheritance of many of the rare sites has been demonstrated (Wenger, 1992), only four such loci, *FRAXA*, *FRAXE*, *FRAXF*, and *FRA16B* have been resolved at the molecular level, in these cases as expanded trinucleotide repeats in DNA (Willems, 1994). None of these repeat sequence sites have been specifically implicated in malignancy; a trinucleotide repeat has however been reported in the *AF9* gene which can partner *MLL1* in leukaemogenic gene fusions (Table 4.3) (Nakamura *et al.*, 1993) and the *FRA11B* locus has been shown to involve a repeat expansion in the *CBL2* proto-oncogene (Jones *et al.*, 1995).

(238) The relationship between heritable fragile site expression and leukaemogenesis in man have been debated for many years (Sutherland and Simmers, 1988; LeBeau, 1988) and while it remains possible that such associations exist in rare families (de Braekeleer *et al.*, 1985; LeBeau, 1988; Hori *et al.*, 1988), no specific support for the hypothesis has come from molecular studies. Also, a survey of cancer in the kindreds of leukaemia patients with chromosomal rearrangements at putative heritable fragile sites failed to demonstrate an increased risk of heritable leukaemia but did suggest the possibility of excess lung cancer in certain families (Mules *et al.*, 1989).

(239) Experimental studies in the CBA mouse have also provided ambiguous results in respect to an association between heritable variation in telomere-like sequences, chromosome fragility and radiation-myeloid leukaemogenesis (Silver and Cox, 1993; Meijne *et al.*, 1996). Nevertheless, it may be relevant that heritability of terminal telomeric length in man has been shown to be high (Slagboom *et al.*, 1994).

4.2.5. *Chromosomal and developmental abnormalities*

Chromosomal abnormalities

(240) The somatic chromosomal exchanges that characterise many sub-types of haemopoietic neoplasia have not been reported in the germ line of patients implying that, as specific entities, they play little or no role in germ line predisposition to disease.

Although studies with animals transgenic for certain leukaemia-associated oncogenes (Adams and Cory, 1991) show that in certain circumstances (i.e genomic context) such gene activation or fusion events (Tables 5.1–5.3) are compatible with murine embryogenesis, it may be concluded that, in man, such events in their normal genomic context are either embryo-lethal or extremely rare. It is also notable that animal studies using gene 'knock-out' techniques have not revealed a correlation between genes contributing to leukaemia and a role for these in normal haemopoiesis (Varmus and Lowell, 1994).

(241) In contrast to this, there is evidence that, in a less specific fashion, certain germ line chromosomal imbalances can predispose to haemopoietic neoplasia. The most important and common of these is trisomy for chromosome 21, Down syndrome (DS) having characteristic mongoloid features, where there is an estimated 18-fold increased risk of leukaemia (Fong and Brodeur, 1987). The overall strength of the association between constitutional chromosomal abnormalities and leukaemia is more difficult to judge. Taylor and Birch (1996) list nine cases from the literature where such associations have been found; these include trisomy, monosomy, Robertsonian translocation, balanced translocation, deletion, insertion, and fragile site expression. Larger surveys of patients are however more instructive.

(242) In a study of 1400 patients with haemopoietic neoplasia Alimena *et al.* (1985) noted ten cases of trisomy 21, five translocations, and six sex chromosomal abnormalities while Benitez *et al.* (1987) reported on 718 patients and noted nine cases of constitutional chromosome abnormality.

(243) Since these chromosomal frequencies are significantly higher than seen in the normal population, it may be concluded that a general but relatively weak association between chromosomal abnormality and haemopoietic neoplasia does exist and that this, as expected, tends to be dominated by DS which has by far the highest birth incidence at around 1 in 700 (Epstein, 1986).

(244) Although the genetic basis of the chromosomal associations described above remains obscure, three possible mechanisms may be rehearsed. First, that as a consequence of some underlying defect influencing both meiotic and mitotic processes, there may be familial tendencies towards germ line chromosomal abnormality and the somatic generation of leukaemia-associated chromosomal events in haemopoietic cells. Second, in a rather non-specific fashion, the presence of germ line chromosomal imbalance itself predisposes to somatic chromosomal instability and hence to haemopoietic neoplasia. Third, there is the additional possibility that there is a more specific association between trisomy 21 and dysregulation of haemopoiesis which may be expressed as 'transient' or overt leukaemia (Rosner and Lee, 1972). It seems likely that many genes contribute to the diverse phenotypic manifestations in DS and a current view is that gene dosage effects are most critical. As yet no specific region of chromosome 21 has been associated with leukaemia development (Korenberg, 1995).

Developmental abnormalities

(245) There are conflicting reports on whether there is or is not an association between birth defects overall and the risk of haemopoietic neoplasia (Mann *et al.*, 1993; Mili *et al.*, 1993). If such risk exists it would appear to be small and it is only for a few rare congenital disorders discussed by Li and Bader (1987) where there is any substantial evidence for this.

4.2.6. *Immunological deficiencies*

(246) Genetically determined immunodeficiency disease results principally in defects in the development and function of effector T- and B-cell subsets in the haemopoietic system; deficiency of natural killer (NK) cells has also been noted in some of these disorders (Robertson and Ritz, 1990). These comprise a rare set of disorders where there is not only a high risk of childhood mortality from microbial infection but also a 10–25% risk of malignancy with, overall, non-Hodgkin lymphoma as the dominant neoplasm (Filipovich *et al*., 1994). Included in this group of disorders are: severe combined immunodeficiency (SCID), X-linked hypogammaglobulinaemia (X SCID), X-linked hyper-IgM (HIM), purine nucleoside phosphorylase (PNP) deficiency, Wiskott–Aldrich syndrome (WAS) and X-linked lymphoproliferative disease (XLP) (Taylor and Birch, 1996). The Immunodeficiency Cancer Registry (Filipovich *et al*., 1994; Filipovich *et al*., 1992) records data from many such patients and includes ataxia-telangiectasia (A-T) in its listing. The highest cancer rates occur in A-T and WAS with WAS showing around 180-fold increased risk of malignancy. A candidate gene for WAS has been isolated (Derry *et al*., 1994) and subsequently also shown to underlie X-linked thrombocytopenia (Villa *et al*., 1995). The mechanism of action of the WAS gene is believed to be complex (Featherstone, 1997).

(247) Importantly, the mechanisms that underlie leukaemia/lymphoma predisposition in these disorders may not always be a direct consequence of a lack of cellular surveillance. Although the notable excess of B-cell neoplasia may principally relate to Epstein–Barr virus (EBV) infection (Klein, 1994) the association seems not to be entirely secure (Filipovich *et al*., 1994; Grassmann *et al*., 1994). The role of infective agents in childhood leukaemia and of viral involvement in human and animal lympho-haemopoietic neoplasia in general has been discussed widely (Greaves, 1988; Zur Hausen, 1991; Aboud, 1992) but with the exception of the EBV association noted above, there is no strong evidence for the interaction of specific genetic and viral factors in human susceptibility to haemopoietic neoplasia.

(248) In certain immunodeficiency genetic disorders a substantially different aetiology may apply since in A-T, SCID, and other isolated cases the immunodeficiency and leukaemia predisposition may not be consequential but instead to share a common root which is believed to centre on an underlying genetic defect in DNA recombination and/or repair (see Section 2). Associations between lympho-haemopoietic neoplasia and variation in immune-involved histocompatibility (HLA) genes have also been considered (Taylor and Birch, 1996). In the mouse such associations, which may be strong in some strains, appear to arise as a consequence of HLA-mediated susceptibility/ resistance to lymphomagenic virus. In man these links are much weaker with conflicting reports in the literature even in the case of the rare leukaemia subtype (ATL) known to be associated with HTLV-1 virus infection where, on the basis of mouse data, HLA involvement might be expected (Tajima *et al*., 1984; Tanaka *et al*., 1984). The possibility exists however that the HLA system might also target pre-malignant cells and that polygenetic factors obscure the true involvement of HLA variation in human haemopoietic neoplasia; Mack *et al*. (1995) have discussed the possible relevance of HLA variation to predisposition to HD.

4.2.7. *Epigenetic factors*

(249) There is very little information on the possible role of endogenous epigenetic factors that might, for example, account for the irregular inheritance of haemopoietic neoplasia in families. Principal of the potential epigenetic mechanisms in neoplasia are parent-of-origin (genomic imprinting) effects on mutant gene expression which although

still largely obscure may play a role in the inheritance of susceptibility to certain solid tumours (Section 3).

(250) With the possible exception of the *NF1* gene (Feinberg, 1993) there is no published information on imprinting-like effects in any of the human disorders or genes directly associated with haemopoietic neoplasia. In particular, the initial claim that the t(9;22) translocation in CML is subject to parent-of-origin effects (Haas *et al.*, 1992) has recently been refuted by molecular analysis of the associated *BCR* and *ABL* genes (Tioretos *et al.*, 1994; Melo *et al.*, 1994). Nevertheless knowledge in this whole area is sparse and a role for epigenetic factors in leukaemia/lymphoma predisposition cannot be discounted; Taylor and Birch (1996) and Breckon *et al.* (1991) have provided speculative comment on this issue.

4.2.8. *The genetic component of lympho-haemopoietic neoplasia*

(251) Given the high birth incidence and increased risk of lympho-haemopoietic neoplasia there is a strong expectation that Down syndrome (DS) will make the greatest contribution to leukaemia/lymphoma in the population, particularly in respect to childhood neoplasms. Assuming that the incidence of childhood leukaemia/lymphoma is around 1 in 2000 (Parkin *et al.*, 1988) the values cited in Section 4.2.5 in respect of incidence and risk imply that DS will contribute up to around 3% of these neoplasms in childhood. Additional data cited in Section 4.2.5 provide, however, no reason to believe that other currently recognised developmental or chromosomal genetic abnormalities make significant contributions to leukaemia/lymphoma in the human population.

(252) It may also be judged that, overall, heritable defects of tumour-associated germ line genes (proto-oncogenes and tumour suppressor genes) are rare features of lympho-haemopoietic neoplasia (Section 4.2.3). Two genetic entities associated with such mutation, Li–Fraumeni syndrome (LFS) and neurofibromatosis type 1 (NF1) do however carry a significant degree of risk of these neoplasms. In the case of LFS, the incidence in the population is very low (see Section 6) and the contribution to population risk will be negligible. NF1 is however far more common ($\sim$1 in 3000), largely as a consequence of a high de novo germ line mutation rate (Huson *et al.*, 1989). However, although NF1 patients constitute up to 10% of cases of myeloproliferative syndrome (MPS) and associated myeloid leukaemias in children (Shannon *et al.*, 1992), MPS is uncommon and NF1 seems likely to under only a small fraction ($< 0.5\%$) of total lympho-haemopoietic neoplasia in childhood.

(253) In the case of the genetic disorders associated with defects of the immune system and/or exhibiting chromosomal instability, e.g. Wiskott–Aldridge syndrome, severe combined immunodeficiency disease, ataxia-telangiectasia, Fanconi anaemia and Bloom syndrome (see Section 4.2.4 and 4.2.6) there is often a high risk to the individual of developing subtype specific neoplasia. These diseases are however rare in the population and on this basis it may be judged that collectively they seem likely to contribute substantially less than 1% to population risk.

(254) Since in the majority of the disorders discussed in this Section of the report there is an expectation that neoplasia will be expressed in childhood, a summation of the estimations of genetic contributions provided above leads us to judge that around 3–4% of childhood leukaemia/lymphoma is associated with known genetic abnormalities (see also Narod, 1990).

(255) Some credence to this indirect estimate is provided by an epidemiological study of childhood cancers (Narod *et al.*, 1991). In this survey of 16,546 cases of neoplasia, there

were 7345 leukaemias/lymphomas of which 159 (2.2%) showed evidence of an underlying genetic condition. Down syndrome contributed the greatest number of genetic cases (n = 134 or 1.8% of the total) while much smaller contributions were made by NF1 (n = 10 or 0.14% of the total) and immunodeficiency disorders (n = 13 or 0.18% of the total).

(256) Since, however, there is no a priori expectation that genetic predisposition to lympho-haemopoietic neoplasia in children will always be accompanied by other recognisable clinical features, the 3–4% value for the genetic contribution noted above may tend to be an underestimate. Nevertheless, the failure of twin studies to reveal clear evidence of a genetic component for anything other than early onset HD, the apparent rarity of familial leukaemia and the conflicting data from studies on consanguinity argue that a value of 3–4% should not significantly underestimate the genetic contribution to childhood haemopoietic neoplasia.

(257) Although a genetic contribution to leukaemia/lymphoma in the adult population is more difficult to estimate, family and consanguinity studies suggest that it is small and almost certainly less than that which applies to children. Thus, on the basis of existing knowledge it is judged that 5% is a reasonable value for the genetic contribution as applied to a population of all ages. In making this judgement it is however recognised that the data base available is subject to considerable uncertainty in respect of leukaemogenic mechanisms and potential variation in the interaction of genetic and environmental factors leading to effects on penetrance/expressivity of mutations; also to clinical factors such as unrecognised disease resulting in pre- or early post-natal mortality.

4.3. Summary and conclusions

(258)

1. Specific chromosomal rearrangements involving changes in the activity of proto-oncogenes are a characteristic feature of the development of lympho-haemopoietic neoplasia; these events involve either gene juxtapositions or gene fusions. Loss of chromosome segments encoding possible tumour suppressor genes, gene mutations and numerical chromosomal changes have also been characterised in some subtypes.
2. Familial lympho-haemopoietic neoplasia is very rare although some subtypes are found in excess in Li–Fraumeni syndrome and neurofibromatosis.
3. Studies of lympho-haemopoietic neoplasia in twins and in children with congenital abnormalities have not revealed a strong genetic component. Twin studies have however provided evidence for a genetic component to early-onset Hodgkin disease.
4. Excess lympho-haemopoietic neoplasia is seen most clearly in rare autosomal recessive disorders associated with chromosomal instability and/or immunodeficiency. Some of these—ataxia-telangiectasia, Fanconi anaemia, and Bloom syndrome—are believed to be deficient in DNA damage processing.
5. It is judged that the genetic component to lympho-haemopoietic neoplasia is poorly defined and probably less than that for solid tumours. It is at its greatest in children where it may account for 3–4% of disease with Down syndrome as the greatest single source.

5. EVIDENCE ON ASSOCIATIONS BETWEEN TUMORIGENIC RADIOSENSITIVITY AND HERITABLE PREDISPOSITION TO CANCER

(259) A central question to be addressed in this report is the extent to which human heritable predisposition to spontaneously arising cancer is reflected in an increase in excess risk after exposure to ionising radiation. As will be seen, there is a paucity of data on which to make direct judgements on this question and, for this reason, attention is first given to the mechanistic aspects of the problem. For this, the fundamental data of Sections 2–4 assume particular importance but also included is discussion on tumorigenic radiosensitivity in animal models of heritable cancer.

(260) The second approach adopted is to seek semi-quantitative evidence of tumorigenic radiosensitivity in patients with known or suspected heritable disorders who have received radiotherapy in respect of a primary tumour. In such cases clinical evidence of excess neoplasia arising within the irradiated field and cumulative mortality data provide some indication of the extent to which the underlying heritable disorder predisposes to second, therapy-related neoplasia.

(261) Finally, evidence of increased tumorigenic radiosensitivity associated with heritable cancer predisposition will be reviewed with emphasis on preliminary re-evaluation of the data from the Japanese A-bomb cohort for evidence of the expression of excess cancer risk in the putative cancer-predisposed subgroups that are expected amongst the many thousands of individuals exposed to ionising radiation in Hiroshima and Nagasaki. Attention will be given not only to current limitations of conventional epidemiological approaches to the problem in hand but also to the opportunities presented by new and powerful strategies that utilise closely coupled molecular genetic and epidemiological techniques.

5.1. Mechanistic aspects of tumorigenic radiosensitivity

(262) In Sections 2–4 of this report the molecular genetics of tumour induction and development were discussed in the context of the germ line mutations that are currently judged to be involved in heritable predisposition to cancer. Three principal categories of tumour predisposing mutations were identified. Genetic defects in DNA damage processing were seen, for example, to apply in the case of ataxia-telangiectasia (A-T) and heritable non-polyposis colon cancer (HNPCC) but may also apply to certain disorders associated with breast cancer susceptibility. Defects in tumour suppressor genes represented the most abundant category and included the majority of known genetically dominant disorders, e.g retinoblastoma (RB), familial adenomatous polyposis (FAP), and Von Hippel Lindau syndrome (VHL). Proto-oncogene defects were judged to be the least common form, represented only by multiple endocrine neoplasia and some cases of familial melanoma.

(263) As noted previously, such categorisation, whilst convenient, is somewhat imprecise. This is best illustrated by the mutations of *p53* in Li–Fraumeni syndrome (LFS) and DNA mismatch repair genes in HNPCC which may be functionally included in both DNA repair and tumour suppressor gene categories; also, by the indications that the *BRCA* breast cancer susceptibility genes may play roles in DNA metabolism. In spite of this imprecision of classification some general projections on the impact of radiation damage on the expression of cancer in these different genotypes are possible.

5.1.1. *Genetic defects in DNA processing*

(264) The data and discussion of Section 2 argue strongly that the sensitivity of mammalian cells to ionising radiation is determined principally by the capacity to correctly repair double strand DNA lesions. A most important observation is that cellular radiosensitivity in human A-T and many rodent somatic cell mutants is not accompanied by equivalent hypersensitivity to other DNA damaging agents. In a similar way cells from most but not all genetic categories of the human sun sensitive disorder, xeroderma pigmentosum (XP) are sensitive to UVR and some chemical agents but not to ionising radiation (see Arlett, 1992; Thacker, 1992). The broad message from these observations is that DNA damage repair processes tend to operate in a 'lesion specific' fashion with perhaps few controlling genes being shared by different repair pathways.

(265) Accordingly, on the basis of an assumed direct relationship between lesion-specific DNA repair capacity and cancer development, there should be no a priori expectation that human cancer predisposition associated with defects in all forms of DNA damage processing will show increased cancer risk after ionising radiation. Although the strength of the link between cellular radiosensitivity and cancer predisposition may be questioned (Section 2) it is however to be expected that there will be some human genetic disorders of DNA damage recognition and repair which will show elevated cancer risk after exposure to a broad range of DNA damaging agents.

(266) At present it is possible to tentatively identify LFS which involves mutation of the *p53* gene as one such case. As described in Section 3, the *p53* protein is intimately involved in DNA damage recognition, cell cycle checkpoint control and apoptotic response. Since the DNA damage recognition functions of the *p53* protein may not depend critically upon lesion type, increased sensitivity to a broad range of carcinogens may be anticipated in *p53*-deficient disorders. Studies with *p53*-deficient mice lend some support to this conclusion (see Section 5.2).

(267) In the case of HNPCC patients who are heterozygous for one of four genes involved in DNA mismatch repair (see Section 3) there are no data on which to judge possible abnormalities of cellular response to ionising radiation. The proposed involvement of such repair deficiency in the formation and resolution of DNA heteroduplexes (Fishel and Kolodner, 1995) could however have implications for radiation-induced mutations in cancer development of HNPCC patients.

(268) In conclusion, while it may be judged that the majority of known human DNA repair deficient disorders are probably not associated with increased cancer risk after ionising radiation, there will be some that are. At present it is possible to identify only individuals carrying the A-T and NBS mutations in their homozygous state as being potentially at increased risk. Since however most repair-deficiency disorders are recessive in their principal clinical manifestations there is the possibility of partial phenotypic effects, including cancer, in heterozygotes who will be more common in the population (Section 6). This may be of particular importance in the case of A-T heterozygote who have been proposed to express increases in both chromosomal radiosensitivity and spontaneous breast cancer risk (Sections 2 and 3). In the same context, it may be that some forms of human DNA–repair deficiency are embryo-lethal in the homozygous state but express as cancer susceptibility in viable radiosensitive heterozygotes.

5.1.2. *Genetic defects in tumour suppressor genes*

(269) Early studies in retinoblastoma led to the formulation by Knudson and coworkers of the two-hit concept of carcinogenesis which was later shown to be the result of the

inactivation of tumour suppressor genes (see Knudson, 1993). According to this model inherited mutation of one of two germ line copies of a given suppressor gene will lead to heterozygosity for that gene in all somatic cells of the carrier. Loss or mutation of the remaining wild type gene in a given target somatic cell of the carrier then creates the potential for monoclonal cancer development. Given the multiplicity of such target cells the probability of such loss/mutation is sufficiently high to provide for an incidence of cancer development in some carriers that approaches 100%. In this way cancer predisposition is inherited as a dominant genetic trait with an incidence within a given family which greatly exceeds that in non-carriers where two independent somatic mutations, involving that same gene pair, is demanded for cancer initiation. In essence, the presence of the first germ line mutation in carriers reduces the number of single cell gene targets for cancer initiation from two to one.

(270) It follows from this that the remaining wild type gene copy (allele) in somatic cells of tumour suppressor deficient individuals is wholly exposed to not only the mutagenic action of endogenous metabolic processes such as DNA thermoinstability, oxidative attack, and misreplication (Ames, 1989) but also the mutagenic action of a broad range of exogenous carcinogens. Given these circumstances there is a firm prediction of elevated tumorigenic radiosensitivity in individuals carrying germ line mutations of tumour suppressor genes. To what extent can this prediction be modelled in cellular mutational systems?

(271) As yet there are no quantitative cellular systems with which to assess specific tumour suppressor gene mutation after radiation. However, from the standpoint of somatic cell genetics such autosomal tumour suppressor gene mutations are wholly analogous to the induced loss of function of the remaining wild type *aprt* and *TK* alleles in rodent and human somatic cell lines that respectively carry single copy gene mutations at these loci; in both these cellular systems there is quantitative evidence on the mutagenic action of radiation that allows comment upon the likely radiosensitivity of different forms of tumour suppressor-deficient genotypes.

(272) These data on radiation mutagenesis of autosomal genes in the hemi- or heterozygous state are discussed by Thacker (1992) and in Section 2 of this report. In essence they show that in some but not all circumstances ionising radiation is a very efficient mutagen in respect of the remaining wild type allele. The most profound mutagenic effects of radiation in these in vitro autosomal mutation systems are seen when the first mutant allele carries an intragenic mutation and there are no genetic losses in genomic regions flanking that gene, i.e. a heterozygous state. The mutagenic action of radiation is, however, far less pronounced in hemizygous cells where the first mutation involves not only complete loss of one of the two genes but also losses to adjacent flanking DNA sequences. As noted in Section 2 it is believed that these differences in induced mutation rates reflect the inability of cells to sustain viability when there is homozygous loss of critical sequences. Thus when the first autosomal mutation (analogous to the germ line mutation of a tumour suppressor gene in a given disorder) is a large, DNA deletion the second, radiation-induced mutation may be restricted to the intragenic region of the remaining wild type allele, i.e. a small target resulting in a commensurately low mutation rate. Conversely, when the first mutation is intragenic the effective target for the second induced mutation can be much larger, extending perhaps to deletion of a large part of the whole chromosome. Induced mutation rates with such a large target are therefore very high.

(273) Extrapolating these in vitro findings to radiation effects in a range of human tumour suppressor gene deficiencies is not straightforward. The genomic position of the target gene in respect of essential flanking sequences and differential effects of hemi- and homozygous gene losses in different cell types will both play roles in determining tumorigenic responses (Section 2). Nevertheless, in conjunction with other data, two principal messages emerge.

(274) First, that since ionising radiation is known to principally exert its mutagenic action in mammalian cells via a molecular mechanism that strongly favours DNA deletion (Thacker, 1992) it may be expected to be an efficient agent for in vivo inactivation of the remaining wild type allele in somatic cells of tumour suppressor gene-deficient humans. Second, the relative effectiveness of such a deletion mechanism is predicted to be at its greatest when the first, germ line mutation is intragenic in nature. Importantly, surveys of the form of heritable tumour suppressor gene mutation in a range of disorders (e.g Table 3.4) provide clear evidence that intragenic germ line gene mutations are most common and that gross deletions are rare. Thus, in most cases, there is the expectation that tumorigenic radiosensitivity will be high. Experimental studies with rodents (Section 5.2) and human radiotherapeutic observations (Section 5.3) lend substantial support to this contention.

5.1.3. *Genetic defects in proto-oncogenes*

(275) From the standpoint of known mechanisms it is somewhat unclear as to whether germ line mutation of proto-oncogenes is likely to strongly affect sensitivity to radiation-induced cancer. Single copy and usually specific mutation of such genes is generally thought to be dominantly expressed and to have substantial effects on cellular phenotypes (Section 3). However, in instances where second somatic events leading to loss of the wild type allele and/or duplication of the mutant allele may increase phenotypic expression, some degree of tumorigenic radiosensitivity may be expected. Equally increased expression of cancer would be expected if somatic loss of an unlinked tumour gene were to increase the probability of the clonal development of neoplasia.

(276) This latter scenario might apply in the case of multiple endocrine neoplasia type 2A (MEN2A) and other closely related disorders which result from germ line mutation of *RET* proto-oncogene and familial melanoma associated with defects in the $p16^{INK4}$ gene (Section 3).

(277) In the absence of any relevant observations this scenario remains however speculative and, since cancer predisposition associated with proto-oncogene mutation appears to be very uncommon in humans, the paucity of guidance on this issue is not judged to be critical.

5.2. Rodent models of cancer predisposition

(278) Rodent models representing disease-associated human germ line mutations are playing an increasingly important role in biomedical research. Such models may be provided by animals carrying natural germ line mutations or, with increasing frequency, by transgenic animals in which specific mutations have been produced in germ cells by recombinant DNA techniques (Gordon, 1993). In the context of cancer predisposition the greatest progress is currently being made in respect of rodent models of specific human DNA repair and tumour suppressor gene disorders (Williams and Jacks, 1996).

5.2.1. *DNA repair deficient rodents*

(279) Using recombinant DNA 'gene knockout' techniques, mouse models of human xeroderma pigmentosum (XP) (Sands *et al*., 1995; Nakane *et al*., 1995; de Vries *et al*., 1995) and HNPCC (Baker *et al*., 1995; de Wind *et al*., 1995; Reitmair *et al*., 1995) have been constructed. These mutant mice show some but not all of the features of their human counterparts but mouse homologues of XP are, as expected, highly sensitive to UVR-induced skin cancer. As yet there are however no data relating to the response of XP and HNPCC mice to ionising radiation. Equally there are no data on the carcinogenic response to radiation in naturally occurring *scid* mice that have a genetic defect in the repair of DNA double strand lesions (Section 2).

(280) Following the molecular cloning and characterisation of a number of important DNA repair and repair-related genes it is however anticipated that radiation carcinogenesis data for a range of relevant gene-knockout mice will become available during the next few years. Of particular note is the recent construction of gene knockouts for *Atm*, *Brca1*, *Brca2*, and *Rad51* (see also Section 2). First, in the case of *Atm* it has been shown that the null mice are radiosensitive, defective in cell cycle control and prone to the spontaneous development of lymphoma and the associated chromosomal rearrangements (e.g Barlow *et al*., 1996; Xu and Baltimore, 1996). Second, embryonic lethality and radiation hypersensitivity in *Brca2 null* mice has been demonstrated to be mediated by Rad51 protein indicating that *Brca* protein may be a critical co-factor in the repair of DNA dsb (Sharan *et al*., 1997).

5.2.2. *Tumour suppressor gene deficient rodents*

(281) Rodent models of familial adenomatous polyposis (Moser *et al*., 1990; Fodde *et al*., 1994), neurofibromatosis type 1 (Jacks *et al*., 1994), Li–Fraumeni syndrome (e.g Donehower *et al*., 1992) and tuberous sclerosis type 2 (Kobayashi *et al*., 1995) have been characterised as showing increased cancer incidence although there are usually differences to their human counterparts in respect of the tumour spectrum and other phenotypic manifestations. Limited data on the tumorigenic response to radiation are available for three of these rodent models.

Mice deficient in p53

(282) Mice heterozygously deficient in germ line *p53* are analogous to the human cancer prone disorder Li–Fraumeni syndrome (LFS). Such mice generated by gene knockout techniques have been shown to exhibit an elevated spontaneous incidence of tumours, principally lymphomas and sarcomas, reminiscent of LFS (Donehower *et al*., 1992; Harvey *et al*., 1993; Kemp *et al*., 1994). These mice are also at increased risk of tumour development after exposure to a chemical carcinogen (Harvey *et al*., 1993).

(283) Most importantly however *p53* heterozygotes ($^{+/-}$) have been shown to be highly sensitive to radiation tumorigenesis (Kemp *et al*., 1994). These data on *p53* heterozygotes, which are based on tumour mortality up to 80 weeks of age following 4 Gy gamma ray exposure, are given in Fig. 5.1. The incidence of spontaneously arising fatal tumours in $p53^{+/-}$ mice was found to be high with a median value for tumour latency of >70 weeks. Spontaneous tumour incidence in *p53* null ($^{-/-}$) mice was higher still but the most dramatic effect of the null *p53* genotype was the shortening, to 21 weeks, of the median latent period for spontaneous tumours.

(284) Irradiation of $p53^{+/-}$ mice increased tumour incidence with no significant change in the tumour spectrum but the most profound effect of irradiation was a shortening of

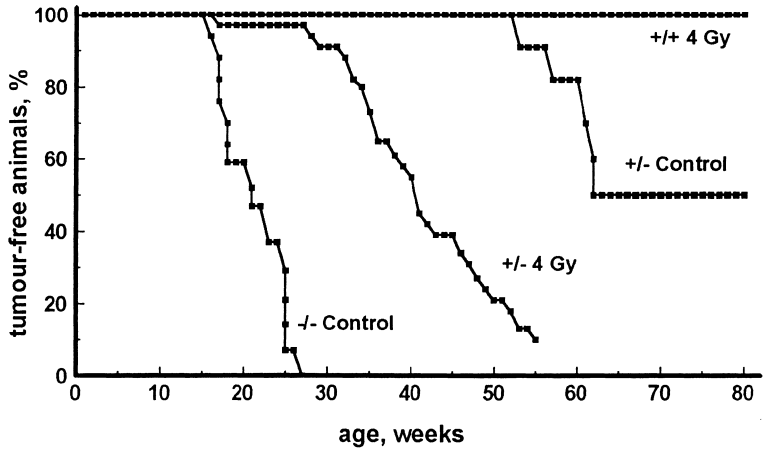

Fig. 5.1. Appearance with time of tumours in irradiated and unirradiated *p53*-deficient mice (from Kemp *et al.*, 1994).

median tumour latency from >70 weeks to 40 weeks, i.e towards the value determined for unirradiated $p53^{-/-}$ mice. Interestingly, irradiation had only a minor effect on tumour incidence and latency in $p53^{-/-}$ mice implying that the presence of a single wild type copy of *p53* was rate limiting for tumorigenesis and, by implication, that in $^{+/-}$ mice the wild type *p53* allele represented the principal target for radiation.

(285) This latter conclusion was supported by molecular analysis of tumours of $p53^{+/-}$ mice which showed that whereas 55–75% of spontaneous tumours had lost the remaining wild type *p53* allele, for radiation induced tumours that figure rose to 96%. More intriguing however was the finding that while only 20% of spontaneous tumours had duplicated the residual mutant *p53* allele, such duplication characterised around 80% of radiation induced tumours. Thus, mutant *p53* gene duplication via mitotic recombination or whole chromosome 11 nondisjunction followed by reduplication appeared to be characteristic of radiation tumorigenesis.

(286) During the 80-week time span of these studies neither control nor irradiated wild type ($p53^{+/+}$) mice developed tumours. Under these circumstances it is not possible to adequately quantify the degree of tumorigenic radiosensitivity associated with *p53* deficiency. Nevertheless, in the absence of tumours in control and irradiated wild type animals, the increase in tumorigenic radiosensitivity in $p53^{+/-}$ animals must be substantial and is also accompanied by a shortening in latency. These features appear to be associated with a tumorigenic mechanism after radiation that involves wild type *p53* gene loss and mutant gene duplication. Follow-up studies on the cytogenetic response of *p53* genotypes in the mouse (Bouffler *et al.*, 1995) allow further comment on these issues.

(287) In these studies mock and 3 Gy in vivo irradiation of $p53^{+/+}$, $^{+/-}$ and $^{-/-}$ mice was followed by analysis for persistent stable structural and numerical chromosomal changes in bone marrow cell populations; the short-term radiobiological endpoints of chromatid damage repair, sister chromatid exchange (SCE) and mitotic delay were also investigated in spleen cells in vitro. The in vivo frequency of spontaneously arising stable structural and numerical aberrations was elevated ~20 fold in $p53^{+/-}$ and $^{-/-}$ compared to $^{+/+}$. No excessive induction of stable structural aberrations was observed in vivo and in vitro studies revealed no evidence of a significant effect of *p53* deficiency in respect of

Table 5.1. Spontaneous and radiation-induced numerical chromosomal aberrations in bone marrow cells of wild-type and *p53*-deficient mice[a] (from Bouffler *et al.*, 1995)

Genotype	Radiation dose (Gy)	% 2n	% <2n	% >2n	Range of chromosome number/cell
++	0	98	2	0	38–40
	3	64	34	2	31–42
+−	0	74	26	0	34–40
	3	32	42	26	35– ~ 180
−−	0	48	46	6	36– ~ 120
	3	30	40	30	26– ~ 160

[a]Each percentage is based on chromosome counts from 50 cells; irradiated samples were taken at 29–30 days after 3 Gy γ-radiation.

chromatid damage repair or SCE induction after radiation. In contrast, in vivo studies showed *p53*-deficient genotypes to be 10–15-fold more sensitive to the induction of numerical chromosomal changes (chromosomal gains≫losses) and in vitro studies revealed a profound defect in the operation of post-irradiation mitotic delay in these *p53*-deficient genotypes implying failure of a G2/M cell cycle checkpoint for repair. It was concluded that this checkpoint failure was the basis of the chromosomal nondisjunction mechanism for increased hyperploidy which subsequently provided the drive for the increased tumorigenic radiosensitivity of *p53*-deficient mice.

(288) On the basis of this conclusion the quantitative data of Bouffler *et al.* (1995) relating to radiation induced hyperploidy may be used as a surrogate to judge the tumorigenic radiosensitivity of *p53*-deficient mice. These data shown in Table 5.1 suggest a factor of 12–13-fold increase in $p53^{+/-}$ mice compared to wild type. If however the degree of hyperploidy, as illustrated by the range of chromosome numbers in Table 5.1 column 6, were to be a positive factor in subsequent tumorigenic development, then the above judged value may underestimate the increase in tumorigenic radiosensitivity associated with *p53* deficiency.

Mice deficient in Apc

(289) Mice that are heterozygously deficient in the *Apc* gene are analogous to the human colon cancer prone disorder, familial adenomatous polyposis (FAP). The first example of this mouse mutant was identified following the observation of multiple intestinal neoplasia, the *Min* phenotype, in the progeny of an F1 animal following paternal exposure to a chemical mutagen (Moser *et al.*, 1990). It was further shown that (a) the *Min* phenotype mapped to mouse chromosome 18 in a region that was syntenic to the human 5q region encoding *APC* (Luongo *et al.*, 1993) and (b) that the *Min* mouse carried a mutant allele of *Apc* characterised by a nonsense mutation at codon 850 (Su *et al.*, 1992). In subsequent studies the *Min* mutation in certain genetic backgrounds has been shown to predispose to spontaneous mammary carcinoma as well as intestinal neoplasia (Moser *et al.*, 1995) and that the penetrance and expressivity of the underlying *Apc* mutation is strongly modified by a chromosome 4 encoded locus termed *Mom1* which may represent the phospholipase gene, *Pla2g5* (Dietrich *et al.*, 1993, MacPhee *et al.*, 1995).

(290) Molecular studies on the spontaneously arising adenomas of *Min* mice have shown them to be characterised principally by loss of the whole of the chromosome 18 that encodes the wild type *Apc* allele while the mutant allele is retained (Luongo *et al.*, 1994, Levy *et al.*, 1994). Such whole chromosome 5 losses have not been observed in the colonic tumours of human FAP patients, a result that may reflect a significant difference in

mechanisms of colon carcinogenesis between mouse and man (Moser *et al.*, 1995). Preliminary experiments with *Min* mice have revealed that the incidence of both intestinal and mammary tumours is increased by exposure of animals to the chemical carcinogen ethyl nitrosourea and that carcinogen sensitivity is critically dependent upon age at exposure (Moser *et al.*, 1995). Ongoing radiation tumorigenesis studies in hybrid Mom^{+} *Min* mice show that a whole body acute x-ray dose of 2 Gy increases the mean incidence of intestinal adenomas from 11 to 22 per animal (Ellender *et al.*, 1997). No adenomas were scored in control wild type mice and only rarely in irradiated wild type mice thus providing evidence of the large increase in absolute tumorigenic risk associated with *Apc* deficiency.

(291) In a similar way Luongo and Dove (1996) assessed the tumorigenic effects of gamma irradiation on Min mice and showed statistically significant increases in adenoma incidence after doses of 0.55 and 0.8 Gy. The different genetic backgrounds of the mice used in the studies of Ellender *et al.* (1997) and Luongo and Dove (1996) provide evidence that the modifying locus *Mom1* has equivalent effects on spontaneous and radiation-induced adenoma incidence.

(292) Importantly, and in accord with mechanistic predictions, studies in both laboratories give evidence of radiation-associated chr18 interstitial deletions in adenomas that encompass the wild type *Apc* gene (Luongo and Dove 1996; Silver *et al.*, 1996).

(293) Overall, given the high spontaneous incidence of intestinal neoplasms in *Apc*-deficient mice, the magnitude of the increases in adenoma incidence after radiation doses up to 2 Gy are modest and are judged to be broadly consistent with the application of relative radiation risk in the case of tumour suppressor gene deficiency (see Section 5.2.5). This view is also supported by data regarding genetic modification of *Apc* penetrance.

Rats deficient in Tsc2

(294) Dominantly inherited predisposition to spontaneously arising renal carcinoma in the rat was originally described by Eker and Mossige (1961). Eker rats typically develop bilateral multiple renal carcinomas with the responsible gene (*RC*) being highly penetrant. The *RC* gene has since been mapped to the proximal (q) region of rat chromosome 10 and, through direct study and lack of syntenic relationships, possible associations with rat homologues of the human *VHL*, *WT1*, and *MTS1* genes have been excluded (Yeung *et al.*, 1993, Hino *et al.*, 1993a). However a new conserved linkage group on rat chromosome 10 and human 16p13.3 has been established within which the Eker *RC* gene and the rat homologue of human tuberous sclerosis type 2 gene (*TSC2*) were shown to be closely linked. Following this, the Eker mutation was characterised as a germ line insertion of a 5kb DNA fragment into the rat *Tsc2* gene (Kobayashi *et al.*, 1995). Thus, the Eker rat provides a rodent homologue of human tuberous sclerosis type 2 (see Section 3) and, like the tumours of such patients, the renal tumours of Eker rats show genomic losses in the region of the predisposing gene (Kubo *et al.*, 1994).

(295) Although the overall phenotypic manifestations in human tuberous sclerosis patients (Section 3) and Eker rats differ in many respects they share a strong predisposition to renal tumours. This allows some comment upon studies conducted on radiation tumorigenesis in this rodent model (Hino *et al.*, 1993b). In these studies male and female rats carrying the Eker mutation were exposed in the renal region to gamma ray doses of 0, 3, 6 or 9 Gy. Animals were sacrificed at 10–11 months, the incidence of kidney tumours was assessed in serial tissue sections and a dose response for tumour induction was obtained.

Table 5.2. Radiation dose-response for renal tumour induction in Eker rats (from Hino *et al.*, 1993b)

Dose (Gy)	Males (affected)	T_m Exp[a]	T_m Obs $\pm$ SD	Females (affected)	T_f Exp[a]	T_f Obs $\pm$ SD
0	17(9)	4.4	5.4 ± 3.7	21(11)	2.8	2.4 ± 1.6
3	17(6)	22.4	21.7 ± 18.4	18(6)	11.8	11.0 ± 6.0
6	16(9)	40.4	38.7 ± 12.9	21(10)	20.9	23.8 ± 13.8
9	19(7)	58.4	59.9 ± 29.9	20(8)	30.0	28.4 ± 10.0
Total	69(31)			80(35)		

Exp, expected; Obs, observed.
[a]Expected numbers from the following equations: $T_m = 6.0D + 4.4$ and $T_f = 3.0D + 2.8$ where T_r = mean number of tumours in males (T_m) and females (T_f).

(296) These dose response data (Table 5.2) show that renal tumour incidence increases linearly with dose, the increase in yield at 9 Gy being 11–12-fold greater than control values. At the time of this study it was not possible to specifically identify heterozygous ($Tsc2^{+/-}$) animals prior to experimentation and therefore all offspring were irradiated. Since the $Tsc2^{-/-}$ genotype is embryo lethal the irradiated population will therefore comprise equal numbers of $^{+/-}$ and $^{+/+}$ genotypes. Nevertheless, given that $^{+/-}$ animals all develop characteristic renal tumours the data of Table 5.2 reflect only the radiation response of $Tsc2^{+/-}$ male and female animals. The authors note, however, that four of the rats judged to be $Tsc2^{+/+}$ genotypes had each developed one or two renal tumours of a non-Eker type after doses of 6 or 9 Gy.

(297) On the basis of the data of Table 5.2 it may therefore be projected that no more than eight tumours had arisen amongst 21 male plus 23 female $^{+/+}$ animals receiving 6 or 9 Gy, i.e $\sim$0.2 tumours per animal. A sum of the mean tumour yields per $^{+/-}$ animal following these doses provides a value of $\sim$38 which reduces to $\sim$34 after subtraction of the average spontaneous yield. Thus, according to the above projections, in the dose range 6–9 Gy, the increase in renal tumorigenic radiosensitivity in $Tsc2^{+/-}$ rats compared to wild type ($^{+/+}$) may, as a first approximation, be judged to be $\sim$170, i.e $34(^{+/-})/0.2(^{+/+})$.

(298) Since, however, $Tsc2^{+/-}$ rats would be expected to develop renal tumours at an earlier age than wild types and the study demanded animal sacrifice at 10–11 months it is almost certain that the wild type response is underestimated and consequently that the above projection significantly overestimates the degree of tumorigenic radiosensitivity in this animal model of human tuberous sclerosis. It is, however, difficult to escape the conclusion that *Tsc2*-deficient Eker rats exhibit renal-specific tumorigenic radiosensitivity that is dramatically greater than that of the wild type.

5.2.3. *Relative tumour risk in different mouse strains*

(299) In the same way that a naturally arising *Tsc2* gene mutation determines the renal carcinoma susceptibility of Eker rats, differences in specific tumour susceptibility between divergent inbred mouse strains probably has genetic origins. On this basis the study of Storer *et al.* (1988) provides data on which to comment upon genetic effects in radiation tumorigenesis. Estimates of natural spontaneous and gamma ray induced cancer risks were obtained for nine neoplasms arising in C3H and C57Bl mice and compared with previous data on BalbC and RFM mice. Two principal messages emerge from this study. First, there was acceptable concordance for most neoplasms for relative radiogenic risk in mice and humans. Second, and of direct relevance to this report, interstrain variation in natural cancer risk was broadly related to the variation observed in radiogenic cancer risk. Assuming that inherited factors are the principal determinants of natural cancer incidence

in different tissues, it follows that tumorigenic radiosensitivity is similarly affected. As yet it is not possible to relate these findings to specific murine genes and hence to human genotypes but the mapping strategies used to uncover the Eker mutation allow for this in the future. Overall, a genetic explanation for the data and conclusions of Storer *et al.*, are consistent with subsequent findings in defined cancer-prone rodent genotypes outlined earlier in this Section and also with the human radiotherapy observations that follow.

(300) The capacity of natural inbred mouse strains to study the influence of genetic factors on induced tumorigenesis has been well demonstrated by the studies of Demant and co-workers (e.g Fijneman *et al.*, 1996). In such studies multiple loci controlling chemical carcinogenesis in the lung have been revealed and evidence provided on the complexity of the gene–gene interactions that determine the incidence of induced tumours.

5.3. Radiotherapeutic observation

(301) Direct observation of increased tumorigenic radiosensitivity in genetically cancer-prone humans has been made in cases of retinoblastoma, nevoid basal cell carcinoma syndrome, neurofibromatosis and Li–Fraumeni syndrome receiving radiotherapy for primary malignant disease. For these disorders and particularly for retinoblastoma and nevoid basal cell carcinoma syndrome there is evidence of increased risk of second, therapy-related cancer.

5.3.1. *Retinoblastoma*

(302) A substantial fraction of children with the heritable (bilateral) form of retinoblastoma (RB) die as a consequence of second primary neoplasms that develop in later life (e.g Mulvihill and McKeen, 1977; Kingston *et al.*, 1987; Meadows, 1988; Eng *et al.*, 1993; Wong *et al.*, 1997). Since unilateral retinoblastoma develops sporadically as a

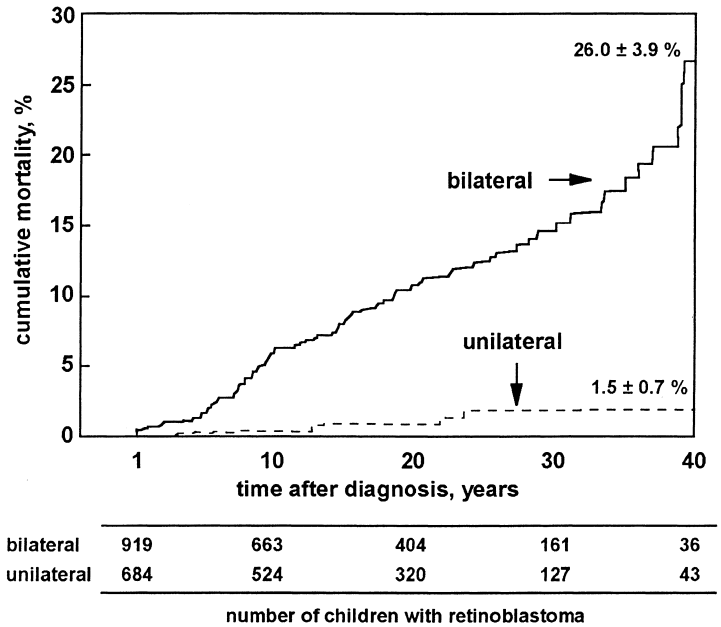

Fig. 5.2. Cumulative mortality from second primary neoplasms during follow-up of the entire cohort of 1603 retinoblastoma patients by laterality; bilateral (heritable) and unilateral (non-heritable) (from Eng *et al.*, 1993).

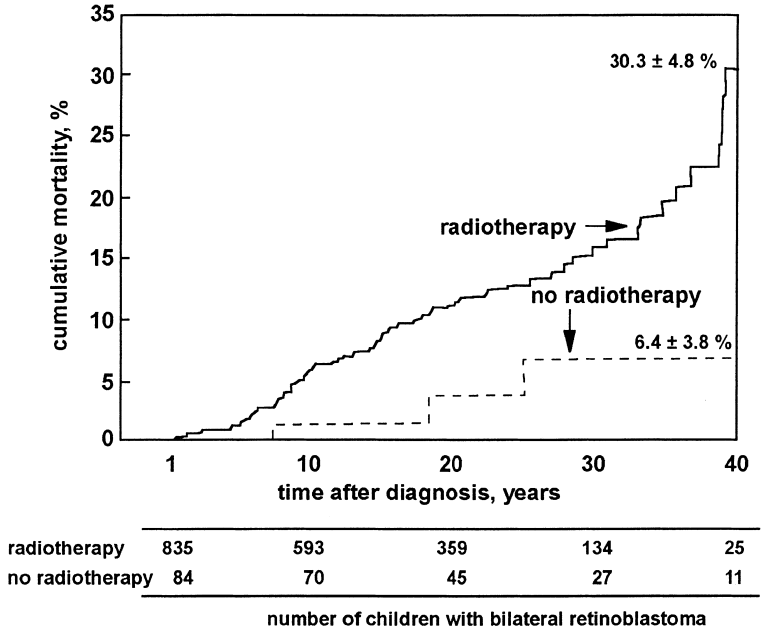

Fig. 5.3. Cumulative mortality from second primary neoplasms during follow-up of bilateral (heritable) retinoblastoma patients by treatment with and without irradiation (from Eng *et al.*, 1993).

non-heritable neoplasm in children it has been possible to compare the cumulative mortality rates associated with the genetic and non-genetic form of this malignancy.

(303) Figure 5.2 and Table 5.3 show a data set on RB compiled by Eng *et al.* (1993). This represents a follow-up of a cohort of 1603 retinoblastoma patients in the USA by

Table 5.3. Mortality from tumours other than retinoblastoma in patients who had been one year survivors of retinoblastoma by laterality and irradiation status (from Eng *et al.*, 1993)

	Observed (O) and expected (E) no. of deaths and O/E ratio[a]											
	Bilateral						Unilateral					
	Irradiated (n = 835)			Non-irradiated (n = 84)			Irradiated (n = 130)			Non-irradiated (n = 554)		
Cause of death	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E
Malignant tumours other than Rb	79	1.3	61[b]	5	0.2	22[b]	2	0.4	5	3	1.2	2
Bone	34	<0.1	630[b]	2	<0.1	270[b]	0	<0.1	0	0	<0.1	0
Connective tissue	15	<0.1	880[b]	0	<0.1	0	0	<0.1	0	0	<0.1	0
Skin melanoma	7	<0.1	180[b]	1	<0.1	120[b]	0	<0.1	0	1	<0.1	25
Brain	8	0.2	45[b]	0	<0.1	0	0	<0.1	0	1	0.1	7
Other cancers	15	1.0	15[b]	2	0.2	11[b]	2	0.4	5	1	0.9	1
Benign tumours	6	<0.1	100[b]	0	<0.1	0	0	<0.1	0	1	<0.1	22
All tumours other than Rb	85	1.3	63[b]	5	0.2	22[b]	2	0.4	5	4	1.2	3
Excess risk per 1000 person-years		5.8			2.5			0.8			0.2	

[a]Deaths are those in the US occurring after 1924. Rb = retinoblastoma.
[b]$P < 0.05$.

laterality and reinforces the conclusion of Section 2 that heritable RB patients are prone to develop a range of tumours, retinoblastoma usually being the first of these. Following diagnosis of retinoblastoma, heritable RB patients experience a rapidly rising risk of tumour mortality which, at 40 years after diagnosis rises to around 26% compared to only 1.5% in patients with non-heritable disease (Fig. 5.2). In the heritable RB group the principal second cancers were of bone and connective tissue although excess melanoma, brain neoplasms and tumours at other sites were also observed (Table 5.3). As noted by Eng *et al.* (1993) these data broadly accord with previous observations although there are differences in tumour types and cumulative mortality estimates which are probably explained by differences in study design, particularly the length of the follow-up period.

(304) For the purposes of this report the principal benefit of the study of Eng *et al.*, is the size of the cohort and the long period of follow-up which allow specific comment on the influence of prior radiotherapy for retinoblastoma on tumour incidence and mortality rates. These data which are reproduced in Table 5.3 and Fig. 5.3 show that the risk of second tumours in heritable RB is further increased by prior radiotherapy to an extent that significantly exceeds that in non-heritable RB. In heritable RB excess risk of mortality per 1000 person years was estimated to be 5.8 in the irradiated cases compared to 2.5 in those not receiving radiotherapy; Fig. 5.3 shows the commensurate divergence of the cumulative tumour mortality curves for these two groups.

(305) By contrast, in non-heritable RB the excess risk of mortality per 1000 person years was estimated to be 0.8 and 0.2 in the irradiated and non-irradiated groups respectively (Table 5.3). Thus, as a first approximation, these tumour mortality data imply that in heritable RB tumorigenic radiosensitivity as measured by cumulative mortality, is increased by around 5–6-fold when compared with non-heritable RB.

(306) Two features of the data suggest however that this simply judged factor may underestimate the true difference in tumorigenic radiosensitivity between normal humans and heritable RB. First, as noted by Eng *et al.*, the risk of second tumours in non-heritable RB appeared to be greater than expected (relative risk = 3.1; 95% CI = 1.0–7.3). The reason for this remains to be determined fully but may, in part, be associated with the presence of occult cases (perhaps 6–12%) of heritable RB in the group presenting with unilateral disease. Second, since the site of the second tumour in relation to the irradiated field could not be specified there is an inherent tendency for overall tumour mortality data to underestimate the true number of radiation-associated malignancies. Simply stated, the extent to which the tumour incidence in heritable RB is increased within the irradiated field is expected to be greater than the increase in their relative contribution to tumour mortality which considers all sites. Relevant to this issue are reports of multiple tumours within the locally irradiated tissues of heritable RB patients (see Traboulsi *et al.*, 1988).

(307) The same USA cohort of retinoblastoma patients have been the subject of a follow-up nested case-control study that considered doses and tumour site (Wong *et al.*, 1997). This study has provided evidence of a dose response relationship for the appearance of second bone and soft tissue sarcoma in heritable RB patients receiving radiotherapy.

(308) For all sarcomas combined the risk was evident at doses >5 Gy and rose to around 11-fold at 60 Gy and above. For soft tissue sarcoma the relative risks showed stepwise increases in all dose categories which were statistically significant at 10–29.9 Gy and 30–59.9 Gy. In this way the further follow-up of this cohort provides confirmation of previous studies showing that the risk of second cancers in heritable RB patients is substantial and is increased further by high dose radiation exposure.

(309) Since the number of second, therapy-related tumours in the qualifying non-heritable RB cases was very small there is, however, almost no information to be gained on the relative tumorigenic radiosensitivity of heritable versus sporadic RB patients. The data are not inconsistent with genetically determined increases in radiosensitivity but serve principally to highlight the uncertainties that attach to any quantitative judgements that may be made.

(310) In an attempt to confirm the increased tumorigenic radiosensitivity of heritable RB patients, analyses are underway to compare radiation risks of bone sarcoma in such patients receiving tumour radiotherapy in infancy and childhood with normal persons treated in childhood, adolescence and adulthood for non-malignant disease (Land, 1997). These analyses are based upon estimated doses to the local tissues in which bone sarcomas were observed.

(311) The preliminary results, while subject to considerable numerical uncertainty, provide further support to the hypothesis that bone tissue of heritable RB patients has an elevated sensitivity to radiation sarcomagenesis. The magnitude of this elevated risk remains most difficult to estimate but it is probable that heritable RB patients are at least 10× more sensitive to radiation sarcomagenesis than members of the general population (Land, 1997).

5.3.2. *Nevoid basal cell carcinoma syndrome*

(312) Evidence that nevoid basal cell carcinoma syndrome (NBCCS) patients are exquisitively sensitive to the carcinogenic effects of ionising radiation dates back to 1949. Scharnagel and Pack (1949) reported on a 5-year-old male patient typical of NBCCS with a history of radiotherapy for thymic enlargement who had developed multiple (>1000) pigmented basal cell skin lesions within the irradiated field (Fig. 5.4).

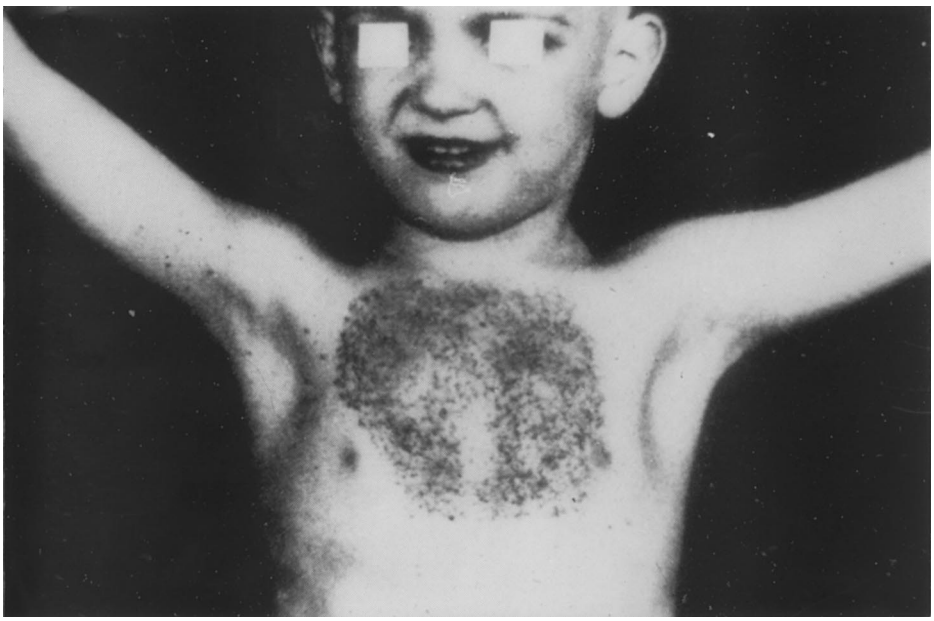

Fig. 5.4. Basal cell nevi after thymic irradiation in nevoid basal cell carcinoma syndrome. Reproduced from Scharnagel and Pack (1949) with kind permission. © 1949, American Medical Association.

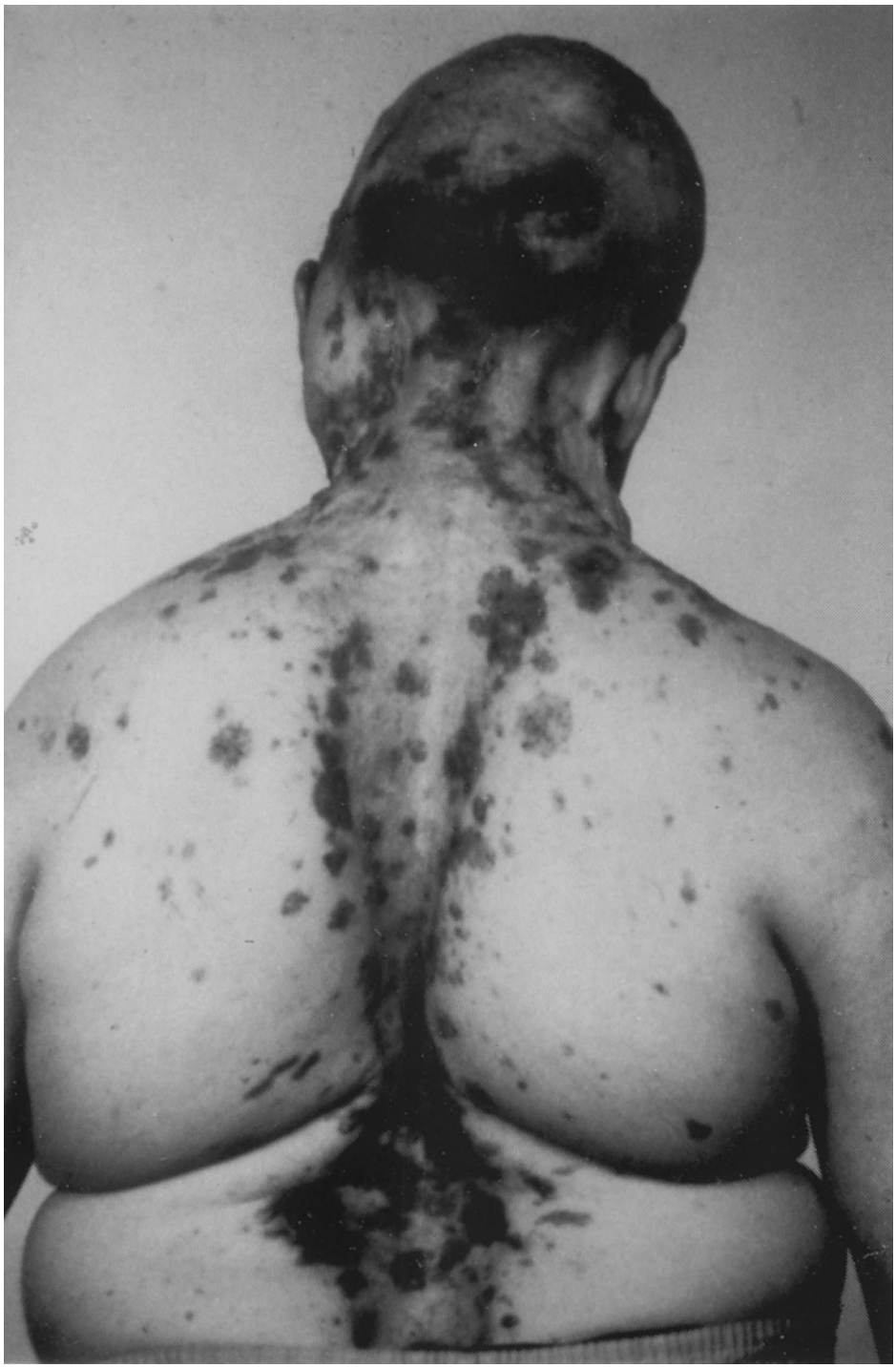

Fig. 5.5. Basal cell nevi and carcinoma after craniospinal irradiation in nevoid basal cell carcinoma syndrome (courtesy of G. Evans).

(313) Subsequently there have been a number of reports detailing the induction of multiple basal cell carcinomas (BCC) in NBCCS patients receiving craniospinal radiotherapy for medulloblastoma, a tumour to which such patients are prone (see Strong, 1977; Hawkins *et al*., 1979; Cutler *et al*., 1968). Similar findings have been made in NBCCS patients treated for rhabdomyosarcoma (Schweisguth *et al*., 1968) and other disorders (Rayner *et al*., 1976). It is, however, evident from the case reports summarised by Southwick and Schwartz (1979) that tumorigenic radiosensitivity in NBCCS may not be uniformly high.

(314) An example of the response of such NBCCS medulloblastoma patients to craniospinal radiotherapy is shown in Fig. 5.5. Many hundreds of basal cell nevi arise within the irradiated field during the first five years post-irradiation with a tendency to cluster on the periphery of the field where the therapy dose was insufficient to sterilise basal target cells in the skin. In subsequent years a much smaller number of new nevi continue to develop outside the irradiated field, principally in sun exposed areas (Strong, 1977) indicating that the dramatic <5-year carcinogenic response is radiation dependent. In some patients, BCC development after radiation is seen as early as 6 months, a latent period substantially shorter than for spontaneous BCC in NBCCS; in normal patients the latent period for radiogenic BCC is reported to be around 20 years (see Strong, 1977).

(315) In addition to BCC, female NBCCS patients are also prone to the development of post-pubertal ovarian fibromas but pre-pubertal neoplasms are rare. Two young (<2.5 years) female patients surviving thoracic and lumbar spine irradiation for medulloblastoma were however reported to have developed multiple pre-pubertal ovarian fibromas <5 years post-irradiation (Strong, 1977; Heimler *et al*., 1979). Thus, unusually short latency for radiation-induced malignancy seems to be characteristic of NBCCS.

(316) None of these data allow for specific quantification of the tumorigenic radiosensitivity of NBCCS and there are insufficient radiotherapy observations to allow for the type of study conducted with hereditary RB by Eng *et al*. (1993). In spite of this, the induction of such large numbers of BCC as illustrated in Figs 5.4 and 5.5 and the incidence of otherwise rare multiple ovarian neoplasms in irradiated young females provides strong evidence that NBCCS is a condition that exhibits a high level of genetically determined tumorigenic radiosensitivity. The tumours arising $\sim$5 years post-therapy in irradiated NBCCS are of the same type that arise spontaneously in the disorder, but there are no reports of extended post-irradiation follow-up in order to judge whether this tumour spectrum changes with age. An important finding is the unambiguously short latency for radiation-induced tumours in NBCCS, a feature that is reminiscent of the response of *p53*-deficient mice.

5.3.3. *Li–Fraumeni syndrome and neurofibromatosis*

(317) A number of reports highlight the possible role of genetic factors in the development of second tumours in children receiving chemo- and radiotherapy (e.g Meadows *et al*., 1977; Meadows *et al*., 1980; Tucker *et al*., 1984; Hawkins, 1990; Heyn *et al*., 1993; Robison and Mertens, 1993). In addition to the most obvious association with heritable RB, a crucial early finding was made in the families of five children where there was a high incidence of breast cancer, acute leukaemia, carcinoma of the lung, pancreas and skin, brain neoplasms, and adrenocortical carcinoma (Li and Fraumeni 1969). Further study culminating in a report of 24 cancer families (Li *et al*., 1988) led to such familial cancer clustering being termed the Li–Fraumeni syndrome (LFS). This disorder is now

known to be associated, albeit not exclusively, with germ line mutation of the *p53* tumour suppressor gene (Section 3).

(318) Similar follow-up studies, discussed by Strong and Williams (1987), of patients developing second soft tissue sarcoma showed that 9/161 eligible patients followed for 3–30 years had developed a second neoplasm compared with an expected 0.5/161. Family studies were consistent with a diagnosis of LFS and the presence of 5/9 of these secondary sarcomas within the irradiated field is strongly suggestive of a relatively high degree of tumorigenic radiosensitivity. More recently, Heyn *et al.* (1993) have added further support to this conclusion by showing LFS-like familial patterns of cancer in 12/13 eligible patients from a total of 22 who developed second cancers (principally bone sarcoma and acute leukaemia) following radiotherapy for rhabdomyosarcoma.

(319) The specific involvement of germ line *p53* deficiency in the tumorigenic radiation response of LFS is more directly evidenced by molecular studies of cancer patients, some of whom had presented with therapy-related bone and soft tissue sarcomas (Toguchida *et al.*, 1992; Malkin *et al.*, 1992). Thus, although these data do not allow for quantification of increased tumorigenic radiosensitivity in humans deficient in *p53*, the responses seen generally accord with those of mice carrying a deficiency in the same gene (Kemp *et al.*, 1994).

(320) A number of studies report that neurofibromatosis is a positive factor for therapy-related second tumours in patients (Kingston *et al.*, 1987; Heyn *et al.*, 1993; Robison and Mertens, 1993). Sznajder *et al.* (1996) found 63 cases of single and seven of multiple neural tumours (schwannomas, vestibular schwannomas, and meningiomas) that developed in a cohort of 3013 people given x-ray beam therapy (skin doses of 750 mGy per course of treatment) before age 16 for benign head and neck conditions, i.e. a highly significant excess. Sixteen of 25 tumours in the seven multiple tumour cases were spinal root schwannomas, compared to 28 of the 63 single tumours, a suggestive difference ($P = 0.07$). There was no radiation dose difference with respect to tumour number (zero, one, or multiple). Only one of the multiple tumour cases met the strict diagnostic criteria for NF2 and follow up molecular studies of candidate germ line mutations in multiple tumour cases are necessary to clarify the possible genetic basis of these observations.

5.3.4. *Other genetic conditions and familial associations*

(321) In a study of 286 sib-pairs who received childhood radiation treatment for benign conditions, within family concordance was statistically significant ($P = 0.05$) for benign and malignant thyroid neoplasia considered as a group with suggestive evidence ($P = 0.18$) of concordance for radiation-associated thyroid cancer (Perkel *et al.*, 1988). These results imply that familial factors either genetic or environmental played a role in the determination of risk in the irradiated cohort but do not provide compelling evidence of specific genetic modification of radiation response.

(322) Since breast cancer is believed to have a relatively large genetic component, genetic modification of radiation response might be evidenced by excess risk of contralateral disease in certain patients receiving scattered radiation from treatment of unilateral breast cancer. Data on this and other breast cancer cohorts discussed by Mark *et al.* (1994) however provide no consistent evidence for an excess risk of radiation-associated breast or other cancers that might have a genetic basis other than LFS and LFS-like conditions (Smith *et al.*, 1993). Boice *et al.* (1992) found that 655 women in whom a second breast cancer developed five or more years after the initial diagnosis were significantly more likely to have received radiotherapy than 1189 matched controls who

did not have a second breast cancer. The excess was limited to women treated before 45 years of age, and the estimated radiation-related risk was similar to that found in other studies of women whose radiation exposure was for reasons other than breast cancer treatment. Storm *et al.* (1992) found no excess of contralateral breast cancer risk in another series of women treated with adjuvant radiotherapy. The absence of risk was attributed to the fact that the ages of treatment for most of the cases were older than those associated in other studies with appreciable radiation-related risk. Tokunaga *et al.* (1994) found 20 bilateral cases, synchronous and asynchronous, among 809 female breast cancer cases observed in the Japanese A-bomb LSS sample during 1950–1985, including 15 of 593 cases among exposed women for whom radiation dose estimates were available. The proportion of bilateral cases did not increase or decrease with radiation dose, and there was therefore no suggestion that the bilateral cases were drawn from a subpopulation with increased radiation sensitivity. There are also data of potential relevance that concern second cancers following therapy for childhood Hodgkins disease (HD) (Donaldson and Hancock, 1996; Bhatia *et al.*, 1996). In the study of Bhatia *et al.* (1996) the risk of therapy-related solid tumours and especially breast cancer was shown to be high (standardised incidence ratio for breast cancer of 75.3; 95% CI = 44.9–118.4). Of particular note was the atypical increased risk within five years of radiotherapy, an observation that finds echoes in the genetically determined early tumorigenic response of other patients discussed in this Section. The extent to which these findings reflect genetic susceptibility in a subset of HD patients remains most uncertain, but it is notable that a proportion of early onset HD in the population probably has a genetic basis (see Section 4).

(323) More information on familial patterns of breast cancer in radiotherapy patients is important to clarify the position. In this context, an excess of breast cancer has been reported in the mothers of children who developed therapy-associated second tumours (Birch *et al.*, 1990). The same need for comprehensive family studies also apply to the possibility of ascertaining the response to radiotherapy of patients who might present with heritable forms of colon cancer (Rustgi, 1994).

(324) In addition, Kony *et al.* (1997) have recently reported on a case control study to determine possible interactions between genetic factors and radiotherapy in childhood for the production of second malignant neoplasms (SMN). This study, which involved 25 children with SMN and 96 children with no SMN, gave a relatively modest odds ratio of 4.7 (95% CI 1.3–17.1) for SMN in those treated patients with a family history of early onset cancer.

(325) Secondary lympho-haemopoietic neoplasia can be a consequence of radiotherapy in childhood (e.g Kingston *et al.*, 1987, Meadows, 1988, Hawkins, 1990, Heyn *et al.*, 1993). Given the judgement that the genetic component of this category of tumours is relatively low (see Section 4) it is not surprising that no clear familial patterns have yet emerged in patients with therapy-associated leukaemia and lymphomas (Kingston *et al.*, 1987; Heyn *et al.*, 1993). Since however *p53* and *NF1* germ line mutation can predispose to acute leukaemia and there is a familial association for early onset Hodgkin's disease (see Section 4), a genetic basis may be expected in a few rare cases.

(326) Finally, while there is unambiguous evidence on the extreme sensitivity of ataxia-telangiectasia (A-T) homozygotes to the deterministic effects of radiotherapy (see Pritchard *et al.*, 1982; Fig. 5.6) too few patients have survived treatment to allow for follow-up studies of tumorigenic outcomes. Levin and Perlov (1971) note however that chronic dermatitis and basal carcinoma developed in the irradiated field of one A-T patient who

Fig. 5.6. Deterministic tissue effects after irradiation in ataxia-telangiectasia. Reprinted by kind permission of Wiley-Liss Inc., a subsidiary of John Wiley & Sons, Inc., from Pritchard *et al.* (1982). © American Cancer Society.

had received irradiation of the scalp for tinea capitis. The uncertain status of epidemiological studies on the tumorigenic response of A-T heterozygotes are discussed later in this section.

5.4. Epidemiological aspects of tumorigenic radiosensitivity

(327) The possibility that genetic variation may be reflected in differential susceptibility among individuals to radiation carcinogenesis is highly plausible by analogy to other carcinogens and other disease experience. The observed excesses of soft tissue sarcoma, osteosarcoma, and basal cell carcinoma in certain cancer-prone individuals receiving radiotherapy (Section 5.3) provide persuasive evidence of a genetically-related susceptibility to radiation carcinogenesis in patients known or strongly suspected to be suffering from cancer-predisposing genetic disorders. Other arguments have often lacked credibility for various reasons, however, especially when the claimed association has been with low-dose exposure (Bross and Natarajan, 1977; Land, 1977; Swift *et al*., 1991; Boice and Miller, 1992; Hall *et al*., 1992; Land, 1992).

5.4.1. *Breast cancer risk in ataxia-telangiectasia heterozygotes*

(328) In respect of breast cancer risk and potential radiosensitivity much attention has been given to carriers of the *ATM* gene—ataxia-telangiectasia heterozygotes. A principal reason for this attention is the relatively high prevalence (~1 in 200) of *ATM* gene carriers in the population which, depending upon breast cancer susceptibility and radiosensitivity, may allow for a significant contribution to population risk. As detailed below it is judged that the association between *ATM* heterozygosity and breast cancer may not be as secure as some commentators have suggested.

(329) As noted in Section 6 of this report, Easton (1994) has reviewed earlier published data regarding the incidence of breast cancer amongst obligate and predicted ataxia-telangiectasia (*ATM*) heterozygotes. Although these analyses broadly supported the contention that such heterozygotes do show increased risk of spontaneously arising breast cancer, considerable uncertainty was recognised. Of particular concern is the unusually low incidence of breast cancer in the controls of the two largest studies (Swift *et al*., 1987; Swift *et al*., 1991).

(330) The cloning and sequencing of the *ATM* gene has enabled direct examination of the hypothesis that breast cancer risk is increased among *ATM* heterozygotes. Two approaches have been used. In the first, DNA from breast cancer cases belonging to cancer families (Vorechovsky *et al*., 1996a) or who were blood relatives of A-T homozygotes (Athma *et al*., 1996) have been assayed for germline *ATM* mutations. In the second, the assays have been applied to breast cancer cases (Vorechovsky *et al*., 1996b) or early-onset breast cancer cases and non-cases (Fitzgerald *et al*., 1997) from the general population. The two approaches have yielded somewhat different results leading to different conclusions; however, the frequency of *ATM* heterozygotes in the general population is not known with sufficient accuracy for these results to be judged statistically inconsistent (Bishop and Hopper, 1997).

(331) Vorechovsky *et al*. (1996a) examined 88 index cases with primary breast cancer, each having a family history of breast cancer and/or a family history of tumours previously associated with A-T homozygosity or heterozygosity. Three instances of heterozygotic germline *ATM* mutations were found, two in members of breast cancer families and the other in a family with multiple cancers other than breast cancer. The study demonstrated the occurrence of germ line *ATM* mutations in cancer families but

provided little insight into the role of *ATM* as a breast cancer risk factor. A more ambitious approach was undertaken by Athma *et al.* (1996) who determined A-T gene carrier status of 775 blood relatives in 99 A-T families. Of these, there were 33 female breast cancer cases in 28 families who could be phenotyped; 25 were *ATM* heterozygotes compared to 14.9 expected on the basis of their known familial relationships to the A-T probands. A statistically significant odds ratio (OR) of 3.8 was obtained, with 95% confidence limits 1.7–8.4, for breast cancer risk among *ATM* heterozygotes as opposed to non-carriers. The odds ratio was non-significantly higher for cases with breast cancer diagnosis at age 60 or older than for younger cases, and the authors used age-specific odds ratios to estimate that, if the US population frequency of *ATM* heterozygotes is 1.4%, 6.6% of all breast cancers may occur in women who are AT heterozygotes. This estimate is several times higher than that estimated for *BRCA1* carriers.

(332) Vorechovsky *et al.* (1996b) screened 38 consecutive breast cancer patients using an exon-scanning PCR single-strand conformation polymorphism assay for mutation detection of *ATM* and found no evidence of either somatic *ATM* genetic changes in breast tumour samples or mutant *ATM* carriers. With this small number of subjects, the upper 95% confidence limit for the frequency of *ATM* carriers among breast cancer patients is 9.7%. Fitzgerald *et al.* (1997) undertook a germ line mutational analysis in a cohort of 401 women diagnosed with breast cancer at age 40 or younger, and 202 healthy women with no prior history of breast cancer, using a cDNA-based protein truncation assay. Definitive chain-terminating mutations were detected in 2/401 women with breast cancer (rate 0.5% with 95% confidence bounds 0.06%–1.6%) and in 2/202 non-cases (rate 1.0% with confidence limits 0.12%–3.6%). The rate ratio is 0.50 (the odds ratio is 0.501) with confidence bounds 0.12–6.95. Thus, although 0.50 is substantially less than the value 3.8 obtained by Athma *et al.* (1996), the two estimates are statistically consistent.

(333) As pointed out by Bishop and Hopper (1997) the confidence limits for the rate ratio estimated by Fitzgerald *et al.*, are wide because the estimated rate for the non-cancer rate denominator is based on only two positive events. If, however, we accept the conclusion of Athma *et al.*, that the prevalence of *ATM* is greater among breast cancer cases than in the general population, it follows that four positive findings among 401 women with and 202 without breast cancer must overestimate the population prevalence of germ line *ATM*, at least after adjustment for the imperfect ascertainment efficiency of the protein truncation assay used by Fitzgerald *et al.*, For example, suppose the ascertainment efficiency to be 70%, and the odds ratio estimate of 3.8 obtained by Athma *et al.*, is known without error and applies to the population studied by Fitzgerald *et al.*, On this basis, the population prevalence of *ATM* in the Fitzgerald population would be estimated as $(4/0.70)/(202 + 3.8 + 401) = 0.33\%$, with 95% confidence limits 0.10%–0.79%.

(334) A more realistic assessment is obtained by adding uncertainty terms for the *ATM* odds ratio among breast cancer cases and for the ascertainment efficiency of the protein truncation method, and obtaining subjective confidence limits for the population prevalence based on a Monte Carlo simulation analysis. The rationale is as follows: For fixed ascertainment efficiency E and fixed odds ratio R, the number of positive findings should be distributed approximately as a binomial random variable with sample size $n = E + (202 + R + 401)$ and unknown binomial probability, p. Thus, the conditional probability of obtaining four positive findings would be predicted to be

$$nP(X = 4) = 4\,p^4(1 - p)^{n-4}.$$

Conversely, the conditional likelihood function for p, given $X = 4$, E, and R, is also proportional to $p^4(1-p)^{n-4}$. Thus, in a Bayesian sense, the (conditional) subjective uncertainty for p follows a beta distribution with 5 and n–3 degrees of freedom. By conducting a large number of trials, in which pseudo-random realisations are generated of E, R, and (conditionally on the realised values of E and R) of p, it is possible to estimate the subjective uncertainty distribution of p conditional only on $X = 4$.

(335) A lognormal uncertainty distribution for the odds ratio R, with median 3.8 and geometric standard deviation (GSD) 1.5, agrees well with the 95% confidence limits 1.7–8.4 obtained by Athma *et al.*, The efficiency E of the protein truncation method might be assigned a rectangular uncertainty distribution with minimum 0.55 and maximum 0.85. A Monte Carlo simulation analysis based on 1600 independent trials produced a central value of 0.39% for the population prevalence of *ATM*, with 95% uncertainty limits 0.10%–1.13% and 90% limits 0.13%–0.97%. Similar results (central value 0.39%, 95% limits 0.11%–1.15%, 90% limits 0.13%–1.01%) were obtained assuming a lognormal uncertainty distribution for $1-E$ with median 0.3 and GSD 1.2, corresponding to subjective 95% confidence limits 0.57–0.79 for E.

(336) This analysis suggests that the findings of Athma *et al.*, and Fitzgerald *et al.*, may be consistent only if a population prevalence less than about 1% is assumed for heritable *ATM* mutations in the population studied by Fitzgerald *et al.*, Alternatively, it is conceivable that the two studies simply may not be comparable, as would be the case if germ line *ATM* were a strong risk factor only for breast cancer diagnosed after 40 years of age.

(337) In respect of a possible association between *ATM* and radiation-induced breast cancer the current position is even more contentious. Swift *et al.* (1991) reported that female heterozygotes have 5.1 times the breast cancer risk of the general population, that 20% of heterozygotes have been exposed to medical radiation, and that those exposed have 5.8 times the breast cancer risk of the non-exposed remainder. Kuller and Modan (1992) and Boice and Miller (1992) have suggested alternative interpretations of the data presented by Swift *et al.*, leading to (statistically non-significant) relative risk estimates of 1.8 and 1.6, respectively, for medically exposed cf. non-exposed heterozygotes, and have pointed out other aspects of the study that require clarification or may violate principles of epidemiological inference. The estimates provided by Swift *et al.*, imply relative risks for the medically unexposed and exposed heterozygotes of 2.6 and 15.1 times, respectively, that of the general population. The ratio of excess relative risks in the two groups, 1.6 to 14.1, is 8.8. Extreme sensitivity to the carcinogenic effects of ionising radiation implies that all heterozygotes must suffer from the effects of the 1 mGy received by everyone, every year, from natural background radiation. If *all* of the excess breast cancer risk among heterozygotes is due to radiation exposure, the ratio of exposure between the medically exposed and non-exposed subpopulations should be about 8.8; otherwise, the ratio must be even higher. Thus, the numerical estimates presented by Swift *et al.*, imply cumulative levels of medical exposure for 20% of the heterozygote population that are impossibly high (Land, 1992). It may be that heterozygotes are indeed sensitive to radiation-related breast cancer, but the current argument presented in support of that inference was flawed. Concerns on the potential tumorigenic radiosensitivity of these heterozygotes should also be tempered by uncertainties noted above on the strength of the association between *ATM* and spontaneous breast cancer. In spite of these uncertainties, clear evidence has been presented that heritable mutations and ionising radiation exposure are both important cancer risk factors and, as discussed earlier in this Section, interaction of these factors

should be expected. As noted earlier in this Section of the report, there is however no expectation that all genetic determinants of cancer will show strong interactions with radiation.

5.4.2. *Contributions from family studies and modifiers of cancer risk*

(338) It has long been recognised that unusually high cancer risk can be familial and it is becoming clear that such patterns often reflect inherited susceptibility as distinguished from shared environments and lifestyles. Studies of hereditary diseases predisposing to cancer have led to the identification of tumour suppressor genes, such as the retinoblastoma susceptibility gene (*RB1*), genes associated with neurofibromatosis of types 1 and 2 (*NF1* and *NF2*), and the (*p53*) gene mutation in the Li–Fraumeni syndrome (see Section 3). Other studies, of kindreds with exceptionally high levels of cancer risk, have led to the identification of other tumour suppressor genes, inherited mutations of which are associated with increased risk of breast and ovarian cancer (*BRCA1* and *BRCA2*) or heritable nonpolyposis colon cancer (*MSH2*, *MLH1*, *PMS1*, and *PMS2*) (Skuse and Ludlow, 1995).

(339) There is substantial evidence that the excess cancer risk associated with exposure to certain carcinogens, like ionising radiation, may vary by sex and age at exposure, and that it may be modified by life events (e.g. reproductive history, tobacco use) prior to or following exposure. Women with heritable mutations in *BRCA1* or *BRCA2* were initially estimated to experience between 5 and 10% of all breast cancers occurring in the US population, and to have individual lifetime risks of 80% or more (Ford *et al.*, 1994; Wooster *et al.*, 1994). More recent estimates suggest however that less than 2% of all breast cancers are determined by genes of high penetrance although that figure rises to around 10% in cases diagnosed before age 40 (Peto *et al.*, 1996). However, although the distribution of that risk over their lifetimes is disproportionately heavier at young ages than in the rest of the population, it is not concentrated there (Ford, 1994). Thus, even for a powerful risk factor present from conception, the expression of risk awaits further developmental events. Thus the timing, if not the likelihood, of the occurrence of familial breast cancer appears to be governed to a large extent by events unrelated to the presence of germ line mutations. These may be presumed to be the same categories of lifetime events that determine the age-specific distribution of sporadic breast cancer risk. There is also the possibility of modification of expression of such mutations in certain genetic backgrounds (see Sections 5 and 7). In contrast, at least for risk after age 35 or so, the timing of radiation-related breast cancer risk in particular, and of most other radiation-related solid cancers in general, is similar to that of non-radiation-related cancer and therefore would appear to be almost wholly determined by events unrelated to exposure (Tokunaga *et al.*, 1994; UNSCEAR, 1994).

(340) Site-specific cancer risks vary by population, and there is a small but growing body of information that allows us to compare different populations, with different site-specific baseline cancer rates, with respect to susceptibility to radiation carcinogenesis. For breast cancer, it appears that radiation-related risks, in *absolute* terms, are similar in the US and Japan, despite the two to seven-fold difference in age-specific baseline rates (Boice *et al.*, 1979; Land *et al.*, 1980; Land, 1992; UNSCEAR, 1994), whereas for stomach cancer, radiation-related *relative* risks appear similar (NCRP, 1997). More generally, results from migrant studies tend to indicate that, although baseline, site-specific cancer rates may vary considerably by population, such differences usually reflect common lifestyle factors that tend to change slowly over time following arrival in the host country,

rather than genetic traits characteristic of a population as a whole (Haenszel and Kurihara, 1968; Buell, 1973; Ziegler *et al.*, 1993). Thus, comparisons of radiation-related risk in different populations seem unlikely to be useful for studying *genetic* variation in radiation susceptibility.

(341) Nevertheless, it is interesting to speculate on the role of heritable mutations in relation to site-specific cancer risk in countries with radically different baseline rates. For example, Japanese women have about one-fourth the lifetime breast cancer risk of US women, whereas rates among their US-born descendants are typical of US rates (Zeigler *et al.*, 1993); moreover, as previously mentioned, excess rates associated with specified radiation exposures appear to be about the same in the two countries. It is therefore conceivable that inherited *BRCA1* and *BRCA2* mutations might be responsible for 10–20% of the much lower level of Japanese breast cancer risk; it is interesting, for example, that rates of early-onset breast cancer (i.e. before age 35 or before age 45) in Japan are about half those among US Whites or among US residents of Japanese descent in Los Angeles or Hawaii (Parkin *et al.*, 1992), whereas rates at older ages differ by factors of 3–7. Alternatively, Japanese environmental and lifestyle factors might reduce the influence of heritable gene mutations in the same way that these factors seem to minimise Japanese baseline breast cancer risk.

(342) Unfortunately, few studies have been published on the role played by *BRCA1* and *BRCA2* mutations in Japanese breast cancer risk. A recent study by Inoue *et al.* (1995) analyzed germline mutations of the *BRCA1* gene in 18 Japanese breast cancer and two breast–ovarian cancer families. The same nonsense mutation, previously not observed, was found in two of the breast cancer families, but nothing in the others. The study results suggest that the spectrum of heritable *BRCA1* mutations may be substantially different in ethnically distinct populations. Also, the relative rarity of breast cancer in Japan may be a major obstacle in finding true breast cancer families, since the numbers commonly used in western studies to define such a family in a high risk population may be simply unattainable, and environmental and lifestyle factors may have more of a role in producing cancer frequencies that satisfy less stringent selection requirements.

5.4.3. *Susceptibility to radiation carcinogenesis in Japanese A-bomb survivors*

(343) Dose-response analyses of breast cancer risk among Hiroshima–Nagasaki A-bomb survivors exposed before age 20 (Land *et al.*, 1993; Tokunaga *et al.*, 1994) found an excess relative risk of 13.5 at one Sv for radiation-related breast cancer diagnosed before age 35, compared to 2.3 among those diagnosed at later ages, a statistically significant, six-fold difference (Fig. 5.7). The finding pertains to women exposed during either childhood or adolescence, but not to women exposed after age 20. Preliminary analyses (Land, 1997), based on incidence data through 1990, demonstrate that the marked excess in radiation-related excess relative risk is seen among women exposed at 0–9 and 10–19 years of age (Fig. 5.8, A and B).

(344) Moreover, the markedly greater excess relative risk for early-onset breast cancer as compared to later-onset risk does not merely reflect a general tendency for excess relative risk to decline with increasing age at diagnosis; there is no evidence of a continuing decline with age for cancers diagnosed after age 35, regardless of age at radiation exposure (Fig. 5.8). While far from conclusive, the finding suggests the possibility of increased susceptibility among a subgroup genetically predisposed to breast cancer in general and to early-onset breast cancer in particular, e.g. women who have inherited mutations in *BRCA1*, *BRCA2*, or other predisposing genes of lower penetrance.

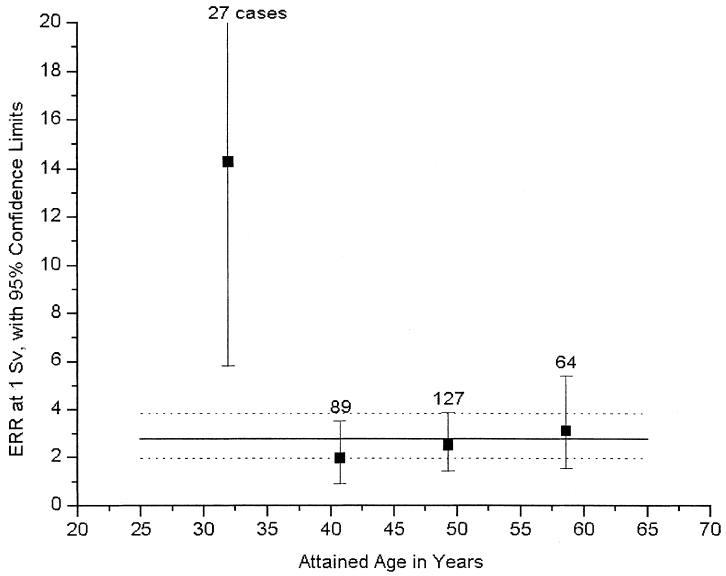

Fig. 5.7. Dose-specific risk of radiation-related breast cancer among female A-bomb survivors exposed before age 20 years of age: overall (horizontal lines) and by age at diagnosis (points with error bars).

5.4.4. *Genetic and molecular studies in irradiated populations*

(345) A search for possible familial aggregations of cancer in irradiated populations under study represents an obvious genetic approach to the problem. One attempt, to explore family history of cancer as a possible modifier of A-bomb radiation as a cause of breast cancer, failed because of a general lack of information on the part of interviewed cancer cases and controls about cancer diagnoses within their families, or even themselves (Land *et al.*, 1994a,b). Traditionally, it is not usual for Japanese physicians to inform their patients that they have cancer. An alternative, now being explored at the Radiation Effects Research Foundation (RERF) in Hiroshima and Nagasaki, is to use existing records to identify relatives of breast cancer cases, find their addresses and, for subjects living in Hiroshima or Nagasaki, match their names against the local tumour registries.

(346) The recent identification of specific genes associated with familial susceptibility to cancers of certain sites, the sequencing of certain of these, and consequent investigations of their mutational spectra in cancer cases promise a more direct method of investigating the question of genetic susceptibility to radiation carcinogenesis. The method presupposes the availability of tissue from cancer cases (and, possibly, controls) in an irradiated population with a strong radiation dose response, known gene sequences for the candidate genes, and (ideally) previously identified familial mutations associated with increased risk. Briefly, the suggested approach is to compare mutation frequencies and spectra in terms of radiation dose and/or estimated probability of radiation causation (excess risk divided by total risk), with attention to modifying factors such as age at diagnosis, age at exposure, and other factors thought to influence cancer risk in general and radiation-related risk in particular.

(347) In the context of the possible involvement of *BRCA1* and *BRCA2* germ line mutations in early-onset breast cancers of the A-bomb survivors it would be of substantial value to have information on their contribution to breast cancer in Japan. There would be great additional benefit if, as in the case of Jewish populations of Ashkenazi descent, there

Fig. 5.8. Dose-specific excess relative risk of radiation-related breast cancer, by age at exposure and age at diagnosis. A, exposure at ages 0–9 years; B, exposure at ages 10–19 years; C, exposure at ages 20–39 years; D, exposure at ages >40 years.

Fig. 5.8. Continued from previous page.

were to be a disproportionate contribution to heritable breast cancer in Japan from specific gene mutations arising through common descent (see Collins, 1996 and Section 6). Other molecular approaches to establishing a genetic basis for radiation-associated cancer risk in the Japanese cohort are available (see Sheffield *et al.*, 1995) but all of these may be compromised by the difficulties already noted in conducting family studies on cancer incidence in Japan. Molecular studies on tumour material from cases can, as in the case of

p53-deficient mice (Section 5.2), be informative for genetic influences on radiation carcinogenesis but, usually, only when a large number of cases are available and when candidate germ line target DNA sequences are known.

5.4.5. *Statistical considerations and modelling*

(348) The question of a genetic basis for sensitivity to radiation carcinogenesis is a second-order problem, as contrasted with the first-order problems of characterising genetic variation and radiation dose as independent risk factors. An *interaction* model describes how the effect of one risk factor may vary according to the level of another. Here, we are concerned with the interaction of inherited genetic mutations and radiation dose in the causation of cancer, just as we might consider the interaction of dose and reproductive history as risk factors for breast cancer, or of dose and smoking history for lung cancer. As with any interaction problem, the likelihood of obtaining estimates of useful precision is increased if both factors, radiation dose and genetic predisposition to cancer, are strongly associated with risk. Thus, suitable populations for study are those known to have a strong radiation dose response for the cancer site under consideration, with many cases for which radiation causation is likely, and a strong likelihood that many of the cases resulted from a genetic predisposition.

(349) The possibility should also be considered that some germ line mutations might be associated with increased risk only at high radiation doses. Such mutations would be unlikely to be identified from studies of non-irradiated populations. An ideal situation would be to have normal tissue from both cases and matched controls, as well as tumour tissue from cases, available for analysis. If the population is well studied and well characterised for radiation-related cancer risk, cases and controls can be matched on radiation dose as well as on sex, year of birth, age at exposure, and other relevant factors, and the existing information on dose response can be incorporated into the analysis with a resultant increase in statistical power. Suppose, for example, that the excess relative risk (ERR) per Sv is estimated to be $\beta = 1.6$. *(For simplicity of notation, we ignore possible variation in dose response by age, sex, etc.) Let D* denote radiation dose in Sv, let X denote presence or absence of a particular mutation or class of mutations, and let α denote the unknown excess relative risk associated with the mutation (i.e. with $X = 1$). If the dose response does not depend upon the value of X, the following (additive) model holds for relative risk RR:

$$RR(\mathrm{D}, X) = 1 + \alpha X + 1.6\mathrm{D}.$$

The multiplicative model corresponds to another simple situation, in which the risk of radiation-related cancer is increased by presence of the mutation in the same ratio as the risk of radiation-independent cancer:

$$RR(\mathrm{D}, X) = (1 + \alpha X)(1 + 1.6\mathrm{D}).$$

(350) It is possible to discriminate between the additive and multiplicative models by embedding them in a more general model, like the following:

$$RR(\mathrm{D}, X) = \{1 + 1.6\mathrm{D}\}\{1 + \alpha X/(1 + 1.6\mathrm{D})^{\theta}\}$$

which reduces to the additive model when $\theta = 1$ and to the multiplicative model when $\theta = 0$. Other general models can be derived with the same properties, e.g.

$$RR(D, X) = 1 + \alpha X + 1.6D + \gamma DX$$

which reduces to the additive model when $\gamma = 0$ and to the multiplicative model when $\gamma = \alpha$. With both of these general models, it is possible to test whether the data are consistent with either of the two simple models. The two general models differ somewhat in how they characterise departures from the two simple models, but with each, it is possible to make inferences about such departures based on the estimated value of the additional parameter (θ or γ).

(351) Although it clearly would be desirable to obtain fresh blood and tissue samples from cases and matched controls, it seems unlikely that sufficient numbers to establish a genetic role in radiation carcinogenesis could be obtained without great difficulty and expense in many of the irradiated populations now under study. With formalin-fixed, paraffin-embedded blocks of tumour and adjacent normal tissue, plus lymph nodes, obtained at surgery from cancer cases only, it is possible to test for departures from the multiplicative interaction model, according to which the proportion of cases with inherited mutations should not vary by radiation dose (all other things being equal). Thus, we can ask whether the increased sensitivity to radiation associated with the mutation is less than, the same as, or greater than that for all other causes of cancer considered as a group, but not whether there is evidence of radiation sensitivity as indicated by statistical inconsistency with the additive model.

(352) The difficulty just mentioned occurs because availability of genetic information only for cases precludes estimation of the effect of a particular heritable mutation, or group of mutations, on cancer risk. Let δ be the probability that a person randomly selected from the study population will have a heritable mutation of the gene in question. Under the additive model, the relative risk of a heritable mutation ($X = 1$) for a case ($C = 1$) with dose D, relative to a case with dose zero, is

$$RR(X = 1|C = 1, D) = (1 + \alpha + \beta D)/(1 + \alpha) \times (1 + \alpha\delta)/(1 + \beta D + \alpha\delta).$$

Thus, with known α, β, δ, it would be possible to test the additive as well as the multiplicative model. The question is whether, in a population that has not previously been studied for cancer risk in relation to mutations of the gene of interest, estimates of α and δ from another population could be used with any confidence.

5.4.6. *Irradiated groups for potential study*

Breast cancer among A-bomb survivors

(353) The A-bomb survivor cohort has a very high signal-to-noise ratio for information on radiation-induced breast cancer, having, in absolute terms, about the same excess risk per unit dose as medically-irradiated Western populations that have been studied, but a much lower baseline rate. Individuals can be identified whose cancers probably would not have occurred in the absence of exposure, and others (many more) whose cancers very probably were not radiation-related. Formalin-fixed, paraffin-embedded tissue blocks are available for most of the 893 cases accepted into the series of 1093 cases on the basis of pathology review by the investigators in a current study. Radiation-related risk depends upon age at exposure in 1945; thus about 80 of 387 cases (with tissue) who were under 20 years of age at exposure were probably caused by radiation, 40 of 378 who were between 20 and 40, and only four of 128 who were older. Formalin-fixed, archival breast tumour tissue is available for about 15 early-onset cases in the group under 20 at exposure, of

whom, if the estimate of 13.5 excess relative risk at 1 Sv can be believed, over half are probably radiation related.

(354) The population seems highly suitable for investigations of *BRCA1* and *BRCA2* mutations. At low doses, we might expect to find, in cases, a decreasing trend in the frequency of heritable mutations with increasing age at diagnosis. If an inherited mutation does not increase susceptibility to radiation carcinogenesis or if susceptibility is increased less than for other risk factors, then (controlling for age at diagnosis) mutation frequency should decrease with increasing dose. If there is a marked increase in susceptibility, mutation frequency should increase with dose. It is possible that the same model may not hold for early-onset and late-onset cases.

(355) There is a clinical sub-sample of survivors who are invited to come to the Radiation Effects Research Foundation (RERF) every two years. Blood samples are taken and lines of immortalised lymphocytes have been stored for 3000 sub-sample members. It is possible that a case-control study might be done restricted to participants in a biennial examination cycle, but a more promising approach may be to match controls from the clinical sample to cases for which only tissue blocks are available, and to use information from cases with both bloods and tissue blocks to estimate the effect of using different kinds of biological materials (and perhaps also the difference between tumour and non-tumour tissue, a possible confounder).

Breast cancer in other irradiated populations

(356) The possibility of radiation-related, early-onset breast cancer occurs in several medically irradiated populations under study in the United States and Sweden. These include women treated during childhood or adolescence for tuberculosis by fluoroscopically monitored pneumothorax (Hrubec *et al*., 1989) or for scoliosis monitored by x ray (Hoffman *et al*., 1989), and women irradiated during infancy for enlarged thymus (Hildreth, 1989) or for hemangioma. Radiation-related excess risks have been observed in all these populations; also, the baseline levels of risk are several times higher than in Japan. Tissue from early-onset cases from the pneumothorax and scoliosis patient populations, plus a cohort of X-ray technologists with long-term occupational exposure to radiation, are being assayed for *BRCA1* mutations in a pilot study (Boice, 1997). It would be very interesting to see if *BRCA1* and *BRCA2* mutation frequencies are similar in US populations and in the RERF cohort, for comparable ages at diagnosis, radiation doses, and estimated probabilities of radiation causation. Of particular interest is whether the proportion of genetically-related breast cancer, both early and late onset but especially the former, is higher among the relatively low-risk Japanese than among Westerners. Assuming no difference in the level of genetic predisposition to breast cancer, the answer to this question should be informative about the extent to which a genetically-determined risk can be modified by environmental and life style factors.

Colon cancer among A-bomb survivors

(357) Colon cancer is about 60% as frequent among Japanese as among Westerners. The number of cases, in both sexes, in the RERF study population is about the same as the number of breast cancers among women, with about half the ERR at 1 Sv (0.72). There is less evidence of a decrease in ERR with increasing age at exposure than for breast cancer. The recent identification, and sequencing, of the *MLH1* and *MSH2* genes believed to be responsible for a high proportion of heritable non-polyposis colon cancer (HNPCC), offers an opportunity to investigate the interaction of these genes with ionising

radiation. In Westerners, HNPCC is reported to occur predominantly in the upper segment of the colon, and cancers occurring there may therefore be the most valuable for study. In the A-bomb survivors, however, ERR per Sv does not appear to vary among the upper, middle, and lower segments (Nakatsuka *et al.*, 1992), a finding that gives little encouragement to the idea that *MLH1* and *MSH2* mutations may be radiosensitising. A new incidence study is planned, which will among other things identify cases with available tissue for molecular assay. A search for unusual patterns of HNPCC-related microsatellite instability amongst such tumours (Section 3) would be a relatively straightforward and potentially rewarding exercise.

Ovarian cancer among A-bomb survivors

(358) Cancer of the ovary, like cancers of the female breast and the colon, is relatively rare among Japanese compared to Europeans and North Americans. Among A-bomb survivors, it is about one-fourth as frequent as breast cancer, but the estimates of ERR at 1 Sv are comparable for all ages combined. Patterns of ERR per Sv with respect to age at exposure are less clear than for breast cancer, however. Because ovarian cancer risk is also tied to *BRCA1* mutations and may also occur in excess in some HNPCC families, it would seem reasonable to include tissue from ovarian cancer cases in investigations of breast and/or colon cancer.

5.5. Overall judgements on genetically determined tumorigenic radiosensitivity

(359) It has been argued on mechanistic grounds that there is good reason to believe that, in many cases, genetically determined risk of spontaneously arising cancer will be accompanied by an increase in sensitivity to the tumorigenic effects of ionising radiation. Based upon somatic cell mutation data it was concluded that the most certain manifestation of increased tumorigenic radiosensitivity would occur in the case of human cancer predisposition associated with inherited deficiency in tumour suppressor genes (Section 5.1.2). Some but not all inherited deficiencies in DNA repair would also be expected to increase radiation cancer risk (Section 5.1.1) but no clear judgement could be made in respect of very rare deficiencies in proto-oncogenes (Section 5.1.3).

(360) The above prediction regarding tumour suppressor gene deficiency is met in the three informative animal data sets currently available in rodent models of Li–Fraumeni syndrome (*p53*-deficiency) familial adenomatous polyposis (*Apc*-deficiency) and tuberous sclerosis (*Tsc2*-deficiency). Although far from being quantitatively definitive it is judged that in *p53*-deficient mice there may be an increase in tissue-specific tumorigenic radiosensitivity of around 13-fold while in *Tsc2*-deficient rats that increase maybe 100-fold or more (Section 5.2.2). Currently there are no animal data on which to comment on the influence of specific DNA repair deficiencies but this position may be expected to change in the near future (Section 5.1.1). Whether these data from animal genetic models may be extrapolated with confidence to humans is however open to doubt since differences in genetic background may have profound effects on the expression of some germ line determinants of cancer and there are specific experimental features that can lead to over- and under-estimation of induced tumour risk. In spite of these problems, the rodent data broadly support the contention of genetically increased radiation risk in certain human disorders.

(361) This conclusion is more directly supported by radiotherapeutic observations in the cancer-prone human genetic conditions retinoblastoma (RB), nevoid basal cell carcinoma

syndrome (NBCCS), Li–Fraumeni syndrome (LFS) and neurofibromatosis (NF); all these disorders are associated with tumour suppressor gene deficiencies.

(362) In the case of heritable RB the post-radiotherapy risk of tumour mortality is five- to six-fold greater than in non-heritable RB and may be greater still when compared with that of normal individuals (Section 5.3.1). It was argued, however, that this simply judged increase in tumorigenic radiosensitivity may underestimate the true effect of *RB* germ line mutation on radiation risk at doses lower than those used in conventional radiotherapy. A further follow-up of these RB cases only served to highlight the uncertainty surrounding the provision of quantitative judgements on relative tumorigenic radiosensitivity.

(363) The case reports on radiotherapeutic response in NBCCS are relevant since they provide evidence of a relatively high level of genetically determined susceptibility to radiation-induced basal cell carcinoma in the low dose periphery of the irradiated field (Section 5.3.2). Post-radiotherapy observations in LFS and, to a lesser extent, NF patients are also consistent with increased radiation cancer risk associated with tumour suppressor gene deficiency but do not allow for any quantitative judgements on these effects (Section 5.3.4). The major strength of studies with LFS patients is that they include familial and molecular investigations which allow direct parallels to be drawn between human and rodent studies on *p53* gene deficiency.

(364) Finally, the study of Kony *et al.* (1997) on the interaction of genetic factors and radiotherapy in the production of second malignant neoplasms (SMN) in children point towards a relatively modest increase in risk of SMN as a result of genetic factors; the odds ratio for those patients having a family history of cancer was around 5.

(365) In addition to the consistent finding of increased tumorigenic radiosensitivity associated with tumour-suppressor gene deficiency in humans and rodents there are strong indications that radiation tumorigenic response is frequently associated with significant and sometimes dramatic reduction in tumour latency. Although no meaningful quantitation of this effect can be made the reduction of tumour latency will tend to further increase the radiation detriment to such cancer-prone individuals. Sankaranarayanan and Chakraborty (1995) have also argued that shortened latency will tend to accompany increased tumorigenic radiosensitivity in most cases of heritable cancer predisposition. Whether such effects have a specific biological basis, e.g accelerated development of existing pre-neoplastic clones following radiation (Strong, 1977) or simply reflect an increased probability of early development amongst an increased number of cancer initiated cells remains to be determined.

(366) Epidemiological studies on Japanese A-bomb survivors have yet to provide unequivocal evidence regarding genetic effects on radiation cancer risks. The finding of a $\sim$6-fold increase in excess relative risk per Sv in respect of early onset breast cancer in women irradiated at ages >20 years is broadly consistent with the presence of a genetic subgroup of radiosensitive cases (Section 5.4.2); the shortening of tumour latency in response to radiation may be characteristic of genetically determined cancer risk. In the absence of information on familial patterns of cancer risk in these cases and on molecular studies of germ line candidate breast cancer genes, other non-genetic explanations of these findings remain, however, possible. The initiation of follow-up studies to resolve these questions should be regarded as a priority.

(367) It may be concluded that although the data discussed in this Section provide consistent evidence for genetically determined increase in radiation cancer risk in a limited set of genetic conditions in humans and experimental animals, confident judgement on the extent of increased radiosensitivity remains most elusive. The experimental and

radiotherapeutic observations discussed in respect of tumour suppressor gene deficiencies imply, as a first approximation, that increases in tissue-specific cancer risk following exposure to moderate to high doses of acute radiation are likely to be >5-fold but probably <100-fold greater than normal. The direct human data from high dose radiotherapy follow-up studies imply that a genetically imposed increased risk in the lower end of this range, say around 10-fold, might be realistic. A further element of radiation detriment may also be introduced through the shortening of tumour latency.

(368) It is however important to emphasise strongly the paucity of the data currently available for any judgements for radiological protection purposes. First, even if the above estimates on the range of genetically imposed increased risk were to be confirmed by study of other genotypes and cancer types, great uncertainty would continue to surround tumorigenic responses at low doses and low dose rates. Second, almost all the data sets discussed relate to tumour suppressor type genetic disorders of relatively high penetrance, i.e those exhibiting a high spontaneous incidence of cancer. Whether radiological protection judgements on these may also apply to genetic conditions of lower penetrance remains a matter of conjecture. Third, as discussed in Section 7, radiation cancer risks are likely to be subject to genetic–genetic and other environmental–genetic interactions. The choice of a single value of tumorigenic radiosensitivity to represent all relevant genetic conditions and tumour types in all affected individuals is a gross simplification of a most complex problem. Although these great uncertainties are fully recognised the choice of a 'best estimate' single value of 10-fold to represent genetically imposed tumorigenic radiosensitivity has been made in order to allow the presentation of illustrative calculations of radiological impact in Section 7. Finally, since epidemiological and experimental studies have yet to clarify the tumorigenic response of ataxia-telangiectasia homo- and heterozygotes to radiation, the whole question of responses associated with DNA repair deficiency remains open. There is however unambiguous evidence of increased skin cancer in sunlight exposed regions of UVR-repair deficient human xeroderma pigmentosum (XP) patients (Kraemer *et al.*, 1994) and in animal models of XP (Section 5.1.1). Thus, it may be reasonably argued that inherited human defects in relevant DNA repair genes will also be accompanied by increased cancer risks after ionising radiation.

5.6. Summary and conclusions

(369)

1. Knowledge of fundamental mechanisms of DNA damage repair, mutagenesis, and carcinogenesis imply that, in most cases, human cancer predisposition associated with DNA repair or tumour suppressor gene deficiency will be associated with increased cancer risk after radiation.
2. Experimental studies with animal genetic models of human cancer-predisposing disorders provide evidence for significantly increased cancer risk after radiation.
3. Post-radiotherapy observations in human patients with the cancer-predisposing disorders, retinoblastoma, nevoid basal cell carcinoma syndrome, and Li–Fraumeni syndrome support the notion of increases in absolute cancer risk after radiation.
4. Recent epidemiological and molecular studies weaken, but do not negate, the association between ataxia-telangiectasia heterozygotes and excess breast cancer.
5. Conventional epidemiological approaches to cancer risk in genetically predisposed sub-populations are unlikely to succeed fully unless they can be coupled with appropriate molecular analyses.

6. The availability of cancer case series of sufficient size and a high probability of radiation causation promises that molecular epidemiology will resolve some aspects of the problem of genetic effects on radiation risk.
7. As an interim judgement based on animal and human data it is suggested that the risk of cancer after radiation may, in principle, be elevated by up to around 100-fold in some heritable cancer disorders; limited human data imply values that are lower than those seen in animal models. Great uncertainties are recognised and stressed but a single best estimate value of 10-fold increased risk is suggested for the purposes of illustrative calculation of radiological impact.

6. COMPUTATIONAL MODELLING OF THE IMPACT OF GENETIC FACTORS IN RADIATION CARCINOGENESIS

(370) In previous sections of the document molecular, genetic, clinical, and epidemiological data relevant to genetic predisposition to cancer have been outlined. From a mechanistic viewpoint it has been argued that cancer predisposition associated with germ line tumour suppressor, proto-oncogene, and many DNA-processing gene defects will tend to be accompanied by increased susceptibility to radiation-induced disease. In reviewing what is, unfortunately, a limited set of informative data, some support for this contention was obtained from clinical, epidemiological, and experimental studies.

(371) In accord with previous discussions it is evident that in order to make estimates of the excess cancer in a given irradiated human population that might be attributed to a genetically predisposed subgroup it is necessary to have knowledge of two critical parameters. First the number of individuals within that population that carry mutant genes that predispose to cancer overall; second the overall degree of tumorigenic radiosensitivity conferred by the mutant gene to these carriers.

(372) At present, informed judgement on a value for the overall frequency of cancer predisposed individuals in the human population is not feasible and, albeit with full appreciation of current uncertainties, we consider only those characterised disorders where estimates of prevalence have already been made (Table 6.1). Equally problematical is the provision of judgements on the specific degree of tumorigenic radiosensitivity associated with any of the genetic disorders that we have considered. From the review of the limited data given in Section 5 an interim judgement of an increase in tumorigenic radiosensitivity for certain tissues of between 5- and 100-fold in a few genetic disorders was made. The uncertainties that attach to such judgements are however sufficiently great at present that it would be most premature to apply a specific factor of tumorigenic radiosensitivity to a specific set of disorders even if the prevalence in the population was well established.

(373) In the light of these difficulties attention in this section of the report is focussed principally on the development of genetically based computational models into which given estimates of disease prevalence, strength of predisposition and tumorigenic radiosensitivity may be introduced as further data accumulate. These models are used to explore different scenarios in respect of the input parameters and also to explore the possible impact of genetic susceptibility to breast and colon cancer.

6.1. Familial cancer genes: estimates of mutant gene frequencies

(374) In general, for a single gene trait, the frequency of the mutant allele in the population can be computed from that of the disease in the population and a knowledge of which genotypes are affected, assuming Hardy–Weinberg equilibrium (see Section 6.2). For example, if there are two alleles A and a at an autosomal locus and if the a allele produces the disease only in the aa homozygotes, then the disease is recessive and the frequency p of the a allele in the population is the square root of the disease frequency. If, however, the disease is dominant and caused by the presence of the A allele with allele frequency p, then the disease will be manifest in homozygotes (AA) and in heterozygotes (Aa); their frequency in the population is $p^2 + 2p(1-p)$. For rare dominant diseases, $(1-p)$ is nearly equal to 1 and the p^2 term can be neglected. For these, therefore, the allele frequency is approximately one-half of the disease frequency.

(375) Table 6.1 updated from the paper of Sankaranarayanan and Chakraborty (1995) summarises approximate estimates of prevalences of some familial cancers. Because of the

Table 6.1. Familial cancer genes in man[a]

McKusick number	Gene symbol	Chromosomal location	Syndrome/disorder	Approximate prevalence per 10^5 live births[b]
A. Cloned genes				
1. Tumour-suppressor genes				
180200	RB1	13q14.2	Retinoblastoma	2
194070	WT1	11p13	Wilms' tumour (nephroblastoma)	10
191170	TP53 (*p53*)	17p13.1	Li–Fraumeni syndrome	2
175100	APC	5q21-q22	Familial adenomatous polyposis coli[c]	10
162200	NF1	17q11.2	Neurofibromatosis, type 1	30
101000	NF2	22q12.2	Neurofibromatosis, type 2	3
193300	VHL	3p25	von Hippel Lindau syndrome (multiple bilateral renal carcinomas)	3
114480	BRCA1	17q21	Familial breast and ovarian cancer	200
	BRCA2	13q12-q13	Female (and some male) breast cancers	
155600	CDKN2	9p21	Familial melanoma (hereditary dysplastic nevus syndrome)	[d,e]
109400	PTC	9q22	Nevoid basal cell carcinoma syndrome	< 2[v]
2. Nucleotide excision repair genes[f]				
278700	XPAC	9q34.1	Xeroderma pigmentosum, complementation group A	< 1[g]
133510	XPBC/ERCC3[h]	2q21	Xeroderma pigmentosum, complementation group B	
278720	XPCC	3p25	Xeroderma pigmentosum, complementation group C	
278730/126340	XPDC/ERCC2	19q13.2	Xeroderma pigmentosum, complementation group D	
133520	ERCC4[i]	16p13.13–p13.2	Excision repair cross-complementing-4	
278780/133530	XPGC/ERCC5	13q32–q33	Xeroderma pigmentosum, complementation group G	
3. DNA mismatch repair genes				
120435	hMSH2[j]	2p16	Hereditary non-polyposis Colorectal cancer (HNPCC)	50[k]
120436	hMLH1[l]	3p21		
	hpMS1[m]	2q31–q33		
	hpMS2[m]	7p22		
4. Other DNA repair genes				
208900	AT[t]	11q23	Ataxia telangiectasia	1[u]
210900	BS	15q26	Bloom's syndrome	< 1
227645	FACC[n]	9q22.3	Fanconi's anemia complementation group C	5[o]
5. Proto-oncogene				
171400/164761	Ret[p]	10q11.2	Multiple endocrine neoplasia types 2A and 2B (MEN 2A and 2B) and familial medullary thyroid carcinoma (FMTC); Hirschprung disease; depends on the nature and position of the mutation	?
B. Uncloned but mapped tumour-suppressor or other genes				
131100	MEN1	11q13	Multiple endocrine neoplasia	2–20
164840	NB1p36	Neuroblastoma[r]	15	
130650	BWS	11p15	Beckwith–Wiedemann syndrome (adrenocortical carcinoma and Wilms' tumour)	?

(continued on next page)

Table 6.1 (*continued*)

McKusick number	Gene symbol	Chromosomal location	Syndrome/disorder	Approximate prevalence per 10^5 live births[b]
144700	RCC1	3p14	Renal cell carcinoma	?
191100	TSC1	9q34	Tuberous sclerosis 1	3–8
191090	TSC2	16p13	Tuberous sclerosis 2[s]	

[a]General references: Harris and Hollstein, (1993); Knudson, (1993), and McKusick, (1994).
[b]Prevalence of heterozygotes for tumor suppressors and of homozygotes for recessive disorders.
[c]Accounts for ≈1% of colorectal cancers in the Western world.
[d]Overall age-adjusted US rates of malignant melanoma for 1986–1990 are: $10.5/10^5$; $12.1/10^5$ among whites and only $0.8/10^5$ among blacks (Miller *et al.*, 1993); approximately 10% of cutaneous malignant melanomas occur in familial setting (Fountain *et al.*, 1990).
[e]Kamb *et al.* (1994a, b); Nobori *et al.* (1993); Hussussian *et al.* (1994).
[f]Hoeijmakers, (1993); Tanaka and Wood, (1994).
[g]Overall incidence of XP: 1/250,000 (Robbins *et al.*, 1974); 1/100,000 in Japan and 1/183,000 in Korea (Satoh and Nishigori, (1988).
[h]Excision repair cross complementing gene; this and other such genes were isolated by transfection of human genomic DNA into UV-sensitive rodent repair-deficient mutants, followed by selection of UV-resistant transformants and retrieval of the correcting sequence.
[i]Thompson *et al.* (1994a).
[j]Fishel *et al.* (1993); Leach *et al.* (1993).
[k]See Section 3.4.2.
[l]Bronner *et al.* (1994); Papadopoulos *et al.* (1994).
[m]Nicolaides *et al.* (1994).
[n]Gibson *et al.* (1993).
[o]Overall incidence.
[p]Mulligan *et al.* (1993, 1994).
[q]Wooster *et al.* (1995).
[r]Less than 1% are familial.
[s]Delineation between TSC1 and 2 not yet available.
[t]Gatti, (1991).
[u]Range: 1/90,000 to 1/300,000 (Heim *et al.*, 1992).
[v]Gorlin (1995).

approximate nature of these estimates, and the fact that with some common cancers (e.g. those of the breast and colon), the proportion due to predisposing germinal mutations are not reliably known (and these can also vary in different populations), the estimates of mutant gene frequencies are also approximate as illustrated on pages 107 and 108.

(376) *Ataxia-telangiectasia* (A-T). The overall observed incidence of A-T in the US is about 1/300,000, with the highest of 1/90,000 in Michigan (Swift *et al.*, 1986). For the UK, the observed incidence is 1/100,000 (Pippard *et al.*, 1988). Based on incidence data, the minimum frequency of the *ATM* gene in the US white population was estimated to be 0.0017 (Swift *et al.*, 1986). The estimate based on the proportion of close relatives of homozygous probands (i.e. pedigree analysis) and on the assumption that A-T is a single homogeneous genetic syndrome is higher, being 0.007, corresponding to a heterozygote frequency of 1.4% in the population (Swift *et al.*, 1986). Recent molecular studies have shown that that A-T is indeed a homogeneous genetic entity (Savitsky *et al.*, 1995).

(377) On the basis of more recent data, however, Easton (1994) estimated that A-T heterozygotes have a frequency of about 0.5% and have a relative breast cancer risk of 3.9 (CI 2.2–7.2). These data imply that A-T heterozygotes might account for between 1 and 13% of breast cancer in western populations but that the *ATM* gene would make only a minor contribution to familial breast cancer. The latter inference rests on the current view that the proportion of familial breast cancers among all breast cancers is already small as discussed below. As discussed in Sections 3 and 5 more recent data raise further questions on the association between *ATM* heterozygosity and excess breast cancer.

BRCA1 *and* BRCA2 *in familial breast cancers*

(378) It is estimated that over 10% of the women diagnosed of breast cancer each year have a family history of the disease (Ottman *et al*., 1986). *BRCA1* is estimated to account for about half of the inherited breast cancer and about three quarters of breast plus ovarian cancer. Earlier segregation analysis suggested that the familial clustering of breast cancer may be accounted for by highly penetrant autosomal dominant mutations with a combined population gene frequency of 0.0006 to 0.0033 (Claus *et al*., 1991; Newman *et al*., 1988). These frequencies correspond to about 1 in 300 to 1 in 800 women in the US being carriers of such mutations.

(379) For non-Jewish Caucasian women in the UK, on the basis of two population-based data on cancer mortality in the first-degree relatives of breast and ovarian cancer patients, Ford *et al*. (1995) and Peto *et al*. (1996) estimate that the *BRCA1* mutant gene frequency is 0.0006 (95% CI, 0.0002–0.001). As stated above, the gene frequency of 0.0006 implies that about 1 in 800 women carry a mutation and, even at the upper confidence limit of 0.001, the carrier frequency would only be 1 in 500. The assumption used here is that both the excess risk of ovarian cancer in the first-degree relatives of breast cancer patients and the breast cancer excess in relatives of ovarian cancer patients are entirely accounted for by *BRCA1*.

(380) The calculations of Ford *et al*. (1995) show that the proportion of all breast cancer cases diagnosed in the general population (due to *BRCA1*) is 7.5% between ages 20–29 years, falling to 5.1% between ages 30–39 years, 2.2% between ages 40 and 69 years and 1.1% between ages 50 and 70 years; considered overall, 1.7% of all breast cancers diagnosed between ages 20 and 69 years is due to *BRCA1* mutations. Ford *et al*. (1995) also note that an important unresolved issue here is the genetic basis of familial breast cancers which is not explained by *BRCA1* or *BRCA2*. They estimate that the combined gene frequency of other highly penetrant genes (including *BRCA2*) may be of the order of 0.0003 i.e. 1 in 1600.

(381) In Ashkenazi Jewish women, the *BRCA1* mutant gene frequency appears to be substantially higher. The current estimate is 0.0047 (corresponding approximately to 1% of these women being carriers); this frequency is for one specific mutation, namely, the deletion of an adenine and guanine residue in codon 185 of exon 2 of the gene and denoted as 185delAG (Struewing *et al*., 1995a,b; Takahashi *et al*., 1995; Tonin *et al*., 1995). On the basis of age-dependent penetrance curves for the observed risk of breast cancer in *BRCA1* families, Collins (1996) has estimated that 38% of Ashkenazi Jewish women with breast cancer under age 30 would be expected to have a *BRCA1* germ line mutation, compared to 7.5% for non-Jewish women mentioned earlier (Ford *et al*., 1995).

(382) Roa *et al*. (1966) have recently published the results of an expanded population-based study, on two *BRCA1* mutations (*185delAG* and *5382insC*) and one *BRCA2* mutation (*6174delT*) in over 3000 Ashkenazi Jewish women, unselected for a personal or family history of breast or ovarian cancer. The carrier frequencies of the *BRCA1* mutations were found to be: 1.09% (34/3108; *185delAG*) and 0.13% (4/3116; *5382insC*) and that for *BRCA2*, 1.52% (47/3085). They note that the *BRCA2* mutation may be one-fourth as penetrant as the *BRCA1 185delAG* mutations. As will be evident, the *185delAG* mutation in *BRCA1* and *6174delT* in *BRCA2* have a combined carrier frequency of 2.6%. In another population study involving 1255 Jewish women, Oddoux *et al*. (1996) recorded a carrier frequency of 0.9% (12/1255) for the *BRCA2 6174delT* mutation and estimated that for this mutation, the RR for developing breast cancer by age 42 was 9.3 as

compared to an RR of 31 for the *BRCA1 del185AG* mutation (i.e. more than a three-fold difference).

Mutations in DNA mismatch repair genes and hereditary non-polyposis colon cancer (HNPCC)

(383) For HNPCC, the estimated range of prevalence in Western populations was originally estimated to be between 1 in 500 to 1 in 200 (corresponding to mutant gene frequencies of 0.001 to 0.0025). HNPCC was believed to account for about 2% to 15% of colon cancers (Mecklin, 1987; Vasen *et al*., 1991; Lynch *et al*., 1993; Rustgi, 1994). As discussed in Section 3, these figures (especially the 15%) remain controversial and depend critically on the criteria used for diagnosis. Further, although as of now, four DNA mismatch repair genes have been cloned, the relative contributions of mutations in these genes to HNPCC have not been established. All this means is that the mutant gene frequency may even be lower than 0.001. In Section 3 a prevalence estimate of 1 in 2000 was given on the basis of a likely contribution of HNPCC to colon cancer in the USA of around 2%.

6.2. Population genetic models of cancer predisposition and radiosensitivity

(384) An important purpose of this report was to develop a genetically realistic computational model for the purposes of judging the population impact of genetic predisposition on radiation cancer risk. Two such models were formulated and published (Chakraborty and Sankaranarayanan 1995; Chakraborty *et al*., 1997). Here, an outline of the most appropriate of these models is provided and its function is illustrated in a simple but artificial numerical exercise. Most importantly, best estimates of prevalence and strengths of cancer predisposition and radiosensitivity (Section 5) are used in order to model the impact on population risk after radiation exposure of specific genotypes associated with predisposition to breast and colon cancer. Judgements developed from this work are carried forward in order to provide comment in Section 7 on the implications for radiological protection.

Hardy Weinberg equilibrium

(385) A basic feature of allele frequency in large populations is its stability over time in the absence of differences in viability or fertility among the genotypes at the locus under consideration. In the further absence of assortative mating, i.e. tendency of like to mate with like, migration, mutation, and geographical subdivision of the population, genotypic frequencies also remain constant from generation to generation. In the case of genetic diseases, this results in their stable prevalences in the population. These properties are summarised in the concept of 'Hardy–Weinberg equilibrium' and are fundamental to theories of population genetics.

6.2.1. *A Mendelian single locus, autosomal dominant model of cancer susceptibility and radiosensitivity: A hypothetical numerical exercise*

(386) Consider a single autosomal locus at which there are two alleles, a, the normal or 'wild-type' allele and A, the dominant cancer-predisposing mutant allele, in a population under Hardy–Weinberg equilibrium. The individuals in the population will have one of the following three genotypes: AA, Aa, and aa.

(387) Let p denote the frequency of the A allele and q = that of a so that $p + q = 1$. The genotypic distribution of the individuals will be as follows:

$$p^2(AA);\ 2pq\,(Aa);\ q^2\,(aa).$$

Assume that $p = 0.01$ and $q = 0.99$. In a population of 10,000 individuals, the different genotypes will be distributed as follows:

$$1\,(AA) : 198\,(Aa) : 9801\,(aa).\ \text{Total} = 10,000.$$

(388) Assume that *AA* and *Aa* individuals are cancer-predisposed ('susceptibles') and to the same extent and the *aa* individuals are not ('non-susceptibles'). Assume further that the background cancer risk for *aa* individuals is 1/1000 and that for the *AA* and *Aa*, 1/10 i.e. the latter genotypes have a 100-fold higher 'normal' cancer risk relative to aa individuals. The expected frequency of cancers will be:

$$\begin{array}{ccccc} AA & & Aa & & aa \\ 1 \times 0.1 & + & 198 \times 0.1 & + & 9801 \times 0.001 \ \sum 30 \\ [0.1] & & [19.8] & & [9.8] \end{array}$$

of which about 20 are from the 'susceptibles' and about 10 from the 'non-susceptibles'.

(389) Assume that the population is irradiated and that all the genotypes have the *same* sensitivity to radiation-induced cancers. Assume further that the dose is such that it causes a 1% risk of inducing cancers. So, in 10,000 individuals, there will be a total of 100 induced cancers ($10{,}000 \times 0.01 = 100$) and will be distributed as follows:

$$\begin{array}{ccccc} AA & & Aa & & aa \\ 1 \times 0.01 & & 198 \times 0.01 & & 9801 \times 0.01 \ \sum 100 \\ [0.01] & & [1.98] & & [98.01] \end{array}$$

Note that nearly all the cancers come from the 'non-susceptibles' because of their high frequency. The total number of cancers one will observe will be $100 + 30 = 130$. The relative risk (RR) of radiation exposure is $130/30 = 4.3$ and excess relative risk (ERR) = RR − 1 = 3.3.

(390) Assume now that the *Aa* and *AA* have a 50-fold *higher* radiosensitivity, namely, 0.5, relative to *aa* (which is 0.01 as before). Under these conditions, the cancers in the irradiated population will be distributed as follows:

$$\begin{array}{ccccc} AA & & Aa & & aa \\ 1 \times 0.5 & & 198 \times 0.5 & & 9801 \times 0.01 \ \sum 198 \\ [0.50] & & [99.00] & & [98.01] \end{array}$$

Note now that the frequency of radiation-induced cancers is 198 of which one-half comes from the susceptibles. The total number of cancers in the irradiated population is $198 + 30 = 228$, the RR is $228/30 = 7.6$ and ERR = 7.6 -1 = 6.6.

(391) There is a four-fold message in the above numerical exercise:

1. In the absence of radiation, the load of excess cancers (due to predisposition) is dependent on the frequency of the susceptible mutant allele (and thus on the frequency of the genotypes *AA* and *Aa* carrying the mutant allele) and the relative risk associated

with the mutant genotypes; in the example, the gene frequency of 0.01 and the relative risk of 100 means that most cancers (i.e. 20/30) occur in the 'susceptibles';
2. If the population has uniform radiosensitivity with respect to the susceptibility gene (i.e. the relative risk for a given dose of radiation is the same for the susceptible and non-susceptible genotypes despite heterogeneity with respect to cancer predisposition alone), then the relative distribution of radiation-induced cancers in the susceptible and non-susceptible groups is dependent on the relative frequencies of these groups. In the present example, most of the induced cancers (98 out of 100) occur in the 'non-susceptibles' because they outnumber the 'susceptibles' (9801 versus 198);
3. If however, the susceptible genotypes are also more radiosensitive, a higher proportion of induced cancers will occur in the 'susceptibles' (100 out of 198 in the example); and
4. With a mutant gene frequency of 0.01 (which corresponds to about 2% of the population being heterozygotes for the mutant gene), a 100-fold difference in cancer susceptibility and 50-fold difference in radiosensitivity assumed in the above calculations, the conventional epidemiologic measures show an increase in risk by a factor of only about 2 (RR of 7.6 from 4.3 and ERR of 6.6 from 3.3).

(392) In the above example, for the sake of simplicity, no account has been taken of the following facts/observations: (i) for most common cancers, the proportion attributable to allelic variation at a single locus is small (e.g. breast and colorectal cancers); (ii) not all mutations which confer cancer susceptibility are fully penetrant and (iii) the increase in radiation risk in susceptibles compared to non-susceptibles may vary with the dose of irradiation and may also be affected by other factors. Further, the example discussed above applies to a dominant mutant gene at one locus conferring cancer predisposition; as is well known, there are autosomal recessive diseases (e.g. A-T) for which homozygotes, and possibly, also heterozygotes, may be at a higher risk for both naturally-arising and radiation-induced cancers.

(393) Autosomal dominant and recessive models taking into account some of these factors have now been developed by Chakraborty and Sankaranarayanan (1995) and Chakraborty *et al.* (1997). The difference between the autosomal dominant models discussed in these two papers is that in the Chakraborty *et al.* (1997) paper, the model incorporates differences in penetrance and dose dependence of radiosensitivity differentials whereas these were not explicitly included in the Chakraborty and Sankaranarayanan (1995) paper. Since incorporation of the above two factors in the model is pertinent in the present context, it is the model given in Chakraborty *et al.* (1997) which is discussed below.

6.2.2. *Autosomal dominant model of cancer susceptibility and radiosensitivity which incorporates differences in penetrance and dose-dependence of radiosensitivity differentials*

Parameters and assumptions.

(394) The model assumptions are the following:

(i) Cancer susceptibility is due to a single autosomal locus with two alleles, one a dominant predisposing allele (A) and the other, a normal allele (a); both the heterozygotes (Aa) and homozygotes (AA) for the mutant allele are more radiosensitive than the normal homozygote;
(ii) Hardy–Weinberg expectations hold for genotype frequencies at the cancer-susceptible locus in the general population;

Table 6.2. Notations and interpretation of parameters

Notation	Definition/Interpretation
AA, *Aa*, *aa*	Genotypes (*A* is the dominant allele, which confers cancer predisposition and radiosensitivity to the genotypes).
p,*q*	Gene frequencies (*p* for the *A* allele, and *q* for the *a* allele; $p + q = 1$).
θ	Penetrance parameter; the fraction of *Aa* genotypes where the dominant allele *A* confers higher susceptibility and radiosensitivity (see Fig. 6.1).
R_0	General cancer risk in the population (which also applies for the low-risk genotypes (*aa* and a fraction $(1 - \theta)$ of *Aa* genotype; see Fig. 6.1).
R_B	Background cancer risk in an unirradiated population in which some genotypes are genetically predisposed ('susceptibles' [S]) while others are not ('non-susceptibles' [NS]).
π	Fraction of cancers in the population that are caused by genotypes at the single locus considered.
R_p	Factor by which the background cancer risk is increased in the [S] relative to [NS] genotypes; in the present context it is the relative risk for *AA* and a fraction of *Aa* genotypes in comparison to the cancer risk (R_0) in *aa* individuals.
D	Radiation dose (Gy).
β	Slope of the radiation dose effect curve; in the context of this paper it denotes the excess relative risk coefficient for genotype *aa* which is also approximately the relative risk coefficient in the general population. Thus, cancer risk in [NS] individuals exposed to a dose D of radiation is $R_0(1 + \beta D)$.
R_i	Strength of radiosensitivity differential; a factor by which the slope of the radiation dose effect curve is increased in [S] individuals; i.e. multiplier of the excess relative risk for [S] individuals, yielding cancer risks in [S] individuals at dose D of radiation equal to $R_0 (1 + \beta D R_i)$.
$R_T(D)$	Total cancer risk at dose D in an irradiated population consisting of [S] and [NS] genotypes.
$AF(D)$	Fraction of cancers at dose D in an irradiated population attributed to [S] genotypes; i.e. $1 - [R_0(1 + \beta D)/R_T(D)]$.
$\alpha(D)$	Proportion of total cancers at dose D in an irradiated population which is due to enhanced radiosensitivity alone.
$RR(D)$	Relative risk $= R_T(D)/[R_0(1 + \beta D)]$.

(iii) Only a proportion of cancers (π) of a given type in the population is due to the locus under study;
(iv) The dominant allele (*A*) in heterozygous condition (i.e. in *Aa* genotypes) has a penetrance θ ($0 < \theta \leqslant 1$); in homozygotes, there is complete penetrance of the mutant allele; and
(v) Differential radiosensitivity is mediated through the same genetic predisposition i.e. predisposed individuals are also more radiosensitive; those who have cancers due to causes other than the susceptibility locus have the same general cancer risk due to radiation exposures.

(395) For easy reference, Table 6.2 presents all parameters definitions of the model and the notations used. Figure 6.1 shows schematically the way in which cancer predisposition and radiosensitivity are assumed to cause risk enhancement under the dominant model. Throughout this chapter, the terms 'genetic predisposition' and 'susceptibility' are used interchangeably and, unless otherwise stated, the term 'radiosensitive' is used to indicate the enhanced sensitivity of the susceptible genotypes to the induction of cancers by radiation.

Definition of the model: incorporation of cancer susceptibility and incomplete penetrance

(396) As indicated in Fig. 6.1, let P_{AA}, P_{Aa} and P_{aa} be the frequencies of the three genotypes *AA*, *Aa* and *aa*. In this form, they would apply to any group of individuals in the population, even if the genotype frequencies do not conform to Hardy–Weinberg expectations. Since of the total cancers, a fraction (π) is assumed to be due to the susceptibility locus and only a fraction (θ) of the *Aa* genotypes express susceptibility due to incomplete penetrance of the dominant allele (*A*), there are two 'risk groups' of

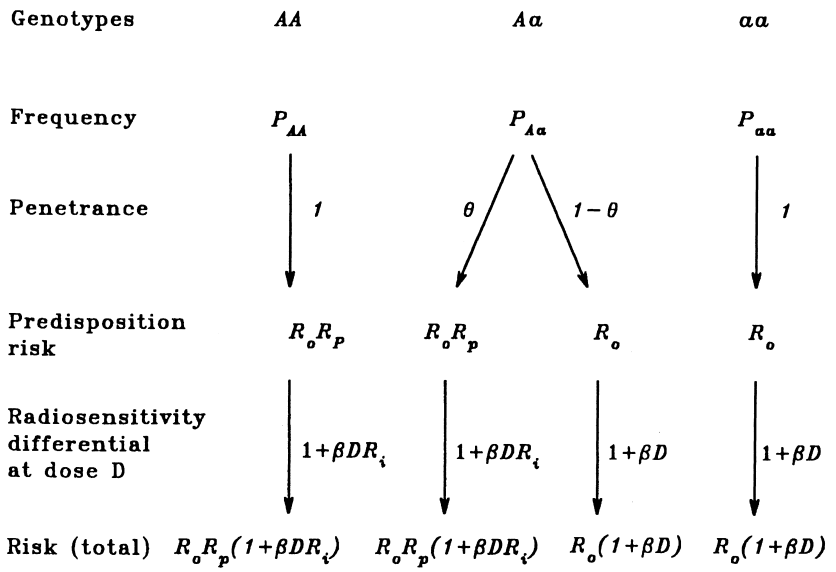

Fig. 6.1. Schematic diagram of the effects of radiosensitivity differentials, strength of predisposition and incomplete penetrance on individuals of different genotypes (see Section 6.2.1.1).

individuals, one with risk R_0R_p and the other with R_0. The total proportion of individuals in the first group is $(P_{AA}+\theta P_{Aa})$, and that in the second group, $(1-\theta)P_{Aa}+P_{aa}$.

(397) In addition, since $(1-\pi)$ fraction of the cancers occurs irrespective of genotype in individuals with risk R_0, the 'background' cancer risk in a population which contains a susceptibility sub-group is given by the weighted average (i.e. weighted by the respective genotypic frequencies in the population):

$$R_B = (1-\pi)R_0 + \pi\left[(P_{AA}+\theta P_{Aa})R_0R_p + \left\{(1-\theta)P_{Aa}+P_{aa}\right\}R_0\right]$$

$$= R_0\left[1+\pi(P_{AA}+\theta P_{Aa})(R_p-1)\right]. \quad (1)$$

(398) For a population under Hardy–Weinberg equilibrium, $P_{AA}=p^2$, $P_{Aa}=2pq$, and $P_{aa}=q^2$ and, under complete penetrance of the dominant allele, $\theta = 1$ so that the above equation becomes

$$= R_0\left[1+\pi(1-q^2)(R_p-1\right]. \quad (1a)$$

(399) It is instructive to note that the polymorphism at the susceptibility locus increases the cancer risk to an extent determined by the contribution of the susceptible genotypes [i.e. $\pi(1\text{-}q^2)$]. Hence, as π approaches 0 or q approaches 1, R_B would approach R_0, namely, the risk in a population without the susceptibility allele, A.

(400) In an unirradiated population, the excess relative risk due to the presence of polymorphism at the susceptibility locus becomes:

$$(R_B - R_0)/R_0 = \pi(P_{AA}+\theta P_{Aa})(R_p-1) \quad (2)$$

and the fraction of cancers due to its presence is given by

$$\frac{(R_B-R_0)}{R_B} = \frac{\pi(P_{AA}+\theta P_{Aa})(R_p-1)}{\left[1+\pi(P_{AA}\theta P_{Aa})(R_p-1)\right]}. \quad (3)$$

Both these measures [Eqs. (2) and (3) above] are dictated by the occurrence of cancers contributed by the susceptibles $[\pi(P_{AA}+\theta P_{Aa})]$ for any fixed value of $R_p > 1$.

Incorporation of dose dependence of radiosensitivity differential in the model

(401) For incorporating dose dependence of radiosensitivity differentials in the model, it is assumed that in the non-predisposed individuals, a dose D of radiation increases cancer risk by a factor $(1 + \beta D)$, in comparison to the background risk (R_0); in the predisposed genotypes (AA homozygotes, and a proportion θ of the Aa heterozygotes), the same dose of radiation increases the cancer risk by a factor $(1 + \beta D R_i)$. In this formulation, the slope of the radiation dose effect curve is β (which can be interpreted as the excess relative risk coefficient) for all non-susceptible individuals, which is also approximately the relative risk coefficient in the general population. In contrast, for a fraction π of individuals in whom the dominant allele (A) is fully penetrant, [i.e. $\pi(P_{AA}+\theta P_{Aa})$], the slope is βR_i. Therefore, the total cancer risk in the irradiated population is given by

$$\begin{aligned} R_T(D) &= (1+\pi)R_0(1+\beta D) \\ &\quad + \pi\big[(P_{AA}+\theta P_{Aa})R_0\, R_p(1+\beta D R_i) + \big\{(1-\theta)P_{Aa}+P_{aa}\big\}R_0(1+\beta D)\big] \\ &= R_0\big[(1+\beta D)+\pi(P_{AA}+\theta P_{Aa})\big\{R_p(1+\beta D R_i)-(1+\beta D)\big\}\big]. \end{aligned} \tag{4}$$

By substituting $P_{AA}=p^2$ and $P_{Aa}=2pq$ in this equation, we obtain the total cancer risk in the irradiated population when incomplete penetrance is incorporated.

(402) Once the total cancer risk is determined, the three epidemiologic measures of interest, namely, the relative risk [$RR(D)$], attributable fraction [$AF(D)$], and the proportion of $AF(D)$ that is due to enhanced radiosensitivity alone [$\alpha(D)$] can be derived. These are given by

$$\begin{aligned} RR(D) &= \frac{R_T(D)}{R_0(1+\beta D)} \\ &= 1+\pi(P_{AA}+\theta P_{Aa})\cdot\left[\frac{R_p(1+\beta D R_i)}{1+\beta D}-1\right] \end{aligned}$$

$$AF(D) = \frac{R_T(D)-R_0(1+\beta D)}{R_T(D)} \tag{5}$$

$$= \frac{\pi(P_{AA}+\theta P_{Aa})\big\{R_p(1+\beta D R_i)-(1+\beta D)\big\}}{(1+\beta D)+\pi(P_{AA}+\theta P_{Aa})\big\{R_p(1+\beta D R_i)-(1+\beta D)\big\}} \tag{6}$$

and

$$\begin{aligned} \alpha(D) &= \frac{R_T(D)-(1+\beta D)R_B}{R_T(D)-(1+\beta D)R_0} \\ &= \frac{\pi(P_{AA}+\theta P_{Aa})\beta D(R_i R_p - R_p)}{\pi(P_{AA}+\theta P_{Aa})\big\{R_p(1+\beta D R_i)-(1+\beta D)\big\}} \\ &= \frac{\beta D R_p(R_i-1)}{R_p(1+\beta D R_i)-(1+\beta D)}. \end{aligned} \tag{7}$$

(403) As can be seen from the formulae, all three measures of risk are dependent on radiation dose. Additionally, the relative risk [*RR*(D)] and attributable fraction [*AF*(D)], are dependent on the strength of predisposition (R_p), radiosensitivity differential (R_i), and the proportion of susceptibles (π). In contrast, [α(D)] is independent of the proportion of susceptibles and the penetrance of the dominant allele.

6.2.3. *Numerical applications*

Rationale for the parameter values chosen for applying the model for breast cancer and hereditary non-polyposis colon cancer

(404) In the case of *BRCA1*-associated breast cancer, the parameter values used in risk computations are some selected combinations of: p (0.0006, 0.0047), π (0.017, 0.075, 0.38) and θ (0.50, 0.75, 1.0). The slope of the dose-effect curve is fixed at $\beta = 2.0$, and three dose levels (D of 0.5, 1.0, and 2.0 Gy) are considered. The choice of p and π values was dictated by more recent data discussed earlier in Section 6.1 (Easton *et al*., 1995; Ford *et al*., 1995; Struewing *et al*., 1995b; Collins 1996; Peto *et al*., 1996).

(405) Estimates for penetrance (θ) used for *BRCA1* mutations encompass the range of values given in Easton *et al*. (1995). The value of $\beta = 2.0$ has been chosen to approximate the excess relative risk coefficient estimated for breast cancer data on A-bomb survivors [all ages: 1.59 (95% CI 1.09–2.19); Thompson *et al*., 1994b]. The ranges of dose levels (D), radiosensitivity differentials (R_i), and predisposition strength (R_p) are arbitrary. However, in view of the computations of (R_p) from cancer risks in relatives (Easton and Peto 1990), and of relative risks of breast cancer in Japanese women exposed to A-bomb radiation (Land *et al*., 1993; Tokunaga *et al*., 1994), the broad ranges of R_p from 10 to 1000 and R_i from 10 to 100 hypothetically describes the contribution of the *BRCA1* locus to cancer susceptibility and radiosensitivity. It is emphasised that no evidence specifically supports such values for heritable breast cancer, nor indeed for heritable colon cancer considered below.

(406) For HNPCC, the parameter values used in computations are the following: p = 0.001, $\pi = 0.02$ and $\beta = 0.72$. The β value is the same as that estimated by Thompson *et al*. (1994b) for colon cancers in A-bomb survivors (all ages and both sexes; 95% CI 0.29–1.28). The R_p and R_i ranges chosen are again arbitrary.

Results: breast cancer

(407) Tables 6.3–6.5 illustrate the numerical results obtained for different combinations of parameter values for breast cancer discussed above. It should be recalled that *RR* quantifies the risk of radiation-induced cancer in a population in the presence of predisposition and radiosensitivity differentials relative to one in the absence of both these factors. The risk measure *AF* denotes the proportion of radiation-induced cancers due to the above heterogeneity and α quantifies the proportion of *AF* that is due to enhanced radiosensitivity alone.

(408) The results shown in Table 6.3 may be deemed applicable to radiation-induced breast cancers in non-Jewish Caucasian women for whom the estimated mutant gene frequency ($p = 0.0006$) and the proportion (π) of breast cancers due to the *BRCA1* mutations (1.7% of all women with breast cancers under age 70) are both small. It is clear that: (i) *RR* values higher than 1 can arise only when both R_i and R_p are very high, e.g. at a dose of 2 Gy, with $R_i = 100$, $R_p = 1000$, and $\theta = 1$, *RR* is 2.64; for other combinations of R_i and R_p shown in Table 6.3, however, the *RR* values are only marginally higher than 1 and (ii) likewise, the dependence of *AF* values on R_i and R_p is clearly seen.

Table 6.3. Estimates of population breast cancer risk obtained using the dominant model of cancer susceptibiity and radiosensitivity for a range of R_i and R_p values, as a function of penetrance (θ) and radiation dose (D) when $\pi = 1.7\%$, $p = 0.0006$, and $\beta = 2.0$ per Gy

	$\theta = 0.50$			$\theta = 0.75$			$\theta = 1.00$		
Dose (D) in Gy	*RR*	*AF*	α	*RR*	*AF*	α	*RR*	*AF*	α
$R_i = 10.0$, $R_p = 10.0$									
0.5	1.00	0.00	0.83	1.00	0.00	0.83	1.00	0.00	0.83
1.0	1.00	0.00	0.87	1.00	0.00	0.87	1.00	0.00	0.87
2.0	1.00	0.00	0.89	1.00	0.00	0.89	1.00	0.00	0.89
$R_i = 10.0$, $R_p = 100.0$									
0.5	1.01	0.01	0.82	1.01	0.01	0.82	1.01	0.01	0.82
1.0	1.01	0.01	0.86	1.01	0.01	0.86	1.01	0.01	0.86
2.0	1.01	0.01	0.88	1.01	0.01	0.88	1.02	0.02	0.88
$R_i = 10.0$, $R_p = 1000.0$									
0.5	1.06	0.05	0.82	1.08	0.08	0.82	1.11	0.10	0.82
1.0	1.07	0.07	0.86	1.11	0.10	0.86	1.14	0.12	0.86
2.0	1.08	0.08	0.88	1.13	0.11	0.88	1.17	0.14	0.88
$R_i = 100.0$, $R_p = 10.0$									
0.5	1.01	0.01	0.98	1.01	0.01	0.98	1.01	0.01	0.98
1.0	1.01	0.01	0.99	1.01	0.01	0.99	1.01	0.01	0.99
2.0	1.01	0.01	0.99	1.01	0.01	0.99	1.02	0.02	0.99
$R_i = 100.0$, $R_p = 100.0$									
0.5	1.05	0.05	0.98	1.08	0.07	0.98	1.10	0.09	0.98
1.0	1.07	0.06	0.99	1.10	0.09	0.99	1.14	0.12	0.99
2.0	1.08	0.08	0.99	1.12	0.11	0.99	1.16	0.14	0.99
$R_i = 100.0$, $R_p = 1000.0$									
0.5	1.52	0.34	0.98	1.77	0.44	0.98	2.03	0.51	0.98
1.0	1.68	0.41	0.99	2.02	0.51	0.99	2.37	0.58	0.99
2.0	1.82	0.45	0.99	2.23	0.55	0.99	2.64	0.62	0.99

(409) The results presented in Table 6.4 may apply for radiation risk of early onset breast cancers in non-Jewish Caucasian women for whom the p is the same as above (0.0006) but π is higher (i.e. 7.5%). As will be clear, for the same R_i, R_p, θ, and D values as those in Table 6.3, the *RR* values are higher (e.g. for $R_i = 100$ and $R_p = 1000$, the *RR* is 8.22 (compared to 2.64 when $\pi = 1.7\%$). Further, a comparison of the α values in Tables 6.3 and 6.4 reveals that α is independent of π.

(410) Other general observations that can be made from Tables 6.3 and 6.4 are the following: (i) an increase in penetrance of the susceptibility allele causes an increase in risk (*RR* and *AF*); and (ii) at any given level of penetrance and combination of R_i and R_p values, the risks (*RR* and *AF*) increase with radiation dose, but the dose-dependence of *RR*, *AF* and α diminishes at higher dose levels; (iii) α is quite high even when *RR* is close to 1 and *AF* is very small, i.e. most of these excess cancers are contributed by the radiosensitivity differential alone and (iv) α is independent of θ, i.e. at any given radiation dose and combination of R_i and R_p, α is the same at all levels of penetrance used.

(411) The model predictions under conditions when both the mutant gene frequency and the proportion of cancers ascribed to the locus under study are higher (as seems to be the case with early-onset breast cancers in Ashkenazi Jewish women), are shown in Table 6.5. As expected, even at moderate levels of R_i and R_p, the risks are higher than those presented in Tables 6.3 and 6.4. As noted earlier, in all cases, with an increase in penetrance, the risks are enhanced, as expected.

(412) Figures 6.2–6.5 depict, for the breast cancer example (early-onset breast cancers in non-Jewish Caucasian women), the predictions of the model when $p = 0.0006$, $\pi = 0.075$

Table 6.4. Estimates of population breast cancer risk obtained using the dominant model of cancer susceptibiity and radiosensitivity for a range of R_i and R_p values, as a function of penetrance (θ) and radiation dose (D) when $\pi = 7.5\%$, $p = 0.0006$, and $\beta = 2.0$ per Gy

	$\theta = 0.50$			$\theta = 0.75$			$\theta = 1.00$		
Dose (D) in Gy	*RR*	*AF*	α	*RR*	*AF*	α	*RR*	*AF*	α
$R_i = 10.0$, $R_p = 10.0$									
0.5	1.00	0.00	0.83	1.00	0.00	0.83	1.00	0.00	0.83
1.0	1.00	0.00	0.87	1.00	0.00	0.87	1.01	0.01	0.87
2.0	1.00	0.00	0.89	1.01	0.01	0.89	1.01	0.01	0.89
$R_i = 10.0$, $R_p = 100.0$									
0.5	1.02	0.02	0.82	1.04	0.04	0.82	1.05	0.05	0.82
1.0	1.03	0.03	0.86	1.05	0.05	0.86	1.06	0.06	0.86
2.0	1.04	0.04	0.88	1.06	0.05	0.88	1.07	0.07	0.88
$R_i = 10.0$, $R_p = 1000.0$									
0.5	1.25	0.20	0.82	1.37	0.27	0.82	1.49	0.33	0.82
1.0	1.31	0.24	0.86	1.47	0.32	0.86	1.63	0.39	0.86
2.0	1.37	0.27	0.88	1.55	0.36	0.88	1.74	0.42	0.88
$R_i = 100.0$, $R_p = 10.0$									
0.5	1.02	0.02	0.98	1.03	0.03	0.98	1.05	0.04	0.98
1.0	1.03	0.03	0.99	1.05	0.04	0.99	1.06	0.06	0.99
2.0	1.04	0.03	0.99	1.05	0.05	0.99	1.07	0.07	0.99
$R_i = 100.0$, $R_p = 100.0$									
0.5	1.23	0.19	0.98	1.34	0.25	0.98	1.45	0.31	0.98
1.0	1.30	0.23	0.99	1.45	0.31	0.99	1.60	0.38	0.99
2.0	1.36	0.27	0.99	1.54	0.35	0.99	1.72	0.42	0.99
$R_i = 100.0$, $R_p = 1000.0$									
0.5	3.27	0.69	0.98	4.41	0.77	0.98	5.54	0.82	0.98
1.0	4.01	0.75	0.99	5.52	0.82	0.99	7.03	0.86	0.99
2.0	4.61	0.78	0.99	6.41	0.84	0.99	8.22	0.88	0.99

and $\beta = 2.0$ over a wider range of arbitrary R_i and R_p values for $\theta = 0.5$ and D = 0.5 Gy; $\theta = 0.5$ and D = 2.0 Gy; $\theta = 1.0$ and D = 0.5; and $\theta = 1.0$ and D = 2.0 Gy. It is worth noting that the top panels of Figs 6.2–6.5 are identical (and are superimposable on each other), but the scales for *RR* are different.

(413) A comparison of the top panels of Figs 6.2 and 6.3 (or of those of Figs 6.4 and 6.5) shows that: (i) changes in *RR* occur as a result of changes in dose; (ii) at any given level of penetrance, when $R_i = 1$ (i.e. no differential radiosensitivity), the *RR* estimates stay the same (i.e. independent of dose) and are driven by predisposition strength; for instance, with $R_i = 1$ and $R_p = 1000$, the *RR* values at 0.5 Gy (Fig. 6.2) and 2 Gy (Fig. 6.3) are the same, namely $< < 5$; (iii) the effect of a given combination of R_i and R_p values on *RR* is more prominent at 0.5 Gy than at 2.0 Gy; for example, when $R_i = 100$ and $R_p = 1000$, the *RR* is about 3 at 0.5 Gy and about 5 at 2.0 Gy (see also Table 6.4), despite a four-fold difference in dose and (iv) with the p and π values used, large increases in both R_i and R_p values are required to result in *RR* values appreciably higher than 1.

(414) A similar comparison of the middle panels of Figs 6.2 and 6.3 (or of those of Figs 6.4 and 6.5), shows that these are *not* identical indicating that for given combinations of R_i and R_p values, the *AF* estimates are dose-dependent, being higher at 2.0 Gy than at 0.5 Gy. For example, when $R_i = 10$ and $R_p = 1000$, *AF* is about 0.2 at 0.5 Gy and 0.3 at 2.0 Gy.

(415) Finally, a comparison of the bottom panel of Fig. 6.2 with that of Fig. 6.3 (or of those of Figs 6.4 and 6.5) shows, again, that these are not identical indicating that α, in addition to being dependent on R_i and R_p also depends on dose. However, at each of the

Table 6.5. Estimates of population breast cancer risk obtained using the dominant model of cancer susceptibiity and radiosensitivity for a range of R_i and R_p values, as a function of penetrance (θ) and radiation dose (D) when $\pi = 38\%$, $p = 0.0047$, and $\beta = 2.0$ per Gy

	$\theta = 0.50$			$\theta = 0.75$			$\theta = 1.00$		
Dose (D) in Gy	*RR*	*AF*	α	*RR*	*AF*	α	*RR*	*AF*	α
$R_i = 10.0$, $R_p = 10.0$									
0.5	1.10	0.09	0.83	1.14	0.13	0.83	1.19	0.16	0.83
1.0	1.12	0.11	0.87	1.18	0.16	0.87	1.25	0.20	0.87
2.0	1.14	0.13	0.89	1.22	0.18	0.89	1.29	0.22	0.89
$R_i = 10.0$, $R_p = 100.0$									
0.5	1.98	0.50	0.82	2.47	0.59	0.82	2.96	0.66	0.82
1.0	2.25	0.56	0.86	2.87	0.65	0.86	3.49	0.71	0.86
2.0	2.46	0.59	0.88	3.19	0.69	0.88	3.92	0.74	0.88
$R_i = 100.0$, $R_p = 10.0$									
0.5	1.90	0.47	0.98	2.35	0.57	0.98	2.80	0.64	0.98
1.0	2.19	0.54	0.99	2.79	0.64	0.99	3.38	0.70	0.99
2.0	2.43	0.59	0.99	3.14	0.68	0.99	3.85	0.74	0.99
$R_i = 100.0$, $R_p = 100.0$									
0.5	10.02	0.90	0.98	14.51	0.93	0.98	18.99	0.95	0.98
1.0	12.96	0.92	0.99	18.92	0.95	0.99	24.87	0.96	0.99
2.0	15.32	0.93	0.99	22.45	0.96	0.99	29.58	0.97	0.99

doses, the bottom panels of Figs 6.3 and 6.5 (or of Figs 6.2 and 6.4) are superimposable on each other showing that α is independent of θ despite differences in penetrance.

(416) The dependence of α on R_i and R_p illustrated in these figures can be summarised as follows: (i) when no cancer-predisposed/radiosensitive subgroup is present in the population (i.e. $R_p = 1$ and $R_i = 1$), all the induced cancers are due to radiation; (ii) when $R_p = 1$ and R_i increases, α rapidly approaches 1 (note that the Z-axis scale is logarithmic) and (iii) for any fixed value of $R_i > 1$, when R_p increases, α decreases. For example, a close scrutiny of Fig. 6.5 will show that when $R_i = 5$ (corresponding to the fourth line above the X-axis) and R_p increases in the range from 1 to 1000 (noting that the R_p scale is to be read from right to left), α progressively decreases to reach a value of about 0.4.

Hereditary non-polyposis colon cancer

(417) Table 6.6 summarises the results of calculations using $p = 0.001$, $\pi = 0.02$, and $\beta = 0.72$. As can be seen, both p and π are small and consequently, even when the penetrance is 100%, with the combinations of R_i and R_p values used, the risks are only marginally increased and are much less when penetrance is low.

6.2.4. *Cancer predisposition and radiosensitivity due to recessive mutations at an autosomal locus*

(418) As may be recalled, with the dominant model, although there are three genotypes at the cancer susceptibility locus, most of the induced cancers are contributed by the heterozygotes. Therefore, when recessive mutations at an autosomal locus are involved in cancer predisposition, even when the mutant homozygotes for the *recessive* allele show substantially large R_p values, the impact on radiation cancer risks to the population will be very small because of the rarity of these homozygotes; the risks will be enhanced only when the heterozygotes show a high degree of cancer predisposition (in which case the model is no longer a recessive model, but a dominant model with incomplete penetrance of the mutant gene). For such a situation, Eqs. (5)–(7) discussed earlier for the dominant

model for prediction of *RR*, *AF*, and α, respectively, can be modified by incorporating a few notational changes.

(419) Since the frequency of heterozygotes (*Aa*) can be approximated by $2q$ (q being the frequency of recessive mutant alleles) the term $[P_{AA}+\theta P_{Aa}]$ representing genotype

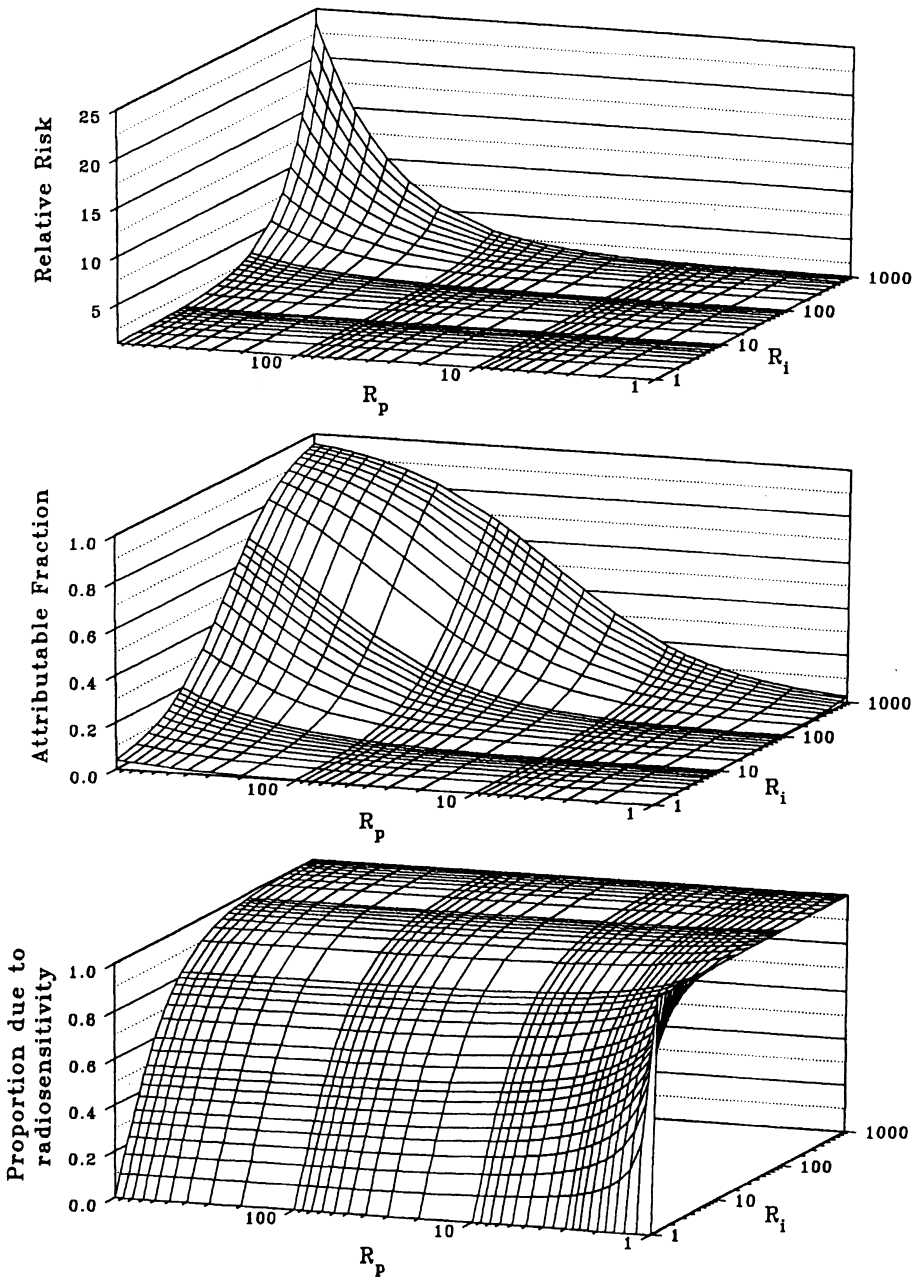

Fig. 6.2. Relationships between *RR*, *AF*, and α when $\theta = 0.5$ and D = 0.5 Gy. For this and Figs 6.3–6.5 the other parameter values used were $p = 0.0006$, $\pi = 7.5\%$ and $\beta = 2.0$ (see Table 6.2).

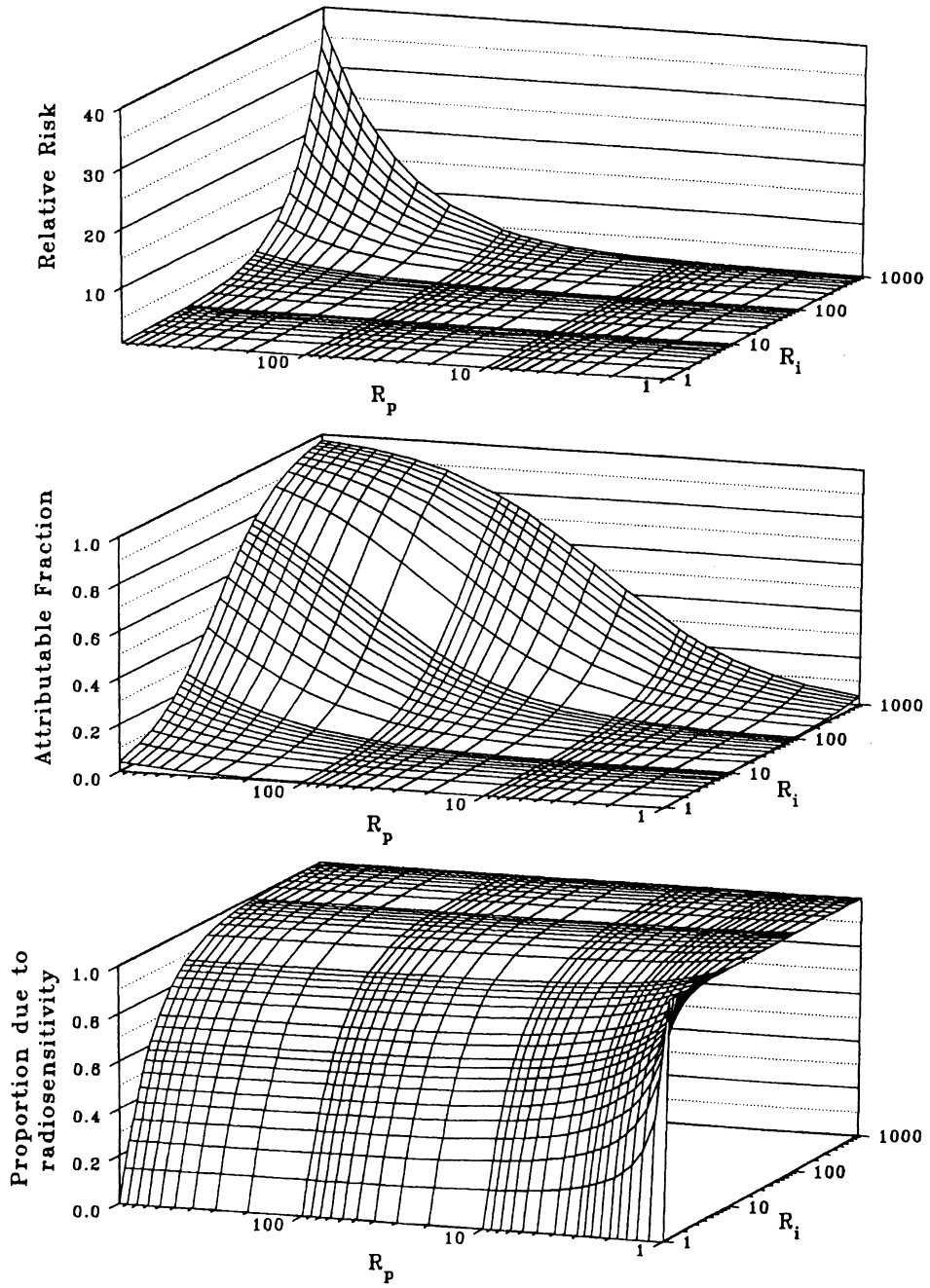

Fig. 6.3. Relationships between *RR*, *AR*, and α when $\theta = 0.5$ and D = 2.0 Gy. See Fig. 6.2 for other parameter values.

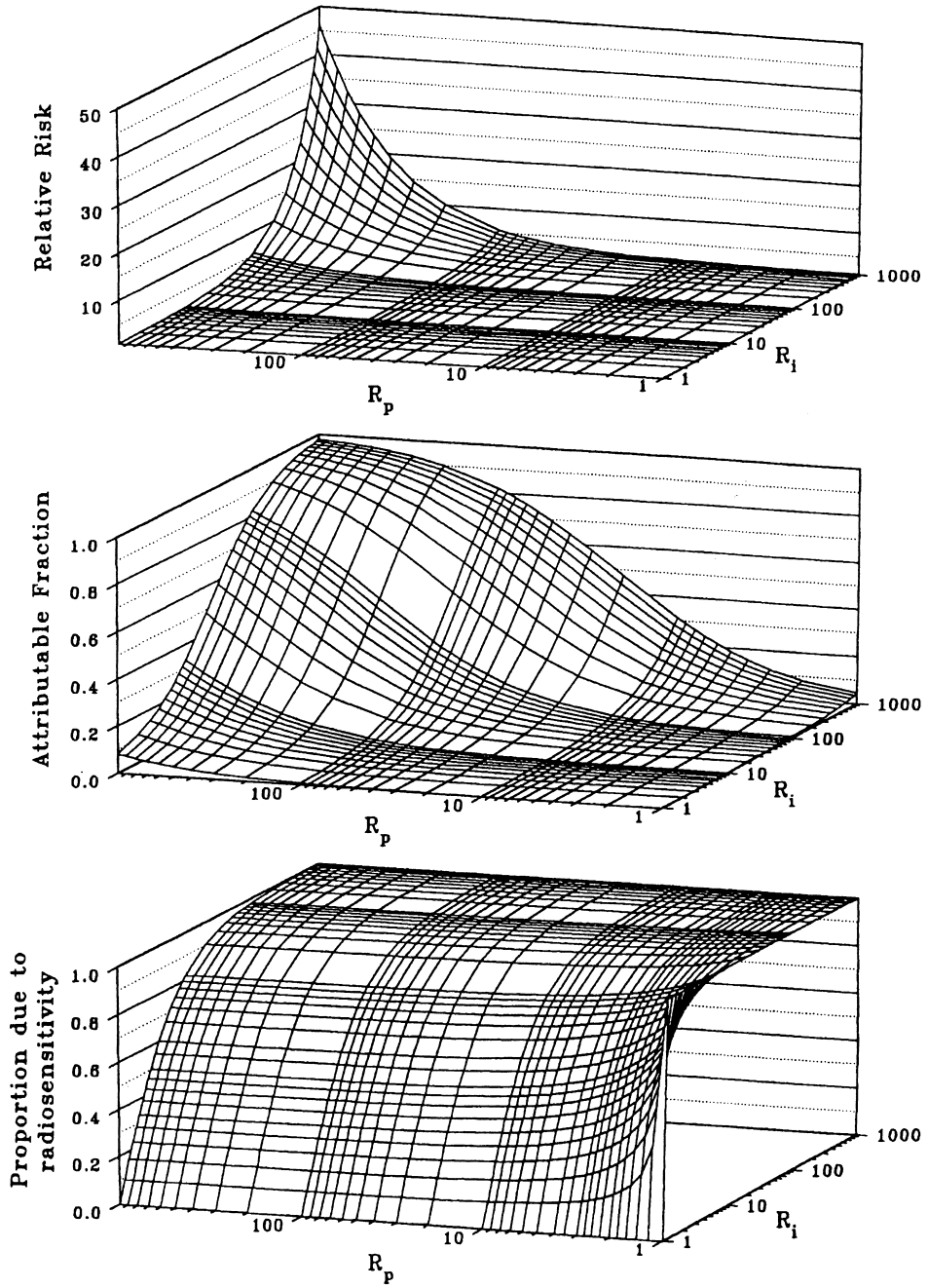

Fig. 6.4. Relationships between *RR*, *AF*, and α when $\theta = 1.0$ and D = 0.5 Gy. See Fig. 6.2 for other parameter values.

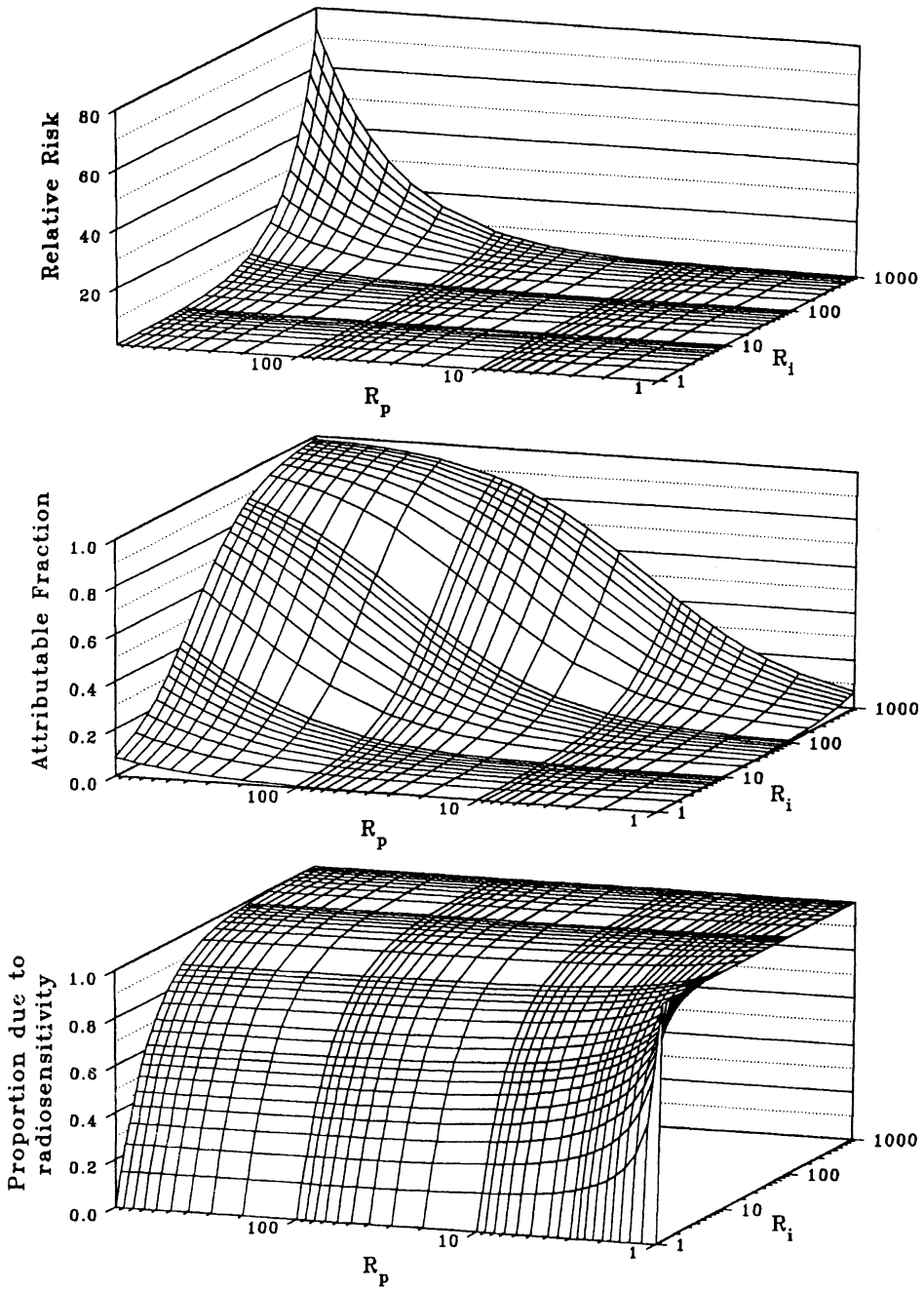

Fig. 6.5. Relationships between *RR*, *AF*, and α when $\theta = 1.0$ and D = 2.0 Gy. See Fig. 6.2 for other parameter values.

Table 6.6. Estimates of population HNPCC risk obtained using the dominant model of cancer susceptibility and radiosensitivity for a range of R_i and R_p values, as a function of penetrance (θ) and radiation dose (D) when $\pi = 2\%$, $p = 0.001$, and $\beta = 0.72$ per Gy

	$\theta = 0.50$			$\theta = 0.75$			$\theta = 1.00$		
Dose (D) in Gy	*RR*	*AF*	α	*RR*	*AF*	α	*RR*	*AF*	α
$R_i = 10$, $R_p = 10$									
0.5	1.00	0.00	0.73	1.00	0.00	0.73	1.00	0.00	0.73
1.0	1.00	0.00	0.81	1.00	0.00	0.81	1.00	0.00	0.90
2.0	1.00	0.00	0.86	1.00	0.00	0.86	1.00	0.00	0.86
$R_i = 10$, $R_p = 100$									
0.5	1.01	0.01	0.71	1.01	0.01	0.71	1.01	0.01	0.71
1.0	1.01	0.01	0.79	1.01	0.01	0.79	1.02	0.02	0.79
2.0	1.01	0.01	0.84	1.02	0.02	0.84	1.03	0.02	0.84
$R_i = 10$, $R_p = 1000$									
0.5	1.07	0.06	0.70	1.10	0.09	0.70	1.14	0.12	0.70
1.0	1.10	0.09	0.79	1.14	0.13	0.79	1.19	0.16	0.79
2.0	1.13	0.11	0.84	1.19	0.16	0.84	1.25	0.20	0.84
$R_i = 100$, $R_p = 10$									
0.5	1.01	0.01	0.97	1.01	0.01	0.97	1.01	0.01	0.97
1.0	1.01	0.01	0.98	1.01	0.01	0.98	1.02	0.02	0.98
2.0	1.01	0.01	0.98	1.02	0.02	0.98	1.02	0.02	0.98
$R_i = 100$, $R_p = 100$									
0.5	1.05	0.05	0.96	1.08	0.08	0.96	1.11	0.10	0.96
1.0	1.08	0.08	0.98	1.13	0.11	0.98	1.17	0.15	0.98
2.0	1.12	0.11	0.98	1.18	0.15	0.98	1.24	0.19	0.98
$R_i = 100$, $R_p = 1000$									
0.5	1.54	0.35	0.96	1.82	0.45	0.96	2.09	0.52	0.96
1.0	1.85	0.46	0.98	2.27	0.56	0.98	2.70	0.63	0.98
2.0	2.19	0.54	0.98	2.78	0.64	0.98	3.38	0.70	0.98

frequencies in Eqs. (5)–(7) needs to be substituted by $2q$ yielding

$$RR(\mathrm{D}) = 1 + 2\pi q \left\{ \frac{R_p(1 + \beta \mathrm{D} R_i)}{1 + \beta \mathrm{D}} \right\} - 1 \tag{8}$$

$$AF(\mathrm{D}) = \frac{2\pi q \left\{ R_p(1 + \beta \mathrm{D} R_i) - (1 + \beta \mathrm{D}) \right\}}{(1 + \beta \mathrm{D}) + 2\pi q \left\{ R_p(1 + \beta \mathrm{D} R_i) - (1 + \beta \mathrm{D}) \right\}} \tag{9}$$

and

$$\alpha(\mathrm{D}) = \frac{\beta \mathrm{D} R_p (R_i - 1)}{R_p(1 + \beta \mathrm{D} R_i) - (1 + \beta \mathrm{D})}. \tag{10}$$

(420) The above formulations thus extend the autosomal dominant model to an autosomal recessive one in which heterozygotes for the recessive mutant gene shows predisposition to cancer. Note that the proportion of *AF* due to radiosensitivity differentials [α(D)] Equation (10) does not depend on genotype frequencies as is the case with the dominant model Equation (7). No numerical illustrations are given because of the fact that, at present, there are no clear examples of this type of cancer predisposition.

6.3. Summary and conclusions

(421) This chapter discusses an autosomal dominant model and its use to assess the impact of the presence of cancer-predisposed subgroups in a population (which are also assumed to be more sensitive to radiation-induced cancers), on radiation cancer risks compared to one which does not have these subgroups. The model predictions are illustrated using current estimates of mutant gene frequencies for, and proportions of, cancers associated with the loci, *BRCA1* (breast cancer) and HNPCC (hereditary non-polyposis colon cancers), and arbitrarily chosen ranges of values for strength of cancer predisposition, radiation dose-response and radiosensitivity differentials. Additionally, the extension the above model to a situation in which cancer predisposition and radiosensitivity are due to recessive mutations at an autosomal locus is also discussed. The main conclusions are the following:

1. Irradiation of a heterogeneous population of the type mentioned above, results in higher cancer risks compared to a population which is not heterogeneous in this regard. However, unless the mutant gene frequencies (p), proportion of cancers attributable to the locus under study (π), radiosensitivity differentials (R_i), and predisposition strength (R_p) are all sufficiently large, the enhancement in risks is small and may not be detectable.
2. All three measures of risk (relative risk [*RR*], attributable fraction [*AF*], and the fraction of *AF* due to radiosensitivity differentials [α] are all dose related. At any given level of penetrance and combination of R_i and R_p values, *RR* and *AF* increase with dose, but the dose dependence of *RR*, *AF* and α diminishes at higher dose levels.
3. Incomplete penetrance of the mutant gene has the effect of 'diluting' the effects of predisposition strength and radiosensitivity differentials; the increase in risks is not linearly related to an increase in penetrance.
4. For non-Jewish Caucasian women for whom a p of 0.0006 (*BRCA1* mutant gene frequency) of 0.0006 and a π of 1.7% (proportion of breast cancers under age 70 due to *BRCA1* mutations) have been assumed, the model predicts that relative risks, *RR*, will be detectably different from 1 only when both the radiosensitivity differential, R_i, and strength of predisposition R_p are very high (e.g. at $R_i = 100$ and $R_p = 1000$, for 100% penetrance, *RR* is 2.64 at 2 Gy). The prediction for the radiation risk of HNPCC in Caucasian populations is similar. In essence, on current knowledge the mutant gene frequencies relating to genetic susceptibility to breast and colon cancer are too low to make a significant impact on radiological risk in typical human populations.
5. When both p and π are higher (as seems to be the case for breast cancers in Ashkenazi Jewish women ($p = 0.0047$ and $\pi = 38\%$ for breast cancers under age 30), the predicted *RR*s are > 1 even at moderate levels of R_i and R_p, its magnitude depending on radiation dose and penetrance of the mutant gene. Thus, in certain atypical sub-populations where mutant gene frequencies for cancer-predisposing disorders are high due to founder effects and inbreeding a genetic impact on population risk after radiation can, in principle, become important.
6. An autosomal recessive model of cancer predisposition and radiosensitivity will be meaningful only when heterozygotes show high R_p values; for such a situation, the autosomal dominant model can be used with a few notational changes.

7. IMPLICATIONS FOR RADIOLOGICAL PROTECTION OF DATA ON CANCER SUSCEPTIBILITY

(422) At the outset, it was recognised that the state of knowledge on the possible interactions between ionising radiation exposure and relevant human genetic factors was such that it would be possible only to paint a broad picture of their impact on radiation-induced carcinogenic risk and only provide some interim judgements in respect of the possible implications for radiological protection.

(423) The data reviews and computational modelling so far presented provide, nevertheless, sufficient information for preliminary comment upon (a) the extent to which current ICRP estimates of cancer risk in typical irradiated populations might be influenced by highly penetrant genetic factors and (b) the radiological implications for individuals with such familial disorders.

(424) A recurring theme in this document has however been the problem of ascertainment of cancer-predisposing human mutations of low penetrance. For these, while some speculative comment is possible, there is currently no firm scientific basis on which to make meaningful judgements. While it is not the intention of this report to provide detailed commentary on future research needs, consideration will also be given to the broad topics that will need to be addressed in order to resolve the most important uncertainties regarding the relationships between human cancer predisposition and radiological risk.

(425) The data reviews presented here have stressed the information that is arising from molecular characterisation of the human germ line mutations that underlie heritable cancer susceptibility. These data and the associated technologies that have been developed have important implications for diagnosis of the relevant disorders. Since such diagnostic techniques are expected to play an increasingly important role in medical genetics and could conceivably impinge on radiological protection, attention is also given briefly to fundamental and technical problems that currently surround their application. Finally, in the light of the considerations noted above a brief interim judgement is provided on the implications of genetic susceptibility to cancer in the context of radiological protection practices.

7.1. Familial cancer involving genes of high penetrance

(426) The vast majority of the information accruing on human cancer susceptibility in humans derives from studies of families showing segregation of genes that substantially increase the lifetime risk of a given set of neoplasms; this feature is often accompanied by an early age-of-onset for the neoplasms in question. That said, environmental factors and co-segregating genes almost certainly play a role in the expression of some of these high penetrance mutations; such mutations can be present in some cases where there is no obvious family history of cancer and, equally, some known carriers remain cancer free (Friend, 1996).

(427) In spite of these uncertainties, the overall incidence of high penetrance cancer-predisposing mutations in the population can be estimated together with their likely contribution to total cancer. In the light of the data reviewed in previous Sections and the views expressed by other commentators, it is judged that the overall incidence of these mutations in Western populations seems likely to be less than 1% and that they probably account for around 5% of total lifetime cancer. As noted earlier, this contribution tends however to be age dependent and will be greater in children and young to middle-aged

adults presenting with cancer. Also evident from the data review is that, in terms of familial cancer, solid tumours appear to be subject to greater genetic influences than lympho-haemopoietic neoplasia. Among well characterised solid tumour categories available data imply that neoplasia of the breast, colon and prostate have the most significant genetic components. Since these cancers are relatively common in the population, the genes responsible may be seen to have the greatest overall impact on cancer mortality.

(428) The data of Section 5 provided evidence that in most, but not all, cancer-predisposing disorders, there is the expectation that genetic susceptibility to spontaneously arising disease will be accompanied by above-normal cancer risk after ionising radiation exposure. It is believed that the tumour types involved will generally be the same for spontaneous and radiation-induced disease.

(429) Quantification of the degree of increased tumorigenic radiosensitivity associated with familial cancer is however highly problematical; the great uncertainties that attach to providing judgements on relevant issues are discussed in Sections 5 and 6. For the reasons given in Section 5 no single factor can be expected to describe this effect in all such disorders; as a first approximation it is judged that the absolute increase in radiation risk may fall between 5- or less and 100-fold as judged from all sources of data with the limited human data being more consistent with increased risk within these disorders of less than around 10-fold. It is stressed at this point however that these judgements are not yet well founded since they depend upon incomplete epidemiological evaluations, radiotherapeutic observations in often isolated cases and somewhat uncertain data obtained in animal genetic models of disease. As a consequence, at this stage in knowledge the judgements provided here should be used with great caution. Nevertheless, they remain useful for the purposes of making calculations that serve to illustrate the possible impact of familial genetic susceptibility to radiogenic cancer at both the population and individual levels.

7.1.1. *Familial cancer and risks in a population*

(430) Assuming a value of $<1\%$ for familial cancer incidence and of values of 5–100-fold to describe the genetically determined increase in radiogenic cancer risk, the computational modelling of Section 6 argue strongly against any major impact of familial cancer on overall risks in the whole population. This modelling exercise showed that even for breast cancer where, on current knowledge, the genetic contribution to risk is relatively high, the gene frequency is far too low to provide for a significant distortion of risk in the whole population. Since the genetic contribution to tumours at other common sites will tend to be lower there can be no reason to speculate that after radiation the risk of excess cancer is concentrated in a genetically predisposed sub-fraction of the population and that the remainder are relatively radio-resistant.

(431) The data considered in Sections 5 and 6 provide no evidence of the widespread and very high degree of sensitivity that would be needed to drive major risk-distorting effects; nor of disorders where the expression of genetically determined cancer is largely dependent upon ionising radiation exposure. The radiotherapy follow-up data cited are of particular relevance to these issues. Cancer patients receiving radiotherapy are a selected sub-population within which the genetic component is expected to be increased to perhaps around 2–5% albeit in an age-dependent fashion. These data provide however no evidence of a major familial component of risk. For breast cancer, Storm *et al.* (1992) report that family history is a positive factor for radiotherapy-related disease (10% of cases in patients

versus 6.4% in controls) but this did not significantly influence the overall estimate of risk. Thus although women with breast cancer carry a 2–5-fold increase in risk of a second breast tumour this was largely independent of radiation. These data tend to argue against a high level of increased tumorigenic radiosensitivity in hereditary breast cancer and support indirectly the lower value of increased risk (no more than 10-fold) judged from other human data. In spite of this failure to identify a major familial component of risk, the radiotherapy data are sufficient to reveal evidence of increased risk of second cancers in known rare disorders—retinoblastoma, Li–Fraumeni syndrome, and nevoid basal call carcinoma syndrome (Section 5).

(432) Overall, and as a further simplification of the problem it is suggested that the available data, although sparse, would not be inconsistent with a familial genetic contribution to cancer in an irradiated population that reflects that which applies to spontaneously arising disease, i.e no more than ~5%. The mechanistic arguments concerning the predominant disorders associated with germ line tumour suppressor gene mutations together with the animal data of Section 5 tend to support this contention. These mechanistic arguments and one set of animal data also allow brief comment on radiation risk relative to that of other carcinogenic agents. Although ionising radiation was framed as a relatively efficient carcinogen in tumour-suppressor gene deficient categories of patients, for the reasons given in Section 5 it is believed that they would also be at increased risk of cancer after exposure to a range of other environmental DNA damaging agents.

7.1.2. *Familial cancer and individual risk*

(433) From the data review of Section 5 it is judged that in many disorders of familial cancer the individual carriers will be subject to an absolute increase in cancer risk; this factor of increase was judged to be in the range 5–100-fold. Direct human observations from radiotherapy follow-up studies suggest that the most appropriate value for this factor might be ~10-fold. In considering individual risk in such cases it is however crucial to relate the extra risk imposed by a given radiation exposure to that which is unavoidably carried, i.e. the elevated risk of spontaneous disease.

(434) In the case of familial cancer disorders which are, by definition, of relatively high penetrance, it is suggested that the spontaneous risk of usually organ-specific cancer is sufficiently high that even large increases in tumorigenic radiosensitivity have only a marginal impact on lifetime risk at low doses of radiation. In order to illustrate this point a theoretical disorder is considered in which there is a spontaneous lifetime risk of fatal breast cancer of 40% and an increase in radiation risk of lifetime fatal breast cancer of 10-fold over normal i.e the value judged to be most consistent with human data.

(435) Table B-17 of *Publication 60* (ICRP, 1991), reproduced here in a modified form as Table 7.1, recommends a low dose risk value of 0.2% Sv^{-1} for lifetime fatal breast cancer in a population of all ages—for the purposes of illustrative calculation this value is taken to represent the risk to a normal individual and since it applies to both sexes the value for females approximates to 0.4% Sv^{-1}. Thus, for a female individual with the theoretical disorder, the radiation-related risk of breast cancer is 4% Sv^{-1} for fatal cancer plus another 4% for non-fatal cancer. In spite of the 10-fold increase in absolute risk, following a protracted dose of 100 mSv, taken to represent an accumulated occupational exposure, the hypothetical risk of fatal breast cancer in that individual rises from 40% to only 40.4% or from 80% to 80.8% for fatal plus non-fatal breast cancer. Thus, unless the interim judgements made have seriously underestimated the increase in absolute risk, low-

Table 7.1. Illustrative example of changes in absolute cancer risk overall in a theoretical heritable disorder having a breast-specific increase in risk of 10-fold over normal[a]

	Fatal probability coefficient (10^{-4} Sv^{-1})	
Organ at risk	Normal	Heritable breast cancer
Bladder	30	30
Bone marrow	50	50
Bone surface	5	5
Breast	40[b]	400
Colon	85	85
Liver	15	15
Lung	85	85
Oesophagus	30	0
Ovary	10	10[c]
Skin	2	2
Stomach	110	110
Thyroid	8	8
Remainder	50	50
Total	520	880

[a]Based upon data of Table B.17 of *Publication 60* (ICRP, 1991) with normal individual risk being assumed to be the same as that for a whole population.
[b]Publication 60 value ×2 to reflect risk in a normal female.
[c]It is recognised that risk of ovarian cancer is also increased in some cases of heritable breast cancer.

dose exposure of occupational magnitude will have only a minor impact on total breast cancer risk associated with familial disorders. Indeed, even if a high nominal value of 50-fold for increased absolute risk is taken the probability of fatal breast cancer in the example given rises from 40% to only 42% or from 80% to 84% for fatal plus non-fatal breast cancer.

(436) In consideration of a 10-fold absolute increase in breast cancer risk the same protracted whole body dose of 100 mSv to the genetically susceptible individual raises the probability of fatal breast cancer from the normal value of 0.04% to 0.4%. Assuming for simplicity that risk to other organs of the affected individual remains normal, the *Publication 60* data of Table 7.1 may also be used to illustrate the true impact on absolute cancer risk overall. Accordingly, although the elevated breast cancer risk of 0.4% means that the breast becomes by far the most radiosensitive organ of the individual, risk of fatal cancer overall rises by less than two-fold, i.e. following a protracted whole body dose of 100 mSv, from around 0.5% to around 0.9%. It should be recognised however that in genetic disorders associated with excess cancer at multiple sites (see Section 3), this increase in absolute risk is expected to be less modest. The same arguments may be applied to the low doses of radiation (usually less than a few tens of mSv) associated with medical diagnostic procedures—the impact on cancer risk overall will be small.

(437) High doses of radiation to normal tissues experienced in radiotherapy or, much more rarely, as a consequence of a radiation accident, present however a potentially greater problem. This may be illustrated by considering the same hypothetical example used above (10-fold absolute increase in breast cancer risk) but, in this case, with the individual presenting for breast cancer radiotherapy. In the course of such fractionated high dose radiotherapy for unilateral breast cancer it is assumed that the unaffected contralateral breast of the predisposed patient will receive a dose of 2 Sv through scattered or incidental exposure. In this hypothetical example after correcting for dose/dose rate effects (×2) and the tissue mass at risk (×0.5 to allow for irradiation of one breast only),

ICRP-based risk in the predisposed patient remains at 4% Sv^{-1} and therefore the 2 Sv dose to contralateral breast will be conditionally associated, given long-term survival from the first breast cancer, with a radiation-related risk of something less than 8% for a fatal second breast cancer and something less than 16% for fatal plus non-fatal breast cancer. The magnitude of the risk of a second breast cancer will also tend to depend upon the age at which the radiotherapy is delivered, i.e. age of diagnosis of the first neoplasm. It is expected that there will be a substantially smaller excess risk for exposure after age 40 or 50 compared with treatment at younger ages (UNSCEAR, 1994).

(438) Although there is as yet no evidence for the strong action of familial factors in contralateral breast disease after radiotherapy (Storm *et al.*, 1992), the principal message of high dose risk that may be taken from the above example broadly accords with radiotherapeutic observations of second cancers of other types in the cancer predisposing disorders retinoblastoma, Li–Fraumeni syndrome, and nevoid basal cell carcinoma syndrome (Section 5). It is stressed however that the examples given are illustrative and are not intended to provide specific guidance on risk.

(439) Also noted in Section 5 was the seemingly characteristic shortening of latency of radiation-associated tumours in human cancer prone disorders and one animal genetic model. The early age of onset of spontaneously arising neoplasms in these disorders is fully expected to be reflected in those induced through exposure to exogenous carcinogens such as radiation. This will tend to add to the detriment that attaches to these disorders. There is as yet, however, insufficient information with which to provide any quantitative judgement on this issue. Also, if as suggested in Section 5, the latency effect in question depends upon the multiplicity of early carcinogenic cellular events then there will tend to be strong dose-dependence. Assuming this to be correct, a further reduction of tumour latency in familial cancer will not be a feature of low dose radiation exposure.

7.2. Cancer involving genes of low penetrance

(440) Whereas germ line cancer-predisposing genes of high penetrance reveal themselves by Mendelian segregation of cancer in family pedigrees, as penetrance decreases so the normal patterns of Mendelian inheritance become obscured. Unless the tumour in question is particularly rare, cancer incidence and distribution within the family approaches that which will occur by chance. Thus, as noted earlier, family studies on cancer incidence are greatly limited in their power to reveal genetic factors for common tumours that have low or variable penetrance. Although it is possible that such mutations are not uncommon in the population there is no firm scientific basis for such a judgement.

(441) In response to this problem attempts have been made to use aggregated family histories and/or large populations to attempt to answer the question as to whether there is a widespread genetic component to human cancer (see Houlston and Peto, 1996). Although there is some positive evidence of this (e.g. Birch *et al.*, 1990; Thompson *et al.*, 1988; Fuchs *et al.*, 1994), studies of the parents of colorectal cancer patients (Sonderjgaard *et al.*, 1991), twin studies (Hrubec and Neel, 1982) and some medical centre-based investigations on first degree relatives of childhood cancer patients (Pastore *et al.*, 1987; Burke *et al.*, 1991; Montou *et al.*, 1994) do not support the concept of general inheritance of cancer susceptibility.

(442) In terms of sheer statistical power the report of Olsen *et al.* (1995) on 11,380 parents of Danish children with cancer is potentially the most important study of this type. The study revealed that the cancer rate of the parents of children (< 15 years) with cancer was not different from that expected in the general population. Although this study

clearly indicates that cancer in children is not a general genetic marker for cancer risk in the parents it does not exclude the presence of low penetrance mutations particularly in respect of adult cancers.

(443) Sorensen (1995) has provided comment on this issue and here it is sufficient to note that uncertainties remain in respect of possible differences in cancer rates between adults with and without children, the relevance of childhood cancer predisposing genes for the whole range of adult cancers and, most importantly, that the study design in not considering risk in siblings, age of onset or histopathological features only allows exclusion of widespread dominant cancer predisposition of moderate to high penetrance. Hence, the low penetrance question remains unanswered and in the absence of heroic epidemiological and molecular investigations is likely to remain so for some time to come. The same rather pessimistic view attaches to determination of the true impact of modifier genes, genomic imprinting and mosaicism on cancer risk in the population (see Sections 3 and 5).

(444) At present it is only $ATM^{+/-}$ genotypes (ataxia-telangiectasia heterozygotes) and an allele of *HRAS* that might qualify as low penetrance determinants or modifiers of cancer risk having frequencies sufficient to potentially impact on risks in the population (see Sections 3, 5 and 6). It should be emphasised, however, that for both *HRAS* and $ATM^{+/-}$ human genotypes, the phenotypic manifestation of excess cancer is not well established. Further candidate low penetrance genes may come to light and included here would be the genetic factors that may determine proliferative breast disease and chromosomal radiosensitivity in unselected breast cancer patients (see Sections 2 and 4).

(445) In the absence of guidance on the overall contribution of low penetrance genes to spontaneous human cancer no firm judgements are possible on their implications for radiological protection. In the light of the arguments presented in Section 5 it is however reasonable to assume that the cancer risk in carriers of these genes would be increased by radiation exposure. With this assumption their possible impact on current estimates of population risk may be framed as follows. If a large number (say 1000 s) of such genes were to make approximately equal contribution to radiogenic cancer and no specific combinations were to confer high sensitivity then risk would be broadly distributed in human populations and no significant distortion of population risk would be expected. Conversely, if a smaller number of genes (say 100 s) were to be involved radiation risk might be expected to be less uniformly distributed. This risk distribution problem for low penetrance mutations will tend to be greatest if specific gene combinations were to be particularly 'sensitising'-resolving such complex multifactorial effects is well recognised as a most difficult task. In general, however, a reduction in penetrance tends to dilute the population impact from cancer predisposing genes (see Section 6) which means that the prevalence of carriers would need to be high in order to significantly affect current judgements on radiological risk.

(446) Finally, on the question of individual risk for proposed low penetrance mutations, it is suggested that the same argument used for familial cancer (see Section 7.1.2) will tend to apply. On the basis of individual radiation risk relative to baseline, low dose effects are expected to be minor and it is only in the case of high dose exposure that there would be cause for concern. It should be recognised however that knowledge in this area is far from complete. The possibility remains that there are rare disorders, probably of DNA damage processing, where the risk of spontaneous cancer is not at all remarkable but where expression is substantially increased by exposure to ionising radiation. Relevant to this is that, in the absence of solar exposures, skin cancer in UVR-repair deficient xeroderma

pigmentosum (XP) patients is uncommon (Kraemer *et al.*, 1994), i.e in respect of skin cancer the XP phenotype is almost wholly dependent upon this specific environmental insult. It remains to be seen whether there are phenotypic variants of the radiosensitive human ataxia-telangiectasia disorder that show XP-like dependence of cancer on ionising radiation exposure. In such cases the individual risk of excess cancer after even low doses of ionising radiation may be high relative to baseline risk.

7.3. Areas of future research in radiological protection

(447) Much of the knowledge necessary to improve judgements on the role and impact of genetic factors in radiation cancer risk is expected to derive from research outside the field of radiological protection. The genetics of the common cancers is developing rapidly, particularly in respect of the highly penetrant genes predisposing to breast and colon cancers. It is important however that a better view is gained, not only of the identity and role of cancer predisposing genes of lower penetrance but also their prevalence and the genetic–genetic and genetic–environmental interactions that guide phenotypic expression. Recent rapid advances in the genetics of breast cancer provide evidence that such information can, in principle, be gained. As well as being directed towards specific goals, radiological protection research may well be able to contribute towards the resolution of some of these fundamental issues.

(448) The degree of tumorigenic radiosensitivity associated with cancer-predisposing disorders was discussed in Section 5. Although few directly informative epidemiological studies are currently available, a number of large potentially informative human groups were identified for further investigations. With the possible exception of UVR, the effects of which are limited to the skin, for no other environmental carcinogen is it possible to achieve this. Thus, the potential studies noted may prove to be an important source of basic information on genetic–environmental interactions in human carcinogenesis.

(449) In any future surveys of radiation-related neoplasms for possible genetic factors it would be of obvious benefit to gain information on age-of-onset, histopathology, and where possible, the family history of cancer. Given ongoing advances, perhaps the most important issue is the availability of normal tissue from which to ascertain whether specific germ line mutations characterised any fraction of the cases.

(450) At present this strategy would be most profitably applied to determine the contribution of *BRCA* and *ATM* genes to radiogenic breast cancer. With knowledge of such contributions in cases and controls, the doses received and the prevalence in the normal population of the respective gene defects it will be possible to better judge the degree to which radiogenic risk is increased in gene carriers (see Section 5). Although this strategy should, in principle, provide a more clear picture of the problem, the work load imposed by the molecular analyses should not be underestimated—some hundreds of unrelated breast cancer cases and controls would need to be analysed at the DNA sequence level (see Section 5, also Section 7.4).

(451) Animal data have contributed significantly towards the judgements made in Section 5 of this report on genetically determined radiogenic risk. There are now considerable opportunities to extend the experimental approaches to mice deficient in, for example, *BRCA*, *ATM*, and DNA mismatch repair genes. Genetic and molecular analysis of the natural interstrain differences in murine tumorigenic radiosensitivity should also be encouraged.

(452) Cellular and molecular studies have played critical roles in understanding the complex relationships between DNA damage repair after radiation, cell cycle control,

genomic instability, and the functions of proto-oncogenes and tumour suppressor genes. Here it is sufficient to highlight a few focal issues for future consideration.

(453) Studies on the cellular and chromosomal radiosensitivity of human and rodent cell strains have proved invaluable in the identification and characterisation of phenotypes relevant to radiation tumorigenesis. There is great scope for further work in this area, particularly in respect of novel mammalian genes controlling radiation response and their structural/functional relationships with those already characterised in lower organisms. More emphasis could perhaps be placed on phenotypes specifically associated with increased sensitivity to the induction of gene and chromosomal mutations rather than simply cell inactivation. In this context the development of more rapid and reliable mutation systems would be invaluable. Associated with the further development of this whole area of research is the need for improved access to relevant cellular material. There is a strengthening case for the establishment of specific banks of cells having known or suspected associations with altered radiation response.

7.4. The application of diagnostic technologies

(454) Although the clinical evaluation of familial cancer aggregation remains a critical tool in the determination of heritable cancer, rapid strides have been made in the development of methods of molecular diagnosis. Following the molecular cloning and DNA sequencing of a given familial cancer gene, it becomes possible to determine the nature and distribution of the mutations that underlie the disorder. The two examples specifically cited in this report relate to *p53* mutation in Li–Fraumeni syndrome and heritable breast cancer associated with *BRCA1* mutation; the issues noted briefly below apply, however, more generally.

(455) Although there may be mutational sequence 'hot spots' in such germ line genes, the mutations tend to be distributed throughout the different exons. Particularly in the case of large genes such as *BRCA1* there is, therefore, the potential for a vast array of different DNA sequence mutations underlying the same or closely related clinical phenotypes.

(456) In the case of a family pedigree segregating a single gene-specific mutation the characterisation of that mutation in one family member allows for ascertainment of inheritance amongst existing family members. Rapid polymerase chain reaction methodologies applied to DNA usually extracted from blood samples is generally favoured—in some instances DNA linkage by microsatellite analysis is used, in others direct mutational analysis is possible (Yates, 1996). In this way carriers of the mutant gene are identified thus allowing for genetic counselling, certain judgements on clinical prognosis and, in a few instances, clinical intervention. Thus, albeit with due attention to the ethical implications which are not uniform (Reilly *et al.*, 1997), the molecular screening by consent for specific gene mutations segregating within family pedigrees is becoming relatively commonplace.

(457) Much more problematical is the feasibility of applying existing molecular technologies to the screening of relevant gene mutations in unrelated individuals. In the context of heritable cancer and radiological protection such screening, if and when judged to be ethically and practically appropriate, might be relevant in the case of planned medical irradiations or of possible occupational exposures (see Section 7.5).

(458) The principal practical difficulties in such screening are that since the individuals to be tested are largely unrelated, first, that linkage studies are by definition ruled out and second, that the site of the mutation in carriers will, in the main, be different. In essence,

DNA conformational analysis or protein characterisation followed by DNA sequencing would be necessary for unambiguous diagnosis and given the multiplicity of different cancer predisposing disorders and their rarity the screening task becomes enormous. Even in an unrelated subgroup substantially enriched for a known set of genes, e.g. *BRCA* genes in early onset breast cancer cases, the technologies noted above simply do not have the speed or degree of automation to make possible the routine mass screening of say thousands of cases.

(459) The single possible exception to this is the gene mutations maintained in whole populations through so called founder effects, i.e ancestral germ line events escaping powerful counter-selection within populations that show a degree of inbreeding. Such single mutations can be shared by a significant fraction of carriers of a single clinical disorder. For example a single *BRCA1* gene mutation is estimated to be shared by around 1% of the Ashkenazi Jewish population (Sections 4 and 6), an incidence that would allow for mass screening using current technology; founder effects for *BRCA2* are also pronounced in this population (see Friend, 1996). The occurrence of founder effects of significant magnitude, although not common, has also been noted for *BRCA2* in Iceland (Thorlacius et al, 1996) and for *hMLH1* mutations underlying hereditary non-polyposis colon cancer in Finland (Papadopoulos *et al*., 1994).

(460) In due course, with increasing technical innovation, the development of methods for mass genetic screening should however be anticipated. Forrest *et al*. (1995) and Mashal and Sklar (1996) provide commentaries on these developing methodologies which include novel DNA mismatch hybridisation methods, conformational analysis and protein truncation testing. Automation of testing will, however, be the key to the problem. In this area the development of so-called 'DNA chips' to detect, by fluorescence analysis, DNA mismatches in multiple micro-arrays of target oligonucleotide sequences constituting a whole gene is a technology that is expected to be fully operational within the next few years (Lipshutz *et al*., 1995; Kreiner, 1996). Laboratory-scale studies utilising oligonucleotide array techniques for *BRCA1* mutation analysis have already been reported (Hacia *et al*., 1996) and further rapid development seems likely.

(461) Irrespective of these developments it is important to recognise, however, that until the full extent of cancer-predisposing genetic determinants is known together with their possible interactions and clinical implications, the true predictive value of such testing cannot be assured. As discussed by Friend (1996), the highly variable penetrance in respect of spontaneous cancer in carriers of the *same* cancer predisposing mutation implies a major potential modification of risk by genetic–genetic and/or genetic–environmental interactions. There is no reason to believe that radiation risks in such kindred would not be similarly variable in which case prognostic judgements on a given individual based upon genetic testing must be open to doubt.

7.5. Summary and conclusions for radiological protection

(462) As a result of the work compiled above, the Main Commission of ICRP has reviewed the rapidly developing field of human cancer genetics with a view to providing an interim judgement on the practical implications for radiological protection. Although critical data on risk of radiation tumorigenesis are limited, it is judged on the basis of current knowledge by the Commission that:

1. In most, if not all, instances of familial cancer predisposition associated with the dominant inheritance of strongly expressing tumour suppressor gene mutations there

will be an absolute increase in the probability of radiation-induced cancer. The range of this probability may vary between familial disorders within which different tumour types will tend to predominate. Overall, a range of increases in absolute cancer probability of 5 or less to 100-fold for some sites is implied by available human and animal data. It is judged however that a 10-fold absolute increase in cancer probability for some sites after radiation encompasses the human data currently available for certain tumours in a few genetic disorders. The detriment associated with this risk increase is at present difficult to quantify. On the one hand because of the high probability of spontaneously arising cancer, relative risk is small at low doses. On the other hand, a shortening of tumour latency will increase the detriment.

2. In the case of cancer predisposition associated with deficiencies in DNA repair it is suggested that some but not all disorders of this type will show elevated cancer risk after radiation. No judgements can be made at present on the degree of such risk but the data regarding increased radiation cancer risks in ataxia-telangiectasia heterozygotes, who are relatively common in the population, were judged to be weak. Data on spontaneous cancer risk in ataxia-telangiectasia heterozygotes indicate, however, that increased radiation risk cannot be discounted.
3. Computational modelling of the contribution of genetically imposed risk of excess cancer in a typical irradiated human population showed that, because of their low prevalence, familial cancer disorders can have a minor impact only. Illustrative calculations were also made with respect to excess cancer in individuals with familial disorders following low and high dose exposures.

(463) On the basis of these data and judgements the practical implications for radiological protection practices may be framed as follows.

7.5.1. *Estimation of risks in whole populations*

(464) Since existing epidemiological measures of risks tend to centre on relatively large and heterogenous populations (ICRP, 1991, UNSCEAR, 1994) a genetic contribution of undetermined magnitude is already included in current recommendations of risk from the Commission which has for some time recognised that risk will not be uniformly distributed. The use by the Commission of baseline cancer data in the extrapolation of risks across populations takes account of possible differences in genetic contributions between populations. The issue is, therefore, not the validity of the overall estimate of risk but how that risk is distributed and whether known genetic factors impose an unacceptable degree of distortion. Since, however, only 1% or less of the population fall into the familial cancer category where significantly increased radiation risk might be anticipated, confidence is gained that existing recommendations from the Commission on cancer risk in irradiated populations are not subject to unacceptable genetic uncertainty.

7.5.2. *Risks in individuals with familial cancer*

(465) Individuals having most, if not all, cancer-predisposing familial disorders are expected to be at increased absolute risk of cancer after radiation exposure. But, as indicated, at low doses that incremental risk is small. Additionally, the inter-individual variation in cancer incidence that increasingly appears to be a feature of many such disorders suggests that there is little value in recommending specific restrictions on low dose exposure.

(466) The true extent of the problem of high dose radiotherapy to cancer patients having familial cancer disorders is far more difficult to judge since it depends critically upon the estimate of enhanced cancer risk (see Section 7.1.2). The data reviewed in this report are insufficient to allow this estimate to be given with real confidence—that there is a potential problem should not, however, be doubted.

(467) Until such time that the therapy related cancer issue is more clearly defined the Commission considers that it is appropriate to suggest only that clinicians faced with the prospect of treating radiotherapeutically patients having familial cancer disorders seek to balance the clinical benefits to the patient against a possible 10-fold or more increased chance of a second cancer arising in irradiated normal tissue. In the context of this problem a number of issues may be identified. First, the minimisation of dose to normal tissues in 'genetic' patients is judged to be more important than in patients with sporadic (non-genetic) neoplasms. Second, where possible, consideration might be given to alternative cancer treatments but bearing in mind that in many instances there will also be enhanced risk following genotoxic chemotherapeutic agents. Third, although not formally established, there is some reason to believe that children and young people having genetic disorders of cancer predisposition would be at particular risk.

(468) In general a family, or indeed an individual, history of cancer should alert radiotherapists to the possibility of a genetic component to the disease. In some instances diagnostic tests for certain of these disorders will, in conjunction with a medical genetics input, be appropriate (see Section 7.5.3).

7.5.3. *Genetic testing for cancer susceptibility in the context of radiological protection*

(469) The Commission is aware of the fundamental and technical problems that currently limit the screening of unrelated individuals for cancer-predisposing disorders (Section 7.4). Irrespective of the gains in knowledge that will follow, the Commission believes that there are critically important ethical, social, and economic considerations that need to be discussed and resolved prior to the employment of such genetic tests in almost all contexts—the possible implications for radiological protection practice is one small part of a much greater debate.

(470) Factors impinging on the use of genetic testing include: the implications of a positive test extend not only to the tested individual but also to a proportion of his/her family; the desire of the individual to have knowledge of the probability of future disease (including the familial element) cannot be assumed particularly if no remedial clinical intervention is available; a positive test has potentially far-reaching economic consequences in respect of life insurance, borrowing capabilities, and employment prospects—hence, the confidentiality of test results is of critical importance. These and other aspects of the problem are being debated widely (Eeles *et al.*, 1996; Masood, 1996; Reilly *et al.*, 1997) and will not be considered further here.

(471) Genetic testing for cancer predisposition and other disorders has or will become a matter for legislation in all developed countries. As a consequence, the Commission does not believe that it is appropriate at this stage to make specific recommendations on the employment of genetic testing in the context of radiological protection. Some general observations on the potential applications may, nevertheless, be useful even at this early point.

(472) The data available suggest to the Commission that the most obvious question posed by cancer-susceptibility to radiological protection arises when high dose radiotherapy is indicated. Since the genetic elevation of risk of a second cancer may be

substantial there is clear potential benefit to the patient in unambiguous genetic diagnosis in order for clinicians to balance the risk of a second cancer against the likely therapeutic gain. As genetic tests become more predictive, automated, and ethically resolved, the Commission does not find it difficult to envisaged that, in a small proportion of cancer cases, they might play an important future role in the formulation of the most appropriate therapeutic strategies.

(473) Even in the most favourable circumstances it is, however, far more difficult for the Commission to project the use of genetic testing in cases of potential low-dose exposure in either medical diagnostic or occupational settings. It is anticipated that, in the main, such testing would be performed on individuals without cancer with a view to judging the potential for risk and whether avoidance of exposure was appropriate. In many countries the projected number of genetic tests of this type and hence the costs would be very great indeed yet a current judgement would be that potential benefit would accrue to around only 1% or less of those tested. It would also appear that the elevated low-dose risk for which avoidance might be sought is small. Thus, the potential benefits of testing are judged by the Commission to be very small when weighed against the great costs involved. On this basis there seems little reason to believe that genetic testing has a significant role in the context of occupational exposure to radiation. In some future circumstances it might be appropriate to consider genetic testing in selected patients prior to certain medical diagnostic exposures where relatively high doses are involved but a widespread application does not appear likely.

7.5.4. *Concluding remarks*

(474) The principal conclusion by the Commission is that, on current knowledge, the presence of familial cancer disorders does not impose unacceptable distortions in the distribution of radiation cancer risk in typical human populations. For individuals with familial cancer disorders, radiation cancer risks relative to baseline are judged by the Commission to be small at low doses and insufficient to form the basis of special precautions. It seems likely however that risks to those with familial cancer disorders will become important at the high doses received during radiotherapy. At present, and even perhaps in future, genetic testing for familial cancer disorders does not appear to have a major role in radiological protection practice—the principal exception may be selected cancer patients for whom radiotherapy is indicated.

(475) It is stressed again that the data currently available for the development of these views are limited and the judgements given should be regarded as being preliminary. Many of the uncertainties attaching to these judgements have been considered by the Task Group and Commission together with possible research strategies that may be used to improve knowledge. Of particular importance is the need for knowledge on the identity, prevalence, and impact of weakly expressing mutations which do not manifest as familial cancer.

(476) Given these uncertainties the Commission will maintain close surveillance on the rapidly developing fields of cancer genetics and genetic epidemiology and, when appropriate, will expand and/or revise the interim views expressed in this report.

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