THE GENETIC BASIS FOR DIFFERENCES IN INDIVIDUAL SENSITIVITY TO IONISING RADIATION

Thesis submitted for the degree of Doctor of Medicine at the University of Leicester

by

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CONTENTS

LIST OF ABBREVIATIONS

AIDS	Acquired immune deficiency syndrome
ANOVA	One analysis of variance
APC	Adenomatous polyposis coli
A-T	Ataxia-telangiectasia
BRCA	Breast cancer susceptibility gene
BS	Bloom's syndrome
CIN	Cervical intraepithelial neoplasia
CO_2	Carbon dioxide
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
dsb	Double strand break
EBV	Epstein-Barr virus
FA	Fanconi's anaemia
FAP	Familial adenomatous polyposis
FC	Failed culture
FRR	Familial relative risk
Gy	Gray
HBV	Hepatitis B virus
HHV 8	Herpesvirus 8
HIV	Human immunodeficiency virus
HNPCC	Hereditary non-polyposis colon cancer
HPV	Human papillomavirus
HTLV 1	Human T lymphotrophic virus type 1
IU	International units
KCl	Potassium chloride
LMI	Low mitotic index
MEN	Multiple endocrine neoplasia
MI	Mitotic index
MN	Micronuclei
NF	Neurofibromatosis

PHA	Phytohaemagglutinin
PCR	Polymerase chain reaction
PTT	Protein truncation test
RB	Retinoblastoma
rpm	Revolutions per minute
ssb	Single strand break
SD	Standard deviation
SSD	Skin source distance
UV	Ultra-violet
WT	Wilm's tumour
XP	Xeroderma pigmentosum

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DECLARATION

No portion of the work referred in this thesis has been submitted in support of an application for another degree or qualification of this or any other University or Institute of learning.

STATEMENT

I declare that this thesis is a result of conjoint work and that I performed a substantial part of this original research. I located, obtained consent and collected blood samples from the following groups of patients; normal donors, breast, cervical, colorectal, lung carcinoma patients. The blood samples from the benign disease group, paediatric/adolsecent groups and *BRCA* screening subjects were collected at different hospitals by Clinicians or phlebotomists.

Cell culture and slide preparation were performed both by the Scientific Officer, Mrs Warren and myself. The cell culture and slide preparation of the paediatric/adolescent controls and malignancy patients as well as a group of normal donors were performed by the Scientific Officer whilst I concentrated on the other groups.

I was scorer 1, refered to on page 54 and scored the following groups; normal donors, benign disease group, breast, cervical, colorectal, lung and those subjects undergoing screening for mutant *BRCA1/2* genes. The Scientific Officer was scorer 2 and scored groups of normal donors, paediatric/adolescent controls and malignancy patients. Clinical details of the patients was collected and recorded by myself. I was responsible for constructing the database of results and clinical data. I performed the statistical analyses after obtaining advice from Dr S. Roberts.

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Preface

Karin Baria MBChB, graduated from the University of Leicester Medical School in 1988 and has pursued a career in Radiotherapy and Oncology. She is a Fellow of the Royal College of Radiologists.

1. INTRODUCTION

Cancer is a major cause of morbidity and mortality world-wide. A large part of many national health care budgets are spent in treating patients with cancer and in the screening of pre-malignant disease. Reducing the mortality rates is a significant challenge both to medical research and medicine and, with respect to this aim, the ability to identify those with an elevated predisposition to malignancy is of interest. Valuable resources could be targeted to those individuals with an increased risk for developing malignancy, e.g., through targeted screening programmes. This should enable the earlier detection of disease with the corresponding prompt treatment to be translated into improved overall survival rates.

1.1 AETIOLOGY OF CANCER

The common factors in malignancy are the uncontrolled proliferation of cells and the infiltration of normal tissues. Several steps are needed to turn a normal cell into a malignant cell. Most involve mutational change. Cancer is a genetic disease at the level of the somatic cell and cancer may also be a genetic disease at the level of the germline. Mutations accumulate throughout life, and the accumulation of a certain amount of damage coupled with environmental and inherited factors may lead to the formation of a malignancy. Cancer risk depends on environmental exposure and also on genetic make-up. In a minority of the population the inherited factor (germline mutation) has a strong effect and is responsible for about 5-10% of tumours (Doll and Peto 1981; Doll 1996). Although environmental factors are responsible for the vast majority of cancers, environmental and genetic factors may interact. For example, the role of polymorphic alleles can increase an individual's susceptibility to an external carcinogen (UV light, cigarette smoking) and may make a significant contribution to the burden of cancer (Doll 1996). Molecular studies have shown that the uncontrolled growth of neoplastic cells is due to the failure of genes to regulate cell division. The genes involved in this process are proto-oncogenes and tumour suppressor genes (see Section 1.1.2.1).

1.1.1 Environmental aetiology of neoplasia

As environmental factors are implicated in the aetiology of the majority of cancers, it has been suggested that many of the common cancers in the Western world are potentially avoidable (Doll and Peto 1981). Evidence linking the most important environmental exposures to cancer risk will be reviewed briefly.

1.1.1.1 Tobacco

Tobacco use is the environmental exposure most widely known to be linked with an increased risk of cancer. It is associated with the development of lung, head and neck, oesophageal, pancreatic, bladder, and cervical carcinomas (Hecht *et al.,* 1994; Levi 1999). It is estimated that up to 35% of human cancers in the West may be due to the effects of smoking (Doll and Peto 1981) and that consumption of tobacco represents the single most important preventable cause of death in all European countries (Franceschi and Naett 1995). Passive smoking is also a cause of lung cancer (Levi 1999; see Section 1.2.4). In addition the effects of smoking are influenced by an interaction with other exposures, e.g., asbestos in lung cancer (Berry *et al.,* 1985). Finally, the chewing of tobacco quid and betal leaves is common in South Asia and is associated with the development of tumours of the oral cavity (World Health Organisation 1997b).

1.1.1.2 Alcohol and diet

Alcohol is associated with the development of tumours of the upper respiratory and digestive tracts, it also interacts with tobacco in the aetiology of these tumours (Levi 1999). Alcohol also increases the risk of breast cancer in women possibly by interfering with the metabolism of oestrogens (Longnecker et al., 1988; Levi 1999). The importance of diet is illustrated by observations that large differences exist in dietary fat intake between countries and these correlate with the incidence of cancer of the breast, colon, prostate, endometrium and pancreas (Willett 1989). In colon cancer there is evidence that dietary fat intake is associated with an increased risk (Willet *et al.,* 1990), and an intake of dietary fibre is protective (Burkitt 1971; Trock *et al.,*1990; Howe *et al.,* 1992) for the disease.

A high consumption of fruit and green and yellow vegetables is associated with a low incidence of several epithelial cancers (Levi 1999). The incidence of the following cancers is increased in those with a low intake: oral cavity, stomach, pancreas, colon, rectum, lung, larynx, breast, cervix, ovary and bladder (Doll 1996; Hunter and Willet

1996). Products of cooking may lead to the formation of carcinogens, e.g. nitrosamines and polycyclic aromatic hydrocarbons, that are associated with an increased risk of gastric cancer (Doll and Peto 1981). Aflatoxin, a carcinogenic metabolite of the fungus *Aspergillus flavus*, a contaminant of foodstuffs, is associated with an increased risk of hepatocellular carcinoma in South East Asia (Doll and Peto 1981).

1.1.1.3 Radiation

It is estimated that ionising radiation causes about 4% of cancers. The greatest contribution of radiation is natural; cosmic rays, radon gas, external radiation from the radionuclides in rocks and soil and internal radiation from naturally radioactive traces of elements in food (Doll and Peto 1981). Another source of radiation is that associated with medical use. This accounts for 14% of the total exposure (Doll and Peto 1981). There is a large body of data on radiation-induced cancer derived from epidemiological studies in irradiated human populations. Long term follow-up of survivors of the atomic bombings of Hiroshima and Nagasaki provided direct evidence that irradiation leads to carcinogenesis (Land and McGregor 1979).

Non-ionising radiation is also important, e.g., UV light. The UVB component of sunlight is responsible for the vast majority of squamous cell carcinomas, basal cell carcinomas and malignant melanomas of the skin. Squamous cell carcinoma is the most closely related to cumulative exposure, while malignant melanoma appears to be related to the incidence of intermittent exposure to sunlight and sunburn (Doll and Peto 1981). Some of the most dramatic examples of skin cancers induced by exposure to sunlight include xeroderma pigmentosum, Cockayne syndrome, basal cell neavus syndrome and dysplastic neavus syndrome. These genetic disorders show increased responses to sunlight often culminating in malignancy (Sanford *et al.*, 1987; Satoh *et al.*, 1993; de Boer *et al.*, 1999).

1.1.1.4 Occupational exposures

Occupational hazards have caused many cases of cancer in the past. Now that the association between exposure to the carcinogen and causation are linked the hazards are stringently controlled. Table 1.3 lists examples of occupational exposures that influence cancer risk.

Table 1.1 Occupational exposures that influence cancer risk (adapted from Doll and Peto 1981).

Occupation	Agent	Tumour site
Dye workers	aromatic amines	bladder
Rubber workers	aromatic amines	bladder
Asbestos mining workers	asbestos	mesothelium, lung
Copper, cobalt smelters	arsenic	skin, lung
Cadmium workers	cadmium	prostate
Chromium workers	chromium	lung
Nickel refiners	nickel	nasal sinuses, lung
Uranium miners	ionising radiation	lung
Coal gas workers, asphalters.	polycyclic hydrocarcbons	skin, lung
PVC manufacturers	vinyl chloride	angiosarcoma of liver
Hard wood furniture manufacturers	unknown	nasal sinuses
Leather workers	unknown	nasal sinuses
Farmers, seamen	UV light	skin
Glue, varnish workers	benzene	marrow
Radiographers, radiologists.	ionising radiation	bone marrow, all sites

1.1.1.5 Viruses

Certain viruses play a role in the causation of malignancies. Links have been established on the basis of epidemiological, clinical and molecular biology studies. An essential step in malignant transformation of normal cells by most tumour viruses is the integration of viral DNA into the host genome, which leads to activation or disruption of cellular genes.

Hepatitis B Virus (HBV)

Compelling evidence exists for a role of hepatitis B virus in the pathogenesis of hepatocellular carcinoma. Hepatitis B virus is a member of the herpesadenovirus family and is highly endemic in many parts of the world, e.g., South East Asia, subSaharan Africa, (Arthur *et al.,* 1984). The majority of individuals infected with the virus suffer an acute transient illness or an asymptomatic infection that leaves them immune. About 10% of infected individuals develop chronic hepatitis which may progress to cirrhosis and hepatocellular carcinoma (Benchimol and Minden 1998). Chronic HBV carriers show a 100-fold higher risk of hepatocellular carcinoma than unaffected individuals (Beral *et al.,* 1990). The precise mechanisim through which HBV acts is not well understood. It is thought that HBV predisposes to cancer indirectly. The virus-infected hepatocytes are destroyed and this stimulates liver regeneration. Integration of viral DNA into regenerating cells can promote genomic instability, increasing the chances of a HBV-infected cell accumulating a series of genetic changes necessary for malignant transformation (CRC Factsheet 25.4, 1996).

Epstein- Barr Virus (EBV)

Epstein-Barr virus is a herpesvirus widespread throughout all human populations. The vast majority of infected subjects remain asymptomatic (Weinreb *et al.,* 1996). EBV infection is associated with lymphoproliferative disorders of B cell origin, infectious mononucleosis, Burkitt's lymphoma and lymphoma of the immunocompromised host (Grufferman *et al.,* 1985; Liebowitz 1994). There is also a strong association between EBV infection and undifferentiated nasopharyngeal carcinoma (Liebowitz 1994) and Hodgkin's disease (Weinreb *et al.,* 1996).

The virus infects B lymphocytes which become immortalised. EBV encodes around 100 genes, of which about 10 are expressed in immortalised B lymphocytes (Sugden 1989). Four genes are thought to be involved in cellular tranformation: EBNA 1 (Epstein-Barr virus nuclear antigen 1), EBNA 2, LMP 1 (latent membrane protein 1) and LMP2. These genes are involved in DNA replication of viral plasmids in EBV infected cells and protein transcription.

Epidemiological evidence for a causal relationship between Epstein-Barr virus and Burkitt's lymphoma was first noted by de-The *et al.* (1978). Burkitt's lymphoma is

the commonest childhood malignancy in subSaharan Africa. The disease is characterised by chromosomal translocations that result in juxtaposition of immunoglobulin (Ig) genes and c-myc. A chromosomal translocation between chromosomes 8 and 14 is present in 80% of cases involving the Ig heavy chain locus on chromosome 14 and c-myc on chromosome 8. The translocations are thought to result in deregulation of c-myc expression (Benchimol and Minden 1998).

EBV is implicated also in the pathogenesis of nasopharyngeal carcinoma (Pallensen *et al.,* 1991) though its role is poorly understood. The incidence of the disease is strongly influenced by racial, genetic and environmental factors and is highest in Southern China (Liebowitz 1994). Four EBV proteins have been detected in nasopharyngeal cells: the nuclear antigen EBNA 1, LMP 1, LMP2A and LMP2B. LMP 1 exerts growth stimulatory effects *in-vitro* and may exert similar effects in the nasopharyngeal epithelium (Liebowitz 1994).

A link has also been noted between EBV and the development of Hodgkin's disease (Kvale *et al.,* 1979; Weinreb *et al.,* 1996). At least 35% of cases carry the EBV genome within each malignant cell (Pallensen *et al.,* 1991). The association of EBV with Hodgkin's disease varies from country to country. Weinreb *et al.* (1996) reported that 100% of Kenyan children with Hodgkin's disease were EBV positive, whilst 51% of children from the United States of America and the United Kingdom showed evidence of EBV in their malignant cells.

Human papillomavirus (HPV)

Papillomaviruses are a family of closely related agents which infect epithelial cells either of the skin or the mucosal surfaces (Reid *et al.,* 1987). The virus matches its own life cycle to that of the epithelial cells and replicates to produce new virus particles as the cells become squamous and reach the surface of the skin or mucosa. The virus is associated with the formation of benign and malignant neoplasms (Lorincz *et al.,* 1992; Munoz and Bosch 1992). HPV types 6, 10 and 11 which infect the genital tract are associated with benign genital warts and low grades of cervical intraepithelial neoplasia (CIN) that often regress spontaneously (Lorincz *et al.,*1992; Munoz and Bosch 1992), HPV types 16, 18, 31 and 33 are associated with higher grades of CIN and invasive carcinoma (Koutsky *et al.,* 1992; Munoz and Bosch 1992).

Epidemiological evidence exists linking the age of first sexual intercourse, number of sexual partners, and numbers of sexual partners of the husband with subsequent cervical cancer risk (Rotkin 1967; Buckley *et al.*, 1981; Brinton *et al* 1987; Brinton *et al.*, 1989). In benign warts the HPV genome is maintained as an episome (nonintegrated, circular form). In malignant cells HPV DNA is randomly integrated into chromosomes resulting in deletions or disruption of the viral genome. The integrated viral DNA retains the capacity to express early genes E6 and E7, and if these become switched on permanently they produce viral proteins, which drive cell growth. Secondary genetic changes are required to produce an oncogenic process as HPV alone is not carcinogenic (Lorincz *et al.*, 1992).

Human T-cell leukaemia virus (HTLV1)

Human T-cell leukaemia virus 1 was the first human retrovirus to be discovered. It is endemic in certain geographic areas: South West Japan (Blattner *et al.*, 1986), the Carribean, parts of Africa and South America (Benchimol and Minden 1998). Transmission of the virus may occur through breast feeding, sexual intercourse and blood transfusion and is a cause of T cell leukaemia (Blattner *et al.*, 1986).

Human immunodeficiency virus (HIV)

HIV is recognised as the causative agent of acquired immune deficiency syndrome (AIDS). The main consequence of infection is depletion of the target cell population leading to immunosuppression and opportunistic infections (Benchimol and Minden 1998). HIV also predisposes to neoplastic conditions, such as Kaposi's sarcoma (Beral *et al.*, 1990), non-Hodgkin's lymphoma, intraepithelial cervical neoplasia and anal tumours (Schultz *et al.*, 1996). Herpesvirus 8 (HHV-8) is present in over 90% of Kaposi's sarcoma tissues obtained from patients with AIDS indicating that HHV-8 may play a role in the development of this disease (Chang *et al.*, 1994).

1.1.1.6 Hormones

Hormones play a major role in the aetiology of several human cancers. The impact of hormones in the causation of some malignancies is discussed below.

Breast carcinoma

Available evidence regarding the hormonal aetiology of breast cancer is most consistent with the hypothesis that oestrogen is the main stimulant for breast cell proliferation (Henderson *et al.,* 1988). The most consistently documented, hormonally related risk factors for breast cancer are early age at menarche, late age at menopause and late age at first pregnancy (Henderson 1993; Lipworth 1995). Substantial literature exists for a relationship between oral contraceptive use and the risk of breast cancer. Oral contraceptives do not confer protection against breast cancer as they do against ovarian cancer (Henderson *et al.,* 1993). The Collaborative Group on Hormonal Factors in Breast Cancer (1996) found a slight increase in the relative risk of having breast cancer diagnosed whilst currently using, or within 10 years of using oral contraceptives. There was no evidence of an increase in risk of breast cancer, 10 years or more after cessation.

Gynaecological malignancies

Established hormonal risk factors for endometrial cancer include unopposed oestrogen replacement (Mack *et al.,* 1976; Henderson *et al.,* 1993), obesity which is linked to circulating levels of oestrogens (Zumoff 1982), sequential oral contraceptives (Henderson *et al.,* 1993) and a late menopause (Henderson *et al.,* 1988). A late menopause (increased number of ovulations) is an established hormonal risk factor for ovarian carcinoma, whilst oral contraceptives, which suppress ovulation exert a protective effect (Henderson *et al.,* 1993). For vaginal adenocarcinoma Herbst *et al,* (1979) described an association with *in utero* diethylstilboetrol (DES) exposure. It was concluded that oestrogens might induce anomalous development *in utero,* which could have neoplastic consequences in the postpubertal period. These neoplasms developed within the ages 15-29 years, the relevant exposure occurred during the first trimester of pregnancy.

1.1.2 GENETIC PREDISPOSITION TO CANCER

The inherited contribution to cancer incidence and development will be considered. Cancers arise via a monoclonal multi-step process, involving multiple genetic alterations (Fearon and Vogelstein 1990). At least three major classes of genes are involved. First, tumour suppressor genes, whose products appear to negatively regulate growth, promote

apoptosis and maintain *in vivo* homeostatic growth and differentiation potential. Second, oncogenes whose protein products act to stimulate cell growth and survival. Third DNA repair genes, defects in which lead to increased genomic instability (ICRP 1999). Both high and low penetrance genes are believed to be involved in cancer predisposition. Penetrance may be defined as the likelihood that a disease will occur as a result of the presence of a predisposing gene.

1.1.2.1 Inherited cancer syndromes

These syndromes account for a small proportion (1-2%) of cancer incidence (Ponder 1990). They comprise malignancies where a genetic effect is clearly apparent. Examples include familial adenomatous polyposis (Kinzler *et al.,* 1991), familial retinoblastoma (Friend *et al.,* 1986), multiple endocrine neoplasia, types I and II and neurofibromatosis types I (Wallace *et al.,* 1990) and II (Rouleau *et al.,* 1993). The pattern of inheritance is consistent with the effect of single highly penetrant autosomal dominant genes. For inherited cancer syndromes predisposition to cancer is confined to certain tissues. For example, in MEN II, three tissues are involved in tumour formation; the 'C' cells of the thyroid, the adrenal medulla and the parathyroid. The characteristic tumour in familial adenomatous polyposis of the colon (FAP) is colorectal carcinoma, some patients also develop duodenal carcinomas (Ponder 1990). Most inherited cancer syndromes can be distinguished from sporadic tumours by their association with specific phenotypes that are caused by the predisposing gene, e.g., multiple colonic polyps in FAP or areas of C-cell hyperplasia in MEN II.

Tumour suppressor genes

The paradigm is retinoblastoma (Knudson 1971). Knudson's hypothesis states that both copies of a critical gene must be lost from a cell for a tumour to develop. In familial cases, the first *RB* mutation occurs in the germline and is present throughout every cell in the body. A further loss (somatic mutation) is needed within a cell of the target tissue for a tumour to develop. As both alleles at the *RB* locus must be inactivated for the tumour to develop, the *RB* mutation is regarded as recessive at the cellular level. The requirement for loss of activity of both alleles of the *RB* gene implies that the normal activity of the gene is to suppress tumorigenesis. The gene is called a tumour-suppressor gene, although

the mutation is recessive at the cellular level it has a dominant pattern of inheritance (Weinberg 1991; Knudson 1993; Cowell 1994). Further examples of tumour suppressor genes are given in Table 1.2.

Table 1.2. Human tumour suppressor genes involved malignancy.

Gene	Inherited syndromes	Tumours	References
RB1	Familial retinoblastoma	Retinoblastoma, osteosarcoma, breast, lung, bladder	Weinberg 1995
TP53	Li-Fraumeni syndrome	High proportion of tumours	Greenblatt 1994, Ko & Prives 1996
WT1	Wilm's tumour	Nephroblastoma	Hastie 1994
NF1	Neurofibromatosis 1	Astrocytoma, colon carcinoma	Viskochil 1993
NF2	Neurofibromatosis 2	Schwannoma	Rouleau 1993, Trofatter 1993
MTS1	Familial melanoma	Several	Sherr & Roberts 1995.
VHL	Von Hippel Lindau	Renal cell carcinoma	Duan *et al.*,1995, Kibel *et al.*,1995
BRCA1	Familial breast and ovarian carcinoma		Miki *et al.*,1994 Stratton & Wooster 1996
BRCA2	Familial breast cancer		Wooster *et al.*, 1995, Stratton & Wooster 1996
DPC4	?	Pancreatic	Hahn *et al.*,1996

High penetrance cancer predisposing genes are those that when inherited lead to a high chance of developing cancer with increasing age. Examples of tumour suppressor genes with high penetrance are *BRCA1, BRCA2, TP53,* adenomatous polyposis coli (APC). The *BRCA1* and *BRCA2* genes will be discussed in detail as a group of subjects with the mutant gene have formed a part of this study. About 5-10% of breast and ovarian cancers occur as a result of highly penetrant germline mutations in cancer predisposition genes (Easton and Peto 1990). Women carrying mutations in the breast cancer susceptibility genes (*BRCA1, BRCA2*) tend to develop breast cancer at an earlier age, have a higher rate of bilateral breast cancer and an increased risk of developing other cancers, for instance ovarian carcinoma (Stratton and Wooster 1996). The *BRCA1* gene has been mapped to chromosome 17q21, cloned (Hall *et al.,* 1990; Miki *et al.,* 1994) and is estimated to confer a breast cancer risk of 54% by 60 years of age and an ovarian cancer risk of 30% by 60 years. The penetrance of the *BRCA1* gene was estimated to be 59% by 50 years of age and 83% by 70 years (Easton *et al.,* 1995). Ford *et al,* (1998) have estimated that the *BRCA1* mutations are responsible for about 50% of families with a clear dominant predisposition to breast cancer and over 80% of families segregating both breast and ovarian cancer. Approximately 32% of the remaining high-risk breast cancer families are due to a second predisposition gene *BRCA2* (Ford *et al.,* 1998). The *BRCA2* gene on chromosome 13q12-13 has been cloned (Wooster *et al.,* 1994; Wooster *et al.,* 1995). *BRCA1* and *BRCA2* make an approximately equal contribution to early onset breast cancer. However, with increasing age the proportion of carriers with *BRCA2* mutations increases and the proportion of carriers with *BRCA1* mutations decreases (Rahman and Stratton 1998).

A greater proportion of male breast cancer is thought to result from genetic susceptibility than female breast cancer and *BRCA2* mutations are involved (Stratton and Wooster 1996; Ford *et al.,*1998). Family based studies have estimated the risk of developing ovarian cancer to be 27% by 70 years in *BRCA2* patients, which is lower than the risks conveyed by *BRCA1* (Ford *et al.,* 1998).

Increased risks of colorectal cancer and prostate cancer have been reported in *BRCA1* mutation carriers (Ford *et al.,* 1994), however, as the cases involved are few the risk estimates remain imprecise. Mutations of *BRCA2* also confer an increased risk of prostate, pancreatic cancer and ocular melanoma (Rahman and Stratton 1998).

The biological and biochemical activities of the *BRCA1* and *BRCA2* genes are not completely understood. *BRCA1* and *BRCA2* are tumour suppressor genes (Stratton and Wooster 1996). This is shown as tumours arising in *BRCA1* and *BRCA2*-linked families show loss of heterozygosity at the relevant loci (Stratton and Wooster 1996; Gonzalez *et al.,* 1999). The products of both genes are large nuclear proteins, with a cell-cycle regulated expression pattern. Both gene products co-localise in multiple tissues during proliferation and differentiation (Zhang *et al.,* 1998). *BRCA1* and *BRCA2* are thought to function in a similar pathway and both are associated with human RAD51 (hRAD51), a homolog of the *E.coli* RecA gene (Scully 1997a). *BRCA2* binds to RAD51, which may have an important role in recombination and repair of double-stranded DNA breaks (Zhang *et al.,* 1998; Rahman and Stratton 1998). It is thought that *BRCA1* interacts indirectly with RAD51 and it is possible that involvement of both proteins in pathways implicated in DNA repair contributes to their role in cancer susceptibility (Scully *et al.,* 1997*a).* *BRCA1* is hyperphosphorylated and undergoes altered subnuclear localisation with hRAD51 in response to DNA-damaging agents (Scully *et al.,* 1997b). *BRCA1* and 2 are proposed to be involved in the response to DNA damage at different levels including cell cycle checkpoints, activation, induction of apoptosis and DNA repair (Zhang *et al.,* 1998; Kote-Jarai and Eeles 1999). Recent studies (Abbott *et al.,* 1999) have demonstrated that human cancer cells containing mutated *BRCA1* are hypersensitive to ionising radiation. *BRCA1* also functions in transcription-coupled DNA repair (TCR). TCR ability correlates with radioresistance, as cells show both increased TCR and radioresistance, whereas without *BRCA1* cells show decreased TCR and radiosensitivity.

Oncogenes

Oncogenes are mutated forms of proto-oncogenes. Proto-oncogenes encode growth factors, growth factor receptors and proteins and possess kinase activity. Intracellular proto-oncogene products are involved in regulating DNA replication and control of gene transcription. Proto-oncogenes may be activated by mutation, amplification or rearrangements that can occur during chromosome translocation (Minden and Pawson 1992). The cells' proliferative signals are switched "on" by the action of oncogenes (Vile *et al.,* 1994). Examples of some oncogenes are given in Table 1 3. Germline mutations of

a proto-oncogene have been reported in the *RET* gene, which predispose to multiple endocrine neoplasia, namely MEN2A and MEN2B (ICRP 1999).

Table 1.3 Proto-oncogenes mutated in human cancer (adapted from Vile *et al.*, 1994).

Gene	Function	Aberration	Tumour
erb-B1	1	amplification	breast, ovary, bladder
erb-B2	1	amplification	breast
PDGF	1	amplification	many tumours
hst	1	amplification	stomach, glioblastoma
abl	2	translocation	chronic granulocytic leukaemia
H-ras	2	point mutations	colon, lung, pancreas
K-ras	2	point mutations	acute myeloid and lymphoblastic leukaemia
N-ras	2	point mutations	thyroid, genitourinary tract malgnancies
c-myc	3	translocation	Burkitt's lymphoma
c-myc	3	amplification	lung, breast, cervix
L-myc	3	amplification	lung
N-myc	3	amplification	neuroblastoma

1. Genes coding for growth factors or their receptors.
2. Genes coding for G-proteins (cytoplasmic relays in stimulatory signalling pathways).
3. Genes coding for transcription factors that activate growth-promoting genes.

DNA processing defects

A number of genetic disorders have been identified which predispose the affected individual to an increased risk of cancer, these conditions exhibit spontaneous chromosomal instability and have been termed cancer-prone syndromes. The syndromes are separate clinical entities with different clinical characteristics and underlying defects, however, they have an autosomal recessive mode of inheritance, e.g., Ataxia-telangiectasia (A-T), Bloom's syndrome (BS) and Fanconi's anaemia (FA).

These disorders have a defect in the processing of DNA. Defective genes may include those whose products normally control replicative DNA synthesis, DNA repair synthesis, recombination, and transcription (Taylor *et al.*, 1994). The cancer-prone

syndromes are used as models for research into cancer predisposition and its link with mutagen sensitivity. The occurrence of spontaneous chromosome instability and cancer predisposition, indicate that genetic instability may be a mechanism for increasing oncogenesis. Chromosomal instability may be used as a marker of cancer proneness. Hsu (1983) stated that "genetic instability can be a hidden trait, expressed only under a particular circumstance or circumstances, such as exposure to a special mutagen".

The cancer-prone conditions show elevated levels of chromosome breakage when exposed to mutagens, e.g. x-rays. It is thought that the increased chromatid damage results from deficient DNA repair during the G_2 phase of the cell cycle (Parshad 1983; Gantt *et al.,* 1986; 1989). Further cancer-prone conditions have been identified which exhibit G_2 chromosomal radiosensitivity, (see Table 1.4, Section 1.4.2).

Ataxia-telangiectasia (A-T)

A good example of a DNA processing defect is A-T. A-T is a syndrome involving a progressive cerebellar ataxia, oculocutaneous telangiectasia, immune deficiency, cancer susceptibility, premature ageing, cellular radiosensitivity, defects in DNA repair and chromosomal breakage (Gatti *et al.,* 1991). A-T is an autosomal recessive condition, with a birth frequency of about 1 in 300 000 (Taylor *et al.,* 1994). The heterozygote frequency is estimated to be 1 in 200 (Easton 1994).

A-T has been viewed as an explicit human model for studying inherited cancer susceptibility (Gatti *et al.,* 1991) as a high proportion of A-T patients develop malignancy. Hecht *et al.,* (1966) noted the development of lymphocytic leukaemia in patients with A-T, indeed the majority of neoplasms affecting A-T patients are non-Hodgkin's lymphomas (41%) and leukaemias (23%), the remaining neoplasms are solid malignancies, e.g., breast, stomach, ovary, bladder (Hecht and Hecht 1990).

When treated with conventional doses of radiotherapy these patients suffer severe normal tissue reactions often leading to death (Morgan *et al.,* 1968; Harnden and Taylor 1978). The clinically observed enhanced sensitivity of A-T patients to ionising radiation is also seen at the cellular level. Higurashi and Conen (1973) noted that leukocyte cultures from individuals with chromosomal breakage syndromes, including A-T sustained high amounts of chromosome damage when exposed to ionising radiation. Taylor *et al.,* (1975) observed that cultured skin fibroblasts from A-T patients were

intrinsically 2-3 times more sensitive to the lethal effects of ionising radiation than those from normal healthy individuals. .

A-T cells are particularly sensitive in the G_2 phase of the cell cycle. Taylor *et al,* (1982) noted that A-T lymphocyte and fibroblast cultures when irradiated during the G_2 phase of the cell cycle sustained more chromosomal damage than control cells. Following irradiation of lymphocytes with 1 Gy of X-rays in the G_2 phase of the cell cycle there was an almost 10 fold increase in chromatid breaks and gaps compared with normals and almost 20 times as much damage following 2 Gy. These findings are in agreement with earlier work by Higurashi and Conen (1973) and Natarajan and Meyers (1979). Subsequent experiments on the lethal effects of radiation on skin fibroblast cell lines from families with A-T confirmed the extreme radiosensitivity of A-T cells and showed that A-T heterozygotes were intermediate between A-T homozygotes and normal cells in their radiosensitivity (Nagasawa *et al.,* 1985).

From cell radiation survival curves it was evident that the D_0 (dose required to reduce the surviving fraction to 37% of a control value) for A-T homozygote cell lines was 0.4-0.45 Gy whereas those for the heterozygotic cells were 1.0-1.1 Gy as compared with the normal range of 1.2-1.4 Gy (Nagasawa *et al.,* 1985). The cell survival curves for the A-T homozygote lines revealed an absent or very small shoulder region compared with those of normal lines. The latter indicates a reduced ability to repair sub-lethal forms of radiation damage.

Heterozygous A-T gene carriers

The heterozygous carriers of the ATM mutation are a source of interest as two of the disease features are manifest to a limited extent; susceptibility to cancer and cellular hypersensitivity to ionising radiation. An elevated risk of developing cancer, e.g., breast carcinoma has been demonstrated repeatedly in A-T heterozygotes (Swift *et al.,* 1982; Swift *et al.,* 1987; Pippard *et al.,* 1988; Athma *et al.,* 1996; Inskip *et al.,* 1999). This area remains a source of controversy and former risk estimates have been challenged. Easton (1994) estimated that A-T heterozygotes would account for only 3.8% of breast cancer cases and that female A-T heterozygotes have a lifetime risk of developing breast cancer of 3.9 times the normal population. Easton (1994) suggested that the A-T gene was unlikely to make a significant contribution to familial breast cancer. The cloning and

sequencing of the *ATM* gene has enabled direct examination of the hypothesis that breast cancer risk is increased among *ATM* heterozygotes. Athma *et al* (1996) estimated that if the United States population frequency of *ATM* heterozygotes was 1.4%, 6.6% of all breast cancers may occur in women who are A-T heterozygotes, an estimate that was higher than that for *BRCA1* carriers. FitzGerald *et al,* (1997) undertook a germline mutational analysis in a group of women with early onset breast cancer, using a protein truncation assay (PTT) and found heterozygous *ATM* mutations in 0.5% of women with early onset breast cancer and in 1% of controls. It was concluded that heterozygous *ATM* mutations do not confer genetic predisposition to early onset breast cancer. The two approaches have yielded different results, 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).

A-T patients are susceptible to developing leukaemia and lymphoma. *ATM* mutations might occur in sporadic lymphoid tumours and recent clinical studies support this prediction. Vorechovsky *et al,* (1997) reported the occurrence of frequent *ATM* mutations in T-cell prolymphocytic leukaemias (T-PLL) and in non-Hodgkin's lymphomas, a finding that has since been confirmed by Stilgenbauer *et al.,* (1997).

For a clinician the identification of A-T heterozygotes is of interest for two reasons. First, radiation doses used in conventional radiation regimes are kept to a level whereby the late normal tissue reactions occur in less than 5% of the patient population. Higher radiation doses could be delivered in order to improve local control and overall survival rates, but unidentified heterozygotes with an increased radiosensitivity might be at increased risk. Identification of this high risk group and the subsequent reduction of therapeutic radiation dose would enable total doses to be administered to the vast majority of cancer patients, with a theoretical improvement in local control (Norman *et al.,* 1988; Hall *et al.,* 1998). Second,is a to the potential problem of increased cancer risk in A-T heterozygotes induced by mutagenic agents; for instance the risk of inducing breast cancer by radiation exposure of mammography in healthy A-T heterozygotes undergoing breast cancer screening (Swift *et al.,* 1987; Swift *et al.,* 1994).

Functions of the ATM gene

The ATM gene has been mapped to chromosome 11q22-23 (Gatti *et al.*, 1988; Wei *et al.*, 1990) and was identified by use of a positional cloning approach (Savistky *et al.*, 1995). Although cells from A-T homozygotes are exquisitely sensitive to the cytotoxic effects of ionising radiation, their ability to repair DNA damage appears to be largely intact (Rotman and Shiloh 1997). Biochemical studies failed to detect gross abnormalities in the kinetics of single and double strand repair in A-T cells (Taylor *et al.*, 1975; Taylor *et al.*, 1978). However, studies have demonstrated increases in the fraction of DNA breaks left unrepaired in irradiated A-T cells (Cornforth and Bedford 1985; Blocher *et al.*, 1991). This may indicate an inability to repair a small but essential fraction of DSBs (Taylor 1978:Cornforth and Bedford 1985; Pandita and Hittleman 1992).

In normal mammalian cells, cell cycle checkpoints are triggered following exposure to ionising radiation. These checkpoints may restrain the cell cycle temporarily in response to strand breaks, shortened telomeres and other DNA damage that occurs during the course of normal DNA metabolism (Hartwell and Weinert 1989; Hartwell 1992; Meyn 1999). The p53-mediated G_1/S damage sensitive checkpoint is absent in A-T cells (Kastan *et al.*, 1992). A-T cells express radioresistant DNA synthesis due to a lack of S-phase checkpoint function (Painter and Young 1980), and their G_2/M and mitotic spindle checkpoints are also defective (Beamish and Lavin 1994). Following exposure to ionising radiation, intracellular concentrations of p53, p21 and the GADD45 gene normally increase. A-T cells show reduced and delayed activation of TP53 after exposure to ionising radiation (Kastan *et al.*, 1992; Khanna and Lavin 1993). Among these defects are reduced or delayed induction of p21, GADD45 and MDM2 by radiation, in A-T cells grown in culture, suggesting that they are *ATM* dependent effects (Kastan *et al.*, 1992; Canman *et al.*, 1994; Meyn 1999).

The genomic instability and cancer proneness seen in A-T may be a result of defective cell cycle checkpoint control, since loss of TP53 dependent response is associated with genome instability and tumorigenesis (Hartwell 1992). The sensitivity of A-T cells to ionising radiation is also due to defective cell cycle response to DNA damage (Rotman and Shiloh 1997).

1.1.2.2 Familial clustering of cancer

This group comprises families that are at a low-medium increased risk of common cancers such as breast, ovary, endometrium, and colon (Easton and Peto 1990; Easton 1999) with incomplete penetrance and a weaker family history than with those described in Section 1.1.2.1. Features suggesting inherited predisposition include: two or more affected close relatives, early age of onset, cancers of specific types occurring together, e.g., breast and ovary, or multiple tumours in one individual (Ponder 1990). The strength of family clustering is expressed in terms of a relative risk for a malignancy in siblings of an affected individual, compared with the risk for the general population. The relative risk usually lies between 2-5 (Easton and Peto 1990). The familial relative risks (FRR) of cancer in relatives of cancer patients can be measured. High FRRs are suggestive of a genetic component and the pattern of FRR by age, type of disease and number affected can provide clues as to the possible genetic models (Easton 1999). A study by Hemminki *et al* (1999) provided a comprehensive analysis of familial risks of all the common cancer sites. The study confirmed that familial risks are mainly in the range 1.5-3 fold and that most common cancers show familial aggregation. Familial cancers resemble inherited cancer syndromes in the involvement of specific tissues, the age of onset of the malignancy and expression of the gene.

A substantial proportion of cancers may present without obvious familial clustering, and yet have an inherited component of risk. This inheritance may be due to more common, low-penetrance genes, for example, candidate genes such as those that encode enzymes with biochemical or physiological activities that have a role in the pathogenesis of cancer. Genetic polymorphisms which alter the susceptibility to carcinogens may be useful in detecting genotype-disease associations (Rebbeck 1999). Genes involved in carcinogen metabolism, such as the glutathione-S-transferases (GSTM1) (Helzlsouer *et al.,* 1998), members of the cytochrome P450 family (Ishibe *et al.,* 1998) and the N-acetyltransferases (Hunter *et al.,* 1997), may have a role in pathogenesis. The *ATM* gene may also confer a low risk to breast cancer development. Swift *et al.* (1987) reported a relatively high risk of breast cancer among A-T heterozygotes. More recent estimates of breast cancer risks in A-T heterozygotes, however, have been lower than formerly reported (Easton 1994; FitzGerald *et al.,* 1997).

Low penetrance genes are thought to be involved in the causation of some benign disorders such as diabetes mellitus, hypertension and ischaemic heart disease. Low penetrant cancer predisposing genes are thought to cause a higher proportion of common cancers, e.g., breast and colorectal carcinoma (Scott *et al.,* 1994a; Teare *et al.,* 1994; Ford *et al.,* 1998; Roberts *et al.,* 1999). As these genes are of low penetrance they rarely produce large numbers of cancers in a single family and methods other than linkage analysis are needed to detect them, e.g., phenotypic or molecular markers of susceptibility (Houlston and Peto 1996). A study by Skolnick *et al,* (1990) found evidence for a common breast cancer gene with low penetrance responsible for a considerable proportion of breast cancer.

1.2 AETIOLOGY OF MALIGNANCIES STUDIED IN THIS PROJECT

1.2.1 Breast carcinoma

A small proportion (5-10%) of breast cancer is due to the high penetrance genes *BRCA1* and *BRCA2,* and mutations of *TP53* in the Li-Fraumeni syndrome (Goldgar *et al.,* 1996). Most hereditary breast cancers can be explained by alterations in *BRCA1* or *BRCA2* (Rahman and Stratton 1998). Increased risk is also attributed to having a positive family history, in particular affected first degree relatives, second-degree relatives with breast cancer confer little or no increased risk (McPherson *et al.,* 1995; Goldgar *et al.,* 1996). Risk also depends on the age of the affected individual.

The aetiology of breast cancer is unknown in most patients, although various risk factors that provide clues to its genesis have been identified, e.g., family history of disease, age at menarche and age at menopause (McPherson *et al.,* 1995). Large variations exist in the incidence of breast cancer between countries (Harris *et al.,* 1992; Lipworth 1995). These observations suggest that environmental determinants of the disease are more important than genetic factors.

Age is a significant risk factor. The incidence of breast cancer increases with age, doubling about every 10 years until the menopause when the rate of increase slows. The majority of cases (80%) occur in post-menopausal women (CRC Factsheet 6.1 1996). Studies also suggest a strong link between the hormone oestrogen and development of breast cancer (Lipworth 1995, see Section 1.1.1.6).

Poor nutrition delays the onset of regular menstrual cycles and may reduce the incidence of breast cancer; this may partly explain international differences in breast cancer incidence. Delayed menopause increases the risk. Menopause at 55 years or older confers a 2 fold increased risk as compared with a natural menopause at 45 years (McPherson *et al.*, 1995). Increasing age (greater than 30 years) at first pregnancy and nulliparity affect the risk adversely. Breast feeding may have a protective effect. The risk of oral contraceptives is uncertain (Collaborative Group on Hormonal Factors in Breast Cancer 1996). The use of hormone replacement therapy is thought to have little effect on risk though the data are inconsistent (Henderson 1993).

Rates of breast cancer are 5-6 fold higher in the USA and in Europe compared to Africa and Asia. Studies of Japanese migrants to the USA have shown an increased risk in subsequent generations, suggesting that environmental factors operate (Henderson 1993; McPherson *et al.*, 1995). Weak associations exist between the development of breast cancer and both a high fat diet and high alcohol use (Schatzkin *et al.*, 1994; Hunter and Willett 1996; Enger *et al.*, 1999).

Patients with a history of benign breast disease, in particular atypical hyperplasia are also at increased risk of developing breast cancer (relative risk = 4.0, CRC Factsheet 6.1 1996). Finally, ionising radiation is implicated in the formation of breast cancer. Evidence for this includes data from survivors of the atomic bombs in Nagasaka and Hiroshima, and from patients who received radiation for post-partum mastitis (Hall 1988).

1.2.2 Cervical carcinoma

Epidemiological studies have identified a number of risk factors for the development of cervical carcinoma. Technological advances such as the polymerase chain reaction (PCR) for the detection of human papillomavirus (HPV) DNA have allowed analysis of the significance of previously described risk factors. Early epidemiological evidence suggested that cervical carcinoma behaves like a sexually transmitted disease (Brinton *et al.*, 1987). It is most frequent in women with multiple sexual partners, amongst women who began sexual intercourse at a young age and among women whose sexual partners are promiscuous (Rotkin 1967; Brinton *et al.*, 1989; Munoz and Bosch 1989).

The sexual behaviour of the male partner has a bearing on the development of cervical carcinioma. Case control studies of sexual behaviour of thousands of women with cervical carcinoma report that the husbands had more sexual partners, venereal disease and intercourse with prostitutes than the husbands of controls; and the partners of women with cervical cancer are more likely to be HPV infected (Buckley 1981; Brinton *et al.,* 1989; Schiffman 1993).

Experimental, clinical and epidemiological evidence is accumulating to show that HPV plays a central role in the aetiology of cervical carcinoma (Koutsky 1991; Munoz and Bosch 1992). Using PCR technology, more than 90% of preinvasive and invasive lesions contain HPV DNA (Cox *et al.,* 1992), 70% of invasive lesions are positive for HPV types 16 or 18. More than 70 phage types of HPV have been isolated and the anogenital HPVs have been divided into three risk groups (Reid *et al.,* 1987; Lorincz *et al.,* 1992). The low risk group includes HPVs 6, 11, 42, 43 and 44. The intermediate group includes HPVs 31, 33, 35, 52 and 58. The high-risk group includes HPVs 16, 18, 45, and 56. The low risk group is associated with low-grade cervical intraepithelial neoplasia (CIN), whereas the intermediate and high risk groups are associated with high grade CIN or invasive carcinoma. High risk HPVs are found in 90-95% of invasive cervical carcinomas (Riou *et al.,* 1990). HPV 16 is the most common type and is present in 50% of CIN and invasive carcinomas. It is the most common HPV type seen in women with normal cytology (Lorincz *et al.,* 1992). As HPV infection is present in up to 46% of cytologically negative women and 94% of cytologically abnormal patients, HPV infection may be necessary but not essential to cause cervical carcinoma. A minority of carcinomas is HPV negative and the disease may evolve through other pathways. Additional risk factors include low socio-economic status, smoking and immunosuppression (Brinton 1992). However, HPV infection is a strong confounding variable and influences the likelihood of exposure to a risk factor under study. For example, women who smoke tend to have more sexual partners and are more likely to be infected with HPV. Recent studies accounting for HPV infection have not provided evidence to support an independent effect of smoking (Schiffman et al 1993). Similarly the greater the number of sexual exposures, the greater the risk of HPV infection (Koutsky *et al.,* 1992; Schiffman *et al.,* 1993). There is no evidence of an inherited risk in cervical carcinoma.

1.2.3 Colorectal carcinoma

The development of colorectal tumours is likely to be a multistep process, involving genetic and environmental factors (CRC Factsheet 18.3 1993). The incidence of the disease varies across countries. This suggests that environmental factors are important. Diet is thought to be the most important environmental factor. Burkitt (1971) noted a relationship between low residue diets and the development of bowel cancer. He demonstrated that geographic differences in the incidence of bowel cancer related to differences in dietary fibre intake. Subsequent studies revealed a broad inverse relationship between fibre intake and rates of colorectal carcinoma (Trock *et al.,* 1990; Howe *et al.,* 1992). Dietary fat, in particular from animal sources, increases the risk of colon cancer. Evidence for this hypothesis is provided by a prospective study conducted by Willett *et al.,* (1990). Dietary composition affects the biochemical composition of faecal content thus altering the milieu for colonic mucosal cells and changing their proliferation rate and pattern (Burkitt 1971). The beneficial effects of dietary fibre (Trock *et al.,* 1990) may lie in reversing the effects of saturated fat and bile acids by reducing faecal bile acid levels (Burkitt 1971).

The majority of colorectal cancers are sporadic. However, for some individuals, predisposition to bowel cancer is an ill-defined increased risk of the disease, indicated by the history of having an affected relative (Slattery and Kerber 1994). One screening study showed that predisposition relates to the development of colorectal adenomas and that all cases of colorectal carcinoma occur on a background of genetic predisposition (Cannon-Albright *et al.,* 1988).

There are two major forms of inherited colorectal cancer predisposition. The first, familial adenomatous polyposis (FAP), accounts for 1% of colorectal carcinoma in the West (Bocker *et al.,* 1999). Familial adenomatous polyposis is an autosomal dominant disorder characterised by the development of hundreds to thousands of adenomatous polyps in the colon and rectum (Wallis *et al.,* 1999). Malignant change occurs within the polyps. The syndrome is associated with extracolonic features, e.g., epidermoid cysts, multiple craniofacial and long bone osteomata, gastroduodenal polyposis and desmoid tumours (Bishop and Hall 1994). Genetic linkage studies demonstrated that the gene responsible is localised to chromosome 5q21 (Kinzler *et al.,* 1991) and it has

subsequently been cloned. The gene, called adenomatous polyposis coli (APC), confers germline susceptibility to colorectal cancer.

A second form of colorectal cancer associated with genetic predisposition is hereditary non-polyposis colorectal cancer (HNPCC). HNPCC accounts for 5-13% of the total colorectal cancer burden (Bocker *et al.,* 1999). HNPCC is an autosomal dominant disorder, with high penetrance, in which colorectal cancer develops in gene carriers, but without the thousands of adenomas seen in FAP (Lynch *et al.,* 1991). Adenomatous polyps are found in HNPCC patients but the numbers are few (usually less than 10). There is a propensity for adenomas and carcinomas to develop in the proximal part of the colon. The expression of the HNPCC phenotype is diverse, in terms of age of onset and the organs affected by malignant change. It may be inherited as a site-specific colorectal cancer susceptibility trait (Lynch type I) or may be associated with uterine, gastric, ovarian, upper urinary tract, small intestinal and other malignancies (Lynch type II; Lynch *et al.,* 1991). A family of genes responsible for HNPCC are caused by defects in the human mismatch repair genes: hMSH2 on chromosome 2p, hMLH1 on chromosome 3p, hPMS1on chromosome 2q, hPMS2 on chromosome 7q and hMSH6 (Farrington and Dunlop 1996; Bocker *et al.,* 1999).

Epidemiological studies provide strong evidence to indicate that other genetic factors must be involved in susceptibility to bowel cancer, even where there is no obvious hereditary component (St. John *et al.,* 1993; Slattery and Kerber 1994). This increased risk of cancer in relatives of colorectal carcinoma patients has been estimated to be a 2-4 fold increase over that of the general population (Bishop and Hall 1994). Inherited susceptibility could explain a greater proportion of colorectal carcinoma than that seen in the high-risk families. Other diseases that carry an increased risk of colorectal cancer include longstanding ulcerative colitis and primary sclerosing cholangitis (Fracasso *et al.,* 1999).

1.2.4 Lung carcinoma

Lung cancer is a leading cause of cancer deaths, for both men and women in Europe and North America (World Health Organisation 1997a). It is recognised that cigarette smoking is the most important cause of lung cancer (La Vecchia *et al.,* 1991). Between 80-90% of lung carcinomas in men and between 55-80% lung cancers in women are

caused by cigarette smoking (Levi 1999). The smoking prevalence in 1994 in the United Kingdom for males was 28% and for females, 26% (World Health Organisation 1997b). This represents a fall in adult smoking from 1980 when 42% of males and 37% of females smoked. The male mortality rate from lung cancer has fallen since the 1970s, whilst the female mortality rate reached a peak in the late 1980s. Lung cancer is the second commonest cause of death in women after breast cancer. In Scotland and Liverpool, deaths from lung cancer have exceeded those from breast cancer (La Vecchia 1991).

Passive exposure to environmental tobacco smoke also increases the risk of death from lung cancer. Trichopoulous *et al,* (1981) performed a case-control study and found that women whose husbands smoked more than one pack of cigarettes per day had a 3-4 fold increased relative risk of lung cancer. The United States Environmental Protective Agency declared in 1992 that " environmental tobacco smoking " or " passive smoking" was a proven lung carcinogen responsible for 3000 lung cancer deaths each year in non-smokers in the United States.

Lung cancer also occurs in association with occupational and environmental exposure to carcinogenic agents from sources other than smoking. These include: arsenic, asbestos, chloromethylethers, chromium, mustard gas, nickel and radiation (Doll and Peto 1981). In areas where shipbulding was a major industry, asbestos exposure is a clear aetiological agent and acts synergistically with cigarette smoking, resulting in lung cancer relative risks that are 1.5-2.5 times that of smokers (Berry *et al.,* 1985).

The vast majority of cigarette smokers do not develop lung cancer (Cinciripini *et al.,* 1997). This suggests that carcinogenesis depends also on either an inherited predisposition or additional carcinogenic cofactors. Studies have compared the risk factors of individuals with histologically verified lung cancer with those of individuals with other smoking-related cancers. They found that having relatives with lung cancer does not increase an individual's risk of developing lung cancer, but it does increase the risk of having cancer at some other site. This suggests a heritable variation in response to carcinogens (Ooi *et al.,* 1986; Cinciripini *et al.,* 1997). Finally, studies of families predisposed to lung cancer showed that the development of lung cancer in younger people (aged 50 years or less) was compatible with a Mendelian codominant inheritance of a rare autosomal gene. This gene was not involved for older persons who developed

lung cancer; for these individuals the aetiology of cancer was long term exposure to tobacco (Sellars *et al.*, 1990).

1.2.5 Paediatric malignancies

Childhood malignancies are rare, affecting 1 in 600 children before the age of 15 years, and represent the second commonest cause of death in children between 1 and 14 years (CRC Factsheet 15.1 1995). In the vast majority of cases the causes of childhood cancer remain unknown, however, the tumours developed in childhood are different from those seen in adult life. Histologically the tumours resemble undifferentiated, foetal counterparts rather than the differentiated structures seen at birth. The genetic events causing the tumours must occur during embryonic life and so prevent normal differentiation (Cowell 1994).

Knudson's (1971) two-hit hypothesis explains the development of some childhood malignancies. The hypothesis suggests that mutation of both copies of a single gene are sufficient for the initiation of tumorigenesis (see Section 1.1.2.1). An example is the retinoblastoma gene, located on chromosome 13q14 (Friend *et al.*, 1986). Another example is *TP53*, located on chromosome 17q13 which causes the Li-Fraumeni syndrome (Malkin *et al.*, 1990; Knudson 1993; Lane 1994) and is characterised by the development of sarcomas, brain tumours and adrenocortical tumours (Li-Fraumeni 1969; Malkin *et al.*, 1990). Further examples of tumour suppressor genes are WT1 on chromosome 11p13 which causes Wilm's tumour (Haber *et al.*, 1990; Hastie *et al.*, 1994), NF1 on chromosome 17q11 which leads to the development of type 1 neurofibromatosis (Wallace *et al.*, 1990) and is associated with the development of neurological tumours. Other genetic conditions predispose affected children to the development of malignancy. For instance the development of leukaemia is associated with A-T (Hecht *et al.*, 1966; Gatti *et al.*, 1991) and Down's syndrome (Shafik *et al.*, 1990). Similarly skin cancers in children are associated with xeroderma pigmentosum (Heddle *et al.*, 1983; de Boer *et al.*, 1999) and nevoid basal cell carcinoma (Bale *et al.*, 1989; El-Zein *et al.*, 1995).

Environmental factors involved in the aetiology of paediatric malignancy are radiation, viruses and drugs. High radiation exposure, e.g., following the atomic explosions at Hiroshima and Nagasaki and radiotherapy to children, increases the risk of

developing cancer (Hall 1988; Tubiana *et al.*, 1990). The importance of viruses has been highlighted by the rising incidence of Kaposi's sarcoma in areas of Africa severely affected by the AIDS epidemic (Wabinga *et al.*, 1993; Chang *et al.*, 1994). Also before the age of 20, non-Hodgkin's lymphoma is over 300 times more common among AIDS victims than the general population (Beral *et al.*, 1993; Schulz *et al.*, 1996). Many children with hepatocellular carcinoma show evidence of hepatitis B infection (Leuscher *et al.*, 1988). Finally, Epstein-Barr virus has been implicated in the aetiology of Hodgkin's disease (Weinreb *et al.*, 1996), Burkitt's lymphoma (de-The *et al.*, 1978) and nasopharyngeal carcinoma (Liebowitz 1994).

Evidence for a hormonal influence in the development of childhood malignancies comes from the use of synthetic oestrogen, diethylstilboestrol by pregnant women to prevent spontaneous abortion. This was linked to the subsequent development of adenocarcinoma of the vagina in teenage daughters (Herbst *et al.*, 1979).

1.3 ASSAYS OF CANCER PREDISPOSITION

1.3.1 Direct assay of known genes

The number of cancer susceptibilities that have been mapped by genetic linkage is increasing. This allows DNA analysis to be carried out and individuals with a susceptibilty gene to be identified before the development of the disease, e.g., *BRCA1* and *BRCA2* genes (Murday 1994). Various laboratory methods exist for the detection of known and unknown mutations (Markham *et al.,* 1994; Offitt 1998). The clinical utility of genetic testing depends on its ability to provide definitive results, whether positive or negative, so identifying those individuals who are likely to benefit from intensified surveillance or cancer risk reduction strategies (Syngal *et al.,* 1999).

1.3.2 Diagnostic phenotypes

Diagnostic phenotypes may indicate which individual may develop a malignancy. For instance A-T homozygotes have a propensity to develop lymphomas or leukaemias (Hecht *et al.,* 1966), Down's syndrome patients frequently develop leukaemia (Shafik *et al.,* 1990), patients with Gorlin's syndrome develop basal cell carcinomas (Featherstone *et al.,* 1983; El-Zein *et al.,* 1995) and those suffering from dysplastic naevus syndrome develop malignant melanoma (Bale 1986; Sanford *et al.,* 1987). In these cases it is clinically apparent that the individual has a particular phenotype which places him or her at increased risk.

1.3.3 Biochemical assays

Biochemical assays may be of use when the gene has not been identified, but where measurable phenotypic markers of the defect exist. For instance it may be possible to measure the activity of enzymes involved in the metabolism of carcinogens. For example, 50% of the population are deficient in the enzyme glutathione S-transferase due to a deletion in the GSTMI locus (Seidegard 1985). Individuals with the deletion are at increased risk of smoking induced lung cancer (Seidegard 1990; Zhong 1991).

1.3.4 Chromosomal assays

Cytogenetic assays are of potential use when the individual has a normal phenotype and the risk of malignancy is not clinically obvious. Cytogenetic assays measure the amount

of chromosome damage produced either spontaneously as in the case of A-T or Bloom's syndrome or by a mutagen and are potentially a useful method to study the relationship between individual sensitivity to the induction of genetic damage and cancer risk. The rationale behind using these assays is that a cell's inability to cope with DNA damage (expressed as chromosome damage) is indicative of an underlying defect in some cellular process that may also predispose the cell to malignant change. A single assay may be able to predict cancer susceptibility in people with different underlying mechanisms of predisposition if an agent induces a wide range of lesions. For example, A-T and XP cells have different molecular defects but both have increased levels of chromosome aberrations after exposure to ionising radiation (Taylor 1982; Parshad 1983). Those assays that are able to detect different forms of cancer predisposition are most likely to be of use in screening for an increased risk of cancer.

1.3.4.1 G_0 assays (metaphase and micronucleus)

G_0 metaphase assays were used in early studies of cancer-prone conditions. Chromosomal sensitivity to ionising radiation has been detected in A-T (Taylor *et al.*, 1976), basal cell naevus syndrome (Featherstone 1983), and retinoblastoma (Heras and Coco 1987). Sensitivities to the genotoxic effects of alkylating agents and crosslinking agents has been identified using the G_0 assays in familial adenomatous ployposis patients (Delahunty and Cooke 1989).

Micronucleus assay

Micronuclei (MN) are formed post-mitotically from the condensation of lagging chromatid/ chromosome fragments or entire chromosomes. The micronuclei are produced during mitosis. The frequency of induced MN has been used as a quantitative indicator of chromosome damage (Evans 1959; Littlefield 1989) and as a means of distinguishing normal and cancer-prone individuals (Countryman and Heddle 1976; Arlett and Priestley 1985). MN are easy to visualise in cells and a large number of cells can be scored. Discrepancies in MN yields were noted in early studies, due to difficulty in distinguishing cells that have undergone one division from cells which have not divided.

Revised MN assays have been used for studies including dose rate effects and dosimetry (Fenech and Morley 1985; Hall and Wells 1988; Prosser 1988), to monitor drugs for clastogenic effects (Autio 1994) and to detect inter-individual differences in sensitivity to environmental carcinogens (Di-Giorgio 1994) and ionising radiation (Huber 1989).

More recent studies have focussed on attempts to predict cancer predisposition and response to radiotherapy in normal individuals and cancer patients respectively (Slonia and Gasinska 1997). Scott *et al.*, (1996b) obtained reasonable discrimination between normal individuals and A-T heterozygotes by measuring MN induction in lymphocytes exposed to low dose rate gamma irradiation in the G_0 phase of the cell cycle.

1.3.4.2 G_2 assays

Taylor (1978) noted that cells, irradiated in the G_2 rather than the G_0 phase of the cell cycle, were more sensitive to the effects of ionising radiation and gave a better discrimination between A-T and normal cells than G_0 cells. Subsequently Sanford and workers have carried out many investigations which involved x-irradiating cells in the G_2 phase of the cell cycle, to obtain a measure of G_2 chromosomal radiosensitivity. Chromatid damage in cells entering metaphase 0.5-1.5 hours after x-irradiation was monitored and high frequencies of chromatid gaps and breaks were noted for cultured fibroblasts or lymphocytes from individuals with cancer-prone conditions, familial cancers as well as approximately 5% of normal healthy donors (Sanford *et al.*, 1989). Sanford *et al*, (1989) and Parshad *et al.* (1990) identified sensitivity for X-P homozygotes and X-P heterozygotes. Similar radiosensitivity was seen in A-T heterozygotes. Other investigators failed to reproduce the findings of Sanford and Parshad. For example, Bender (1985)
was unable to discriminate between A-T heterozygotes and normal controls using the G_2 assay. The inability to reproduce results mayhave resulted from the use of different protocols for the assay. Inter-experimental variability in the level of radiation induced aberrations is a recognised problem (Taylor 1982; Natarajan *et al.*, 1983; Bender *et al.*, 1985). Factors such as serum batch, bacterial contamination, temperature, cell density

and pH also affect G_2 chromosomal radiosensitivity (Sanford *et al.*, 1989; Bosi *et al.*, 1991).

Scott *et al.*, (1996) attempted to reproduce the findings of Sanford's group at the National Cancer Insititute (NCI). With some modifications of the NCI protocol, the Paterson G_2 assay was developed. Scott *et al.*, (1994) were then able to confirm and expand on many of the findings of the NCI group. Table 1.4 lists cancer-prone conditions which exhibit G_2 chromosomal radiosensitivity.

A-T heterozygotes

A number of studies have confirmed G_2 sensitivity in cells from A-T heterozygotes (Parshad *et al.*, 1983; Parshad *et al.*, 1985; Shiloh *et al.*, 1989; Sanford *et al.*, 1990; Scott *et al.*, 1993; Scott *et al.*, 1994b). The elevated sensitivity of A-T heterozygotes is significant as such individuals are believed to comprise 0.5-1.5% of the general population and about 4% of the breast cancer patients (Easton 1994). Good discrimination between A-T heterozygotes and normal donors has been produced using the G_2 assay. Sanford *et al.*,(1990) found that 5% of normal donors overlapped with the range of chromosomal radiosensitivity for A-T heterozygotes, whilst Scott *et al.* (1994b) found an overlap of 9%.

Table 1.4 Cancer-prone conditions exhibiting chromosomal radiosensitivity in the G_2 phase of the cell cycle.

Diagnosis	Reference
A-T homozygotes	Parshad *et al.*, 1985
	Sanford *et al.*, 1990
	Scott *et al.*, 1994b
A-T heterozygotes	Rary *et al.*, 1974
	Taylor *et al.*, 1978
Blackfan-diamond syndrome	Van Diemen *et al.*, 1997
Bloom's syndrome	Parshad *et al.*, 1983
Common variable Immune deficiency	Vorechovsky *et al.*, 1993
Down's syndrome	Chudina 1968
	Sanford *et al.*, 1993
Dyskeratosis congenita	Debauche *et al.*, 1990
Familial adenomatous polyposis	Parshad *et al.*, 1983
Familial melanoma	Sanford *et al.*, 1987
Fanconi's anaemia	Bigelow *et al.*, 1979
	Parshad *et al.*, 1983
Hereditary retinoblastoma	Sanford *et al.*, 1989
Li-Fraumeni syndrome	Parshad *et al.*, 1993
	Mitchell and Scott 1997
	Varley *et al.*, 1998
Nijmegen breakage syndrome	Taalman *et al.*, 1983
Rothmund-Thomson syndrome	Kerr *et al.*, 1996
Wilm's tumour	Sanford *et al.*, 1989
Xeroderma pigmentosum	Parshad *et al.*, 1990

Choice of mutagen to induce chromatid damage

The best example of hereditary hypersensitivity to an environmental mutagen and subsequent cancer predisposition is xeroderma pigmentosum (XP). These individuals show increased susceptibility to sunlight-induced cutaneous damage resulting from acute sun sensitivity and predisposition to develop cutaneous tumours (Hsu 1987). The frequency of spontaneous chromosome damage is not high in XP, but after irradiation with UV light the frequency of chromosome aberrations is higher than cells of normal individuals similarly irradiated (Heddle *et al.,* 1983). As different mutagens have different mechanisms for inducing DNA damage, it is logical that different genetic defects exist amongst individuals in response to one type of DNA damage but not all types. It is known that cells of A-T patients are sensitive to ionising radiation and bleomycin (Higurashi and Conen 1973; Taylor *et al.,* 1976; Taylor *et al.,* 1994), but are not sensitive to UV light (Hsu 1987). Spontaneous chromosome instability and mutagen hypersensitivity are separate phenomena and people with one syndrome may be hypersensitive to one mutagen but not to another (Heddle *et al.,* 1983; Hsu 1987). A degree of mutagen susceptibility may exist in some people because of mild defects in DNA repair systems.

Many agents have been used to explore the possibility of differential susceptibility to mutagens in order to induce chromatid damage in both lymphocytes and fibroblasts from cancer patients. Lymphocyte hypersensitivity to bleomycin-induced chromosome damage has been reported for cancers of the colon, lung (Hsu 1987) and head and neck (Hsu *et al.,* 1989; Hsu *et al.,* 1991; Cloos *et al.,* 1994; Ankathil *et al.,* 1996; Cloos *et al.,* 1999). Hsu (1987) also found a low bleomycin sensitivity in breast cancer patients that was almost identical to controls. It may be that the G_2 assay employing ionising radiation has a greater discriminatory power as the challenge agent. Recently more effort has concentrated on the use of the G_2 radiosensitivity assay in cancer patients. Parshad *et al.,* (1984; 1985) found a significantly higher incidence of chromatid aberrations following G_2 phase irradiation in fibroblasts derived from human tumours versus normal tissue origin. Elevated sensitivity has been demonstrated in the lymphocytes from breast cancer patients (Scott *et al.,* 1994a; Helzlsouer *et al.,* 1995; Parshad *et al.,* 1996; Patel *et al.,* 1997; Scott *et al.,* 1999). Sanford *et al.* (1997) was also able to demonstrate elevated chromosomal radiosensitivity in blood lymphocytes from

patients with malignant melanoma, whilst Bondy *et al.* (1996) suggested that sensitivity to gamma radiation is significantly associated with the risk of developing gliomas.

G_2 chromosomal radiosensitivity in breast cancer patients

Using the G_2 assay, Scott *et al,* (1994a) found 21 of an unselected series of 50 sporadic breast cancer patients had a chromosomal radiosensitivity within the A-T heterozygote range. This series of patients was expanded to 135 and the results confirmed that approximately 40% of breast carcinoma patients show elevated radiosensitivity (Scott *et al.,* 1999). Parshad (1996) obtained similar results detecting abnormally high sensitivity in 6 out of 12 breast cancer patients with no family history of the disease, and 6 out of 7 patients with a positive family history.

Easton (1994) estimated that approximately 4% of breast cancer cases are A-T heterozygotes and it is clear from several studies that the proportion of patients found to be sensitive in the G_2 assay are higher than can be accounted for by A-T heterozygosity alone (Scott *et al.,* 1994; Parshad *et al.,* 1996; Patel *et al.,* 1997). Low penetrance genes have been postulated as a cause of these cancer cases (Scott *et al.,* 1994a). Epidemiological studies tend to support the existance of low penetrance genes (Teare *et al.,* 1994; Ford *et al.,* 1998; Easton 1999) as high penetrance genes (*BRCA1, BRCA2* and *TP53)* contribute to a minority (5%) of breast cancer cases (Ford and Easton 1996; Goldgar *et al.,* 1996).

Further studies (Scott *et al.,* 1998) examined the use of the MN assay amongst normal individuals and breast cancer patients. Good assay reproducibility was obtained using a high dose rate protocol, which identified 31% of breast cancer patients compared with 5% of healthy controls as having elevated radiation sensitivity. A direct comparison was made by Scott *et al.*. (1999) of the G_0 and the G_2 assays using normal donors and breast cancer patients. Using the MN assay, 25% of breast cancer patients were sensitive compared with 40% of breast cancer patients assayed by the G_2 method. No correlation between MN and G_2 data was seen for the 80 patients tested. Only 4% of patients were sensitive to both assays. It was concluded that different mechanisms of chromosomal radiosensitivity operate in G_2 and G_0 cells.

Heritability studies

Several studies (Parshad *et al.,* 1996; Helzlsouer *et al.,* 1996; Patel *et al.,*1997) indicated that the chromosomes of female relatives of breast cancer patients were on average more radiosensitive than normal controls without a family history of breast cancer. Roberts *et al.* (1999) studied the chromosomal radiosensitivity of family members of patients with breast cancer who demonstrated both elevated or normal levels of chromosomal radiosensitivity. Sixty-two percent of first-degree relatives of sensitive index cases were sensitive to the assay, compared with 7% of first-degree relatives of normal responding breast cancer cases. These results provide clear evidence of heritability of chromosomal radiosensitivity. A segregation analysis was performed and this suggested evidence of Mendelian heritability of chromosomal radiosensitivity; the inheritance being dominated by one or more major genes with large and additive effects. If low penetrance genes do exist, their contribution to the development of cancer would be significant, and it is possible that they may contribute to the development of many malignancies (Easton 1999; Rebbeck 1999). The use of the G_2 assay in other sporadic malignancies, could lead to the identification of low-penetrance genes. Elevated G_2 sensitivity has been shown in patients with sporadic melanoma and sporadic dysplastic naevi (Sanford *et al.,*1997).

Mechanisms of G_2 chromosomal radiosensitivity

DNA damage induced by G_2 x-irradiation produces mainly chromatid breaks and gaps in metaphase cells (Savage 1975) and it is the summation of the aberrations which provides an estimate of the chromosomal radiosensitivity. The expression of chromatid damage depends on the initial level of aberrations induced, the degree of DNA repair and the cells ability to pass through cell cycle checkpoints. Defects in any of these processes may lead to elevated levels of aberrations.

Several models have been proposed to explain the mechanism of chromatid breaks, these include the Classical breakage-first model by Sax (1938), Revell's exchange model (1955) and a recombinational model (Chadwick and Leenhouts 1978). Bryant (1998) proposed the "signal model", in which a single double strand break (dsb) is converted into a chromatid break by initiating a recombinational exchange, involving a large chromatin domain either within or between chromatids. Thus a chromatid break results from a cell-mediated rearrangement. Ionising radiation induces three major types

of DNA lesions: base damage, single strand breaks (ssb) and double strand breaks (Ward 1990; Ward 1994). The failure of chromosome break rejoining is likely to be the result of a cells inability to rejoin DNA dsbs (Natarajan and Obe 1984; Cornforth 1998), however the mechanisim underlying the failure to rejoin dsbs is not understood. There is little evidence to suggest that the rate of induction of dsbs is wholly responsible for the variation seen in G_2 chromosomal radiosensitivity.

Defects in the processing of DNA damage have been identified in cells from individuals exhibiting elevated chromosomal radiosensitivity. For instance A-T cells have been shown to have higher initial levels of chromatid damage compared to those of normal donors immediately after irradiation (Pandita and Hittleman 1992). Studies on cultured cells using inhibitors of DNA repair (Mozdarani and Bryant 1989; MacLeod and Bryant 1992) suggest that differences in the rate of conversion of DNA double strand breaks into chromatid breaks may be due to altered chromatin structure (Pandita and Hittleman 1995) and this could account for some of the variation seen in G_2 sensitivity. The frequency of chromatid breaks in metaphase cells reaches a maximum approximately 30 minutes after irradiation (Macleod and Bryant 1992; Scott *et al.*, 1996a). The frequency of breaks then falls with time in normal cells or remains elevated in cells from individuals with cancer-prone conditions (Parshad *et al.*, 1983; Parshad *et al.*,1993a; Sanford *et al.*, 1987; Sanford *et al.*, 1990). Studies using inhibitors of DNA repair, suggest that it is the lack of DNA repair which leads to the presence and persistance of aberrations following irradiation (Preston 1980; Preston 1982; Preston 1983; Sanford *et al.*, 1993). Studies of fibroblasts from cancer-prone conditions (Parshad *et al.*,1983) and of lymphocytes from breast cancer patients (Parshad *et al.*, 1996) have demonstrated that cells from these individuals are deficient in DNA excision repair.

The mitotic inhibition of cells from A-T homozygotes is significantly reduced compared to cells from normal donors (Scott *et al.*, 1994b). It is reported that A-T fibroblasts (Scott and Zampetti-Bossler 1982a), SV-40 transformed fibroblasts (Mozdarani and Bryant 1989) and lymphocytes (Scott *et al.*, 1994b) suffered less G_2 delay than cells from normal individuals. It is evident that there are two separate G_2 responses of A-T cells that differ from controls. A primary event in which cells in G_2 at the time of irradiation suffer less delay in progress to mitosis and a secondary event in which cells irradiated at earlier stages of the cell cycle are more delayed or arrested when

they pass into G_2. The secondary event may be due to misrepaired or mis-replicated DNA (Painter 1993).

It is known that A-T cells pass through three checkpoints more rapidly than do normal cells. The primary G_2 checkpoint, the checkpoint in S cells which controls initiation of replication and chain elongation (Painter and Young 1980) and a third at the transition from G_1 into S phase (Nagasawa *et al.,* 1985). It is thought that the A-T gene product acts at all three checkpoints and behaves as a transcription factor influencing cell cycle genes (Beamish and Lavin 1994), possibly p53 which is involved in a G_1 to S transition (Lane 1994) and whose radiation activation was reported to be defective in A-T cells (Kastan *et al.,* 1991; Khanna and Lavin 1993). It is feasible that the same defects may be involved in cells from malignancies, i.e., failure to repair DNA damage leads to a low mitotic inhibition and this is manifest as chromosomal radiosensitivity.

Defects in the cell cycle are associated with variation in G_2 chromosomal radiosensitivity. Consistent with the hypothesis that the chromosomal radiosensitivity of A-T cells reflects a failure of these cells to undergo the mitotic delay necessary for repair of DNA lesions (Painter and Young 1980) it has been shown that A-T cells irradiated in the G_2 phase of the cell cycle show less mitotic delay than normal cells (Scott and Zampetti-Bossler 1982; Scott *et al.,* 1994b). This inverse correlation between mitotic delay and chromosomal radiosensitivity has been reported in tumour cell lines (Schwartz *et al.,* 1996). Defects in cell cycle control may lead to genomic instability (Hartwell 1992; Kaufman and Paules 1996; Paulovich *et al.,* 1997). Recent studies by Terzoudi *et al.,* (1999) have suggested that upregulation of the cdk1/ cyclin B complex impairs DNA repair processes leading to increased chromatid aberrations. Such anomalies might be reflected in diferences of G_2 chromosomal radiosensitivity between individuals.

1.4 AIMS OF THIS STUDY

The hypothesis to be tested was that malignancies associated with a genetic aetiology are associated with G_2 radiosensitivity, whilst those linked to an environmental aetiology are not associated with elevated radiosensitivity. The hypothesis was tested using a G_2 chromosome damage assay. The chromosomal response to radiation exposure was to be studied using metaphase analysis of cultured peripheral blood lymphocytes from the following groups: breast, cervical, colorectal, lung and paediatric/ adolescent

malignancies. The results were compared to a group of healthy normal individuals, a group of paediatric/ adolescent controls and a group of patients with benign disease. A group of subjects with a strong family history of cancer who underwent screening for the mutant *BRCA1* and *BRCA2* genes were also tested in order to determine if high penetrance cancer predisposing genes were associated with chromosomal radiosensitivity.

The cervical, colorectal and lung carcinoma groups were studied as their aetiology is *predominantly* environmental. A group of children/adolescents with paediatric malignancies were investigated as the aetiology is uncertain and little work has been performed in this area. In order to investigate the genetic predisposition of paediatric malignancies it was necessary to study the radiosensitivity of healthy children and adolescents. This allowed a comparison with existing data on healthy adults and the effects of donor age on chromosomal radiosensitivity.

A group of patients with benign disease (diabetics and patients with chronic lung disease) were studied to assess the impact of chronic disease on the results of the assay. The purpose of studying the benign group was to evaluate whether chromosomal radiosensitivity is specific for cancer risk. A group of normal individuals was studied for control purposes.

The ultimate aim of the project was to evaluate whether the G_2 assay may have a role in identifying low penetrance cancer predisposing genes. The aim of identifying individuals with low penetrance cancer predisposing genes is to reduce their risk of cancer. This could be achieved through enhanced screening programmes where appropriate (e.g., breast, colorectal) or by limiting exposure to environmental and industrial carcinogens.

Finally, the degree of mitotic inhibition produced by the irradiation was evaluated and correlated with chromosomal radiosensitivity. Checkpoint genes are involved in the cell cycle and their function may be disrupted in malignancy, e.g., mutations in *p53* are found in the majority of tumour types, leading to alterations in G_1 checkpoint, due to failure to properly transactivate the cdk inhibitor $p21^{WAF1}$ (El-Deiry *et al.*, 1994).

2. MATERIALS AND METHODS

2.1 Patient details

The study was performed after South Manchester Medical Research Ethics Committee approval. Informed consent was obtained from patients providing blood samples prior to treatment at the Christie Hospital NHS Trust, Manchester, or at the Royal Manchester Children's hospital. Parental consent was obtained for blood samples from the paediatric groups. The following clinical data were recorded when available: date of birth, age, gender, diagnosis, tumour stage, histological type and tumour grade.

Blood (5ml) was obtained via venepuncture by a clinician or registered phlebotomist. The blood samples were transferred to plastic universal containers, containing 10 IU sodium heparin (Monoparin 1000 IU/ml CP Pharmaceuticals Ltd. Wrexham, UK) and stored at room temperature for a maximum of 24 hours prior to culture. Samples from cancer patients were obtained prior to treatment with radiotherapy or chemotherapy. Samples from patients with a previous history of treatment of neoplasia, i.e., second primary were excluded from the study. Blood samples from local hospitals other than the Christie Hospital were transported immediately via a courier.

The samples were obtained from several groups of individuals. Healthy control subjects comprised staff from the Paterson Institute for Cancer Research and healthy members of the general public. Samples were also obtained from Christie Hospital patients with breast, cervix, colorectal or lung carcinomas. The paediatric malignancy group consisted of children and adolescents attending either the Young Oncology Unit at the Christie Hospital or the Royal Manchester Childrens's Hospital. Paediatric aged- matched control samples were obtained from patients attending the Royal Manchester Childrens's Hospital for treatment for benign conditions, e.g., elective surgery. A group of diabetics and patients with chronic lung disease attending diabetic and chest clinics, respectively at the Wythenshawe Hospital, Manchester provided blood for the benign disease group of patients. Subjects with a strong family history of breast cancer who were undergoing screening for *BRCA1/ BRCA2* gene mutations at St. Mary's Hospital, Manchester provided a blood sample at their initial consultation.

2.2 Materials

Culture Medium

The culture medium consisted of 83% RPMI 1640 (Flow Laboratories), 15% foetal calf serum (Sera-Lab; batches 1060, 169, 71F) and 2% L-Glutamine (Gibco BRC).

Phytohaemagglutinin (PHA)

Cultures were stimulated with PHA (Wellcome Laboratories) a lectin that binds to B and T lymphocytes but acts primarily as a mitogen for T lymphocytes. A vial of lyophilised PHA was reconstituted by dissolving in 5ml of Hank's balanced salt solution (ICN Biomedicals). Reconstitution gave a stock solution of 1μg/ ml that was stored at 4°C for up to one month.

Colcemid

Cycling cells were blocked in metaphase using colcemid (Sigma), a microtubule spindle inhibitor. Colcemid solution at a concentration of 10 μg/ ml was stored at 4°C.

Fixative

Cultures from which metaphase chromosome preparations were made were fixed in a mixture of 3 parts methanol (BDH Chemicals Ltd) to 1 part glacial acetic acid (BDH Chemicals Ltd). This mixture was freshly prepared immediately prior to use.

Hypotonic Solution

A hypotonic solution of 0.075M KCl was used. This was used prior to cell fixation in order to lyse erythrocytes and to complement colcemid treatment in aiding dispersion of chromosomes.

Stain and slides

A 2% Giemsa solution, buffered to pH 6.8 was used to stain the prepared slides. DPX Mountant (BDH Chemicals Ltd) was used for permanently mounting coverslips on slides. SuperFrost 76x26mm microscope slides (Menzel-Glaser, Germany) were used with 22x50mm coverslips (Chance Propper Ltd, England).

Handling conditions

Initial culture manipulation was carried out in a class II microbiological safety cabinet. Blood samples were then incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. Further incubation of cultures was carried out in a walk-in hot room maintained at 37°C. Transportation of cultures to the x-ray machine was undertaken using a portable incubator maintained at 37°C.

2.3 Methods

2.3.1 Initiating cultures of whole blood

Whole blood cultures were set up within 24 hours of venepuncture. Two ml of whole blood was added to 18ml of pregassed, prewarmed culture medium in a T30 tissue culture flask (Corning Ltd) and 0.2ml of PHA added (final concentration of 1μg/ ml). Two flasks were set up per donor; one for irradiation, one for sham irradiation. The flasks were placed upright with their caps loose in the CO_2 incubator for 3 hours. The caps were then tightened and the flasks wrapped in tin foil and placed in a walk-in hot room.

2.3.2 Assaying G_2 chromosomal radiosensitivity (metaphase analysis)

Culture

From the time of PHA stimulation until the cultures were placed on ice during harvesting, all culture processing was carried out at 37°C. To ensure these conditions were satisfied during any manipulation, culture incubation and processing were carried out in a walk-in hot room. Cultures were only removed for the purposes of irradiation and then only in a portable incubator maintained at 37°C.

Irradiation

Culture medium was renewed 71 hours after the addition of PHA. The medium (15 ml) was removed by pipette, care being taken not to disturb the cell layer at the bottom of the flask. Fifteen ml of pre-warmed, pre-gassed medium was then added and the flasks agitated thoroughly by hand. After 72 hours the cells were irradiated with 0.5 Gy X-rays

using an orthovoltage X-ray set operated at 300 kV, 10 mA with 2.3 mm Cu filtration and a dose rate of 1.2 Gy/min.

Cultures were removed from the portable incubator for the duration of irradiation only (usually 25 seconds). These precautions were taken to ensure that the culture conditions were affected minimally during the critical G_2 period. During the irradiation set-up the flasks were placed on a perspex applicator, which rested on a wooden phantom. The skin source distance (SSD) was 15 cm.

Harvesting

Following irradiation the cultures were incubated at 37°C for 30 minutes before 0.2 ml colcemid was added to a final concentration of 0.1 μg/ ml and the cultures mixed. The contents of each flask were then split between two 12ml centrifuge tubes; i.e., two tubes contained irradiated cells and two contained mock-irradiated cells. Exactly one-hour after the addition of the colcemid, the culture tubes were placed in ice and the ice- bucket was agitated to ensure rapid cooling of the tubes.

Previous experimental work at the Paterson Institute revealed that continuation of cell metabolism and DNA repair during harvesting produced variability in the results obtained from the G_2 assay (Scott *et al.,* 1996). To reduce such variability culture temperatures were lowered during harvesting to levels inhibitory to cellular metabolism. Therefore the harvesting temperature was maintained at approximately 4°C, using pre-chilled solutions and a refrigerated centrifuge. Processing was carried out with culture vessels held on ice.

The tubes were centrifuged at 1000 rpm for 7 minutes in a pre-chilled refrigerated centrifuge. After decanting the supernatant, the cell pellet was re-suspended in 5 ml hypotonic KCl and the tubes kept on ice for 20 minutes. The tubes were then centrifuged again at 1000 rpm for 7 minutes at 4°C and the supernatant removed leaving 1 ml above the cell pellet. Gentle pipetting and re-suspension in 10 ml of 3:1 fixative mixed the sample. The cells were then centrifuged at 1000 rpm for 5 minutes, the supernatant decanted, another 10 ml of fixative added and the cells refridgerated at least overnight (e.g., 16-36 hours) prior to slide preparation.

2.3.3 Preparation of metaphase chromosome spreads

Following 16-36 hours refrigeration the fixative was decanted and fresh 3:1 fixative added. The cells were then centrifuged at 1000 rpm for 5 minutes, the supernatant was removed and the cell pellets resuspended in 1ml of fixative to produce a cloudy suspension. Chromosome spreads were produced by dropping a few drops of the suspension on to a wet slide. The suspension was quickly blown along the length of the slide and passed through a flame for around 15 seconds. A phase-contrast microscope was used to ensure adequate spreading of cells and metaphases along the slide. At least three slides were made from each tube of suspension. Once dry, the slides were immersed in a 2% Giemsa solution for 5 minutes. The slides were then rinsed in tap water, dried, cleared in xylene and mounted in DPX Mountant.

2.3.4 Analysis of metaphase spreads

Two scorers analysed separate groups of metaphase cells. Scorer 1 analysed preparations from the following groups; normal donors, benign disease group, breast, cervical, colorectal and lung carcinoma and a small group of subjects who underwent screening for the mutant *BRCA1/2* genes. Scorer 2 scored preparations from adult normal donors, paediatric/ adolescent controls and paediatric/ adolsecent malignancies.

Microscopy was carried out with a binocular research microscope using x19 and x63 planapo objectives and a x10 eyepiece giving final magnifications of x190 and x630, respectively. Slides were randomised and coded prior to analysis to avoid scorer bias. Fifty metaphases were examined per subject for both irradiated and un-irradiated cultures. Good quality metaphases were scored. The spontaneous frequency of aberrations in the un-irradiated (control) cultures was subtracted from the frequency of aberrations of the irradiated cultures to give the induced number of aberrations. The aberration yields were expressed as the number of aberrations per 100 cells.

The most frequent aberrations were chromatid breaks and gaps (Figure 1.1). Discontinuities were scored as breaks if the achromatic region was clearly misaligned with respect to the sister chromatid or as gaps if the achromatic region was wider than the width of the chromatid. Achromatic lesions of less than the width of a chromatid were classed as small gaps and were not scored.

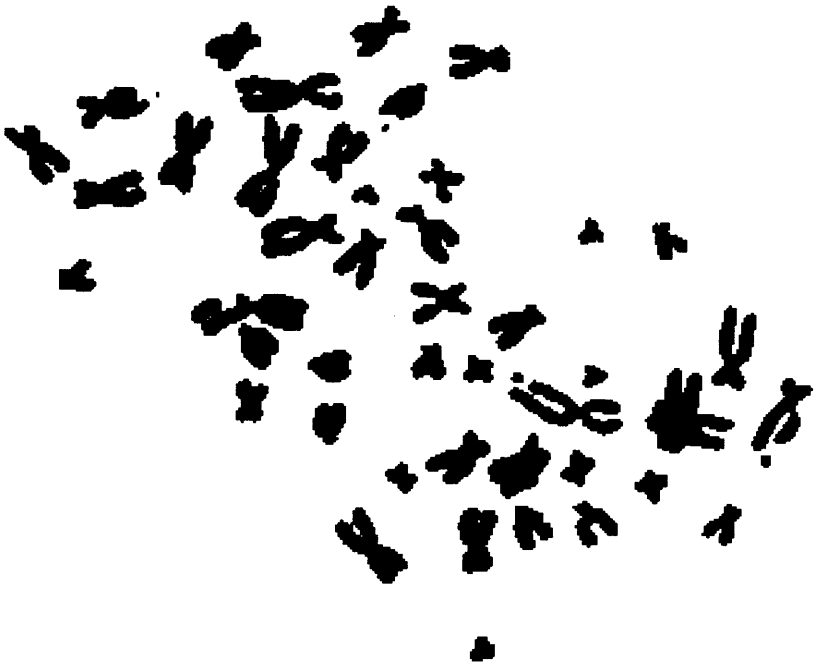

Figure 1.1 Metaphase of a peripheral blood lymphocyte, indicating breaks and gaps.

Mitotic index and inhibition

One thousand mononuclear cells were scored from coded slides to obtain the mitotic index (MI) for both irradiated and non-irradiated cells. Mitotic inhibition was calculated as the percentage reduction in MI in irradiated samples compared with the non-irradiated samples.

2.3.5. Statistical analysis

Statistical advice was sought from Dr Steve Roberts at the Paterson Institute for Cancer Research and statistical analyses were carried out using SPSS software. Correlations between biological data and continuous patient variables (e.g., age) were obtained using Spearman's correlation coefficients. Categorical data were compared using the Kruskall-Wallis one way analysis of variance or Mann-Whitney U test, as appropriate. All tests were 2-tailed and 0.05 was taken as the level of statistical significance.

3. RESULTS: ADULT MALIGNANCIES

3.1 Success rates

A total of 447 blood samples was assayed. However, a high proportion of samples (35%) did not yield data due to either failed cultures or a low mitotic index (Table 3.1). For the 97 cultures that failed, the stimulation and proliferation of T-lymphocytes was so poor that few metaphases were found on slides prepared from both irradiated and unirradiated cell cultures. Another fifty-nine samples failed because of a low mitotic index. In these cases a radiation-induced reduction of mitotic index led to less than 50 scorable metaphases in irradiated samples. The dose of 0.5 Gy x-rays produced a mean mitotic inhibition (percentage reduction of MI compared to unirradiated cultures) of 93.3% in normal donors.

Table 3.1 Yields of successful samples per donor group.

Group	Total no.	Success no.	Success %	Fail no.	FC	LMI
Normal donors	150	112	75	38	11	27
Benign	57	34	60	23	19	4
Breast	40	31	78	9	5	4
BRCA	17	15	88	2	1	1
Screening						
Cervix	34	27	80	7	6	1
Colorectal	57	37	65	20	9	11
Lung	92	35	38	57	46	11
Total	447	291	65	156	97	59

FC= failed culture
LMI= low mitotic index

For the normal donors the majority of failures (35/38) occurred during the first six months of the study. This reflected the inexperience of the experimenter involved in the initiation and harvesting of cultures, in slide preparation and in scoring aberrations. With further experience the failure rate was reduced markedly. All samples of the benign disease group were received during the early part of the study. Of those breast samples

that failed, two-thirds did so early on and one third later on during the study. Samples from subjects undergoing BRCA1/2 screening were received during the latter part of the study. Four of the seven failures of cervical samples occurred during the early experiments, whilst the majority of colorectal failures (12/20) occurred during the latter part of the study, 8/20 occurred early on. The lung carcinoma group posed problems; 24/57 of the early samples failed, whereas 33/57 of the samples received later on in the study failed. A high proportion (46/57 samples) was due to failure of the cultures to grow adequately. Because of the relatively high failure rate with early cultures, those that were successful were re-scored when the scorer was confident of analysis.

Table 3.2 Characteristics of normal donors.

	Mean± SD	Median	Range
Age (years)	37.5± 11.3	35.0	20-61
Induced aberrations/ 100 cells	85.8± 10.8	86.0	64-118
Spontaneous aberrations	1.4± 1.4	1.5	0-6.0
Mitotic inhibition (%)	93.9± 3.0	93.0	83-99

3.2 Results of the G_2 assay on normal donors

Results were obtained for 66 normal donors (Table 3.2,). For 41 of these the assay was performed on one occasion. For the remaining 25 donors, at least two and up to six blood samples were taken so that the total number of assays was 112 (Table 3.1). Thirty-six samples (32%) were obtained from males and 76 (68%) from females. For each sample the spontaneous aberration yield was subtracted from the yield for irradiated cells to give an induced yield. The mean induced yield was 85.8± 10.8 aberrations per 100 cells with a range of 64-118 (Figure 3.1, top panel). Using Spearman's correlation there was a significant positive relationship between the G_2 score and the spontaneous aberration yield ($r=0.42$, $p<0.001$). There were no correlations between the induced aberration score and either the percentage mitotic inhibition ($r=-0.086$, $p=0.49$) or normal donor age ($r=-0.11$, $p=0.39$). There was no significant difference in aberration yields between male and female donors (Table 3.3).

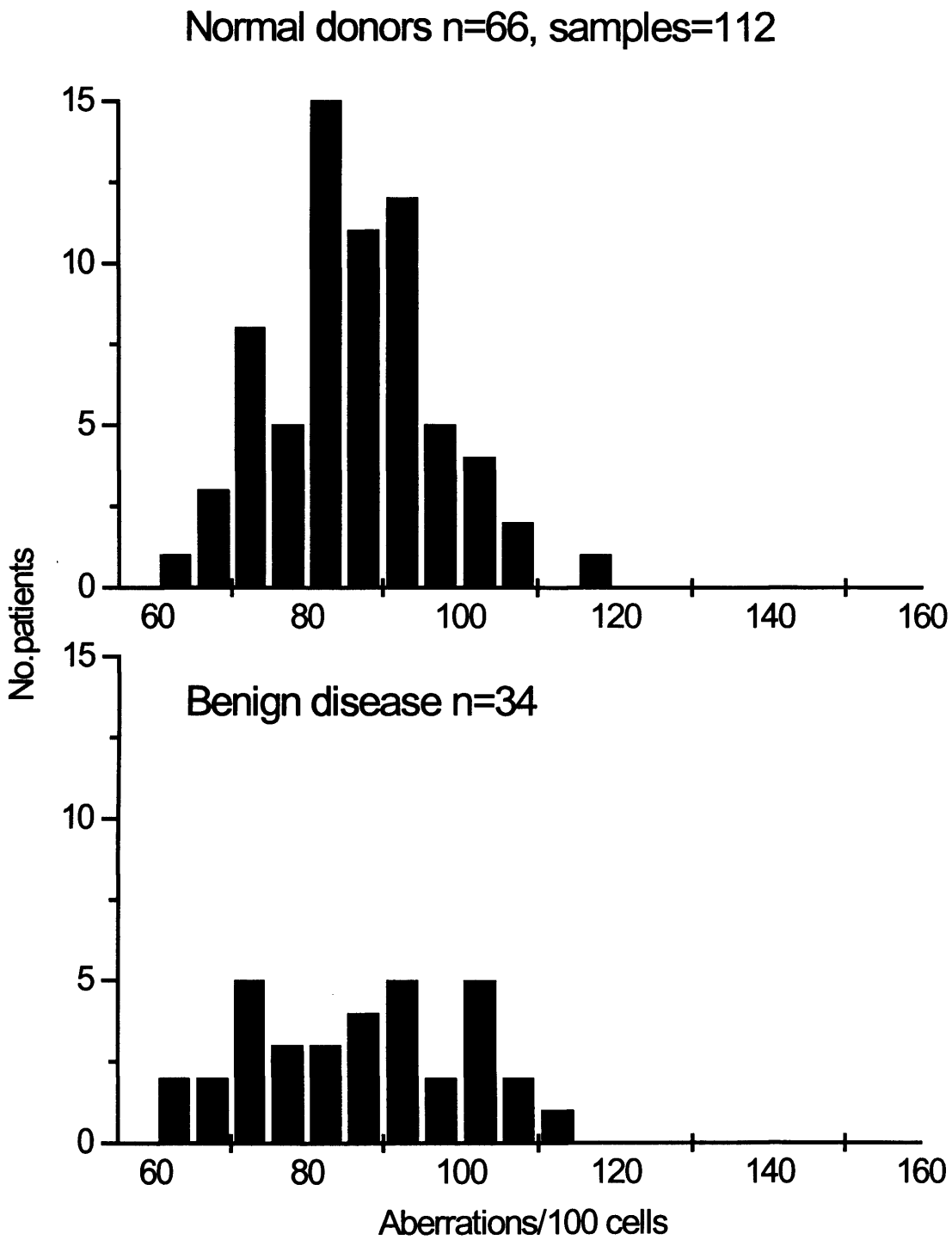

Figure 3.1 Number of patients versus aberrations/ 100 cells. Normal donors top panel. When repeat samples for individuals were available the mean yields are plotted. Benign disease group lower panel.

Table 3.3 Comparison of metaphase aberration yields in male and female donors.

Sex	No. donors	Means± SD	Median	p
F	44	85.8± 10.81	86.0	-
M	22	85.2± 11.3	85.5	0.96

p value, level of significance using the Mann-Whitney U test.

Assay reproducibility

Assay reproducibility was determined using the data for repeat assays on the same individuals by using standard one way analysis of variance (Table 3.4). The coefficients of variation for the levels of induced aberrations within and between individuals were 10.3% and 15.1%, respectively. The assay detected significant inter-donor variability between normal donors (p=0.004).

Table 3.4 Coefficients of variation

	No. donors	CV	p
Inter-individual	70	15.1%	-
Intra-individual	25	10.3%	0.004

p value, the level of significance using ANOVA.

Normal donors - defining a cut-off point

The assay results were expressed in terms of a sensitive/ non-sensitive dichotomy by introducing an arbitrary cut-off value to define sensitivity. A statistician examined the distribution of results from normal donors (Figure 3.1), and chose a cut-off point at the 90th percentile (100 aberrations per 100 cells). Using this value, the proportion of sensitive donors was 9% (6/66; 95% confidence limits = 3.4-18.7).

3.3 Benign disease group

Results were obtained for 34 blood samples from patients with benign disease (Table 3.5). There were no significant correlations between the induced aberration scores for the benign disease group, and either the levels of spontaneous aberrations (r=0.20, p=0.26),

the percentage mitotic inhibition (r=0.23, p=0.18) or donor age (r=0.03, p=0.88). Eighteen samples (53%) were obtained from males and 16 samples (47%) from females and there was no influence of gender on the assay results (Table 3.6). Using 100 aberrations/ 100 cells as the cut-off point the proportion of sensitive cases was 4/34 (12%, 95% CI=3.3-27.5; Figure 3.1).

Table 3.5 Characteristics of 34 samples from the benign disease group.

	Mean± SD	Median	Range
Age (years)	60.2± 15.2	60.5	21-82
Induced aberrations/ 100 cells	85.8±13.7	86.0	60-112
Spontaneous aberrations	0.71±1.41	0.0	0-6.0
Mitotic inhibition (%)	90.6±7.7	93.3	70-99.6

Table 3.6 Comparison of metaphase aberration yields in male and female donors.

Sex	No. donors	Means± SD	Median	p
F	16	84.9± 12.9	85.0	-
M	18	86.6± 14.7	89.0	0.72

p value, level of significance of Mann-Whitney U test.

3.4 Breast carcinoma group

Results were obtained from a total of 31 patients, all of whom were female.

There was no correlation between induced score and either spontaneous aberration rate (r=0.09, p=0.62) or patient age (r=-0.004, p=0.98). A weak though significant inverse correlation was seen between induced score and percentage mitotic inhibition (-0.44, p=0.01). Using the cut-off point, 12/31 (39%) of cases were radiosensitive (95% CI=21.9-57.8, Figure 3.2).

Table 3.7 Characteristics of the breast carcinoma group

	Mean± SD	Median	Range
Age (years)	56.2± 9.4	56	36-79
Induced aberrations/ 100 cells	96.5± 23.9	92	62-154
Spontaneous aberrations	0.77±0.99	0.0	0-2.0
Mitotic inhibition (%)	95.3±2.17	95.8	90-99

The Kruskall-Wallis non-parametric analysis of variance was used to examine differences in induced aberration scores with T stage of disease and histological grade. No significant difference was detected between induced score and T stage of disease (p=0.19). There was some indication for an influence of tumour grade on induced aberration score (p=0.048), but this was not ordered and using Spearman's correlation the association was not significant (r=0.09, p=0.62).

Subjects undergoing BRCA1/ 2 predictive screening.

Blood samples were obtained from subjects with a strong family history of breast cancer who underwent BRCA1/ 2 gene testing (2 males and 13 females). Nine of the above cases possessed the mutant BRCA1 gene, the other cases were normal. Using the cut-off point of 100 aberrations/ 100 cells, 1/9 (11%) cases were sensitive to the assay (p=0.56, Mann Whitney U test; p=1.0 Fisher's exact test). The numbers in this group were too small to perform meaningful statistical analyses and therefore were not included in the comparisons of different patient groups.

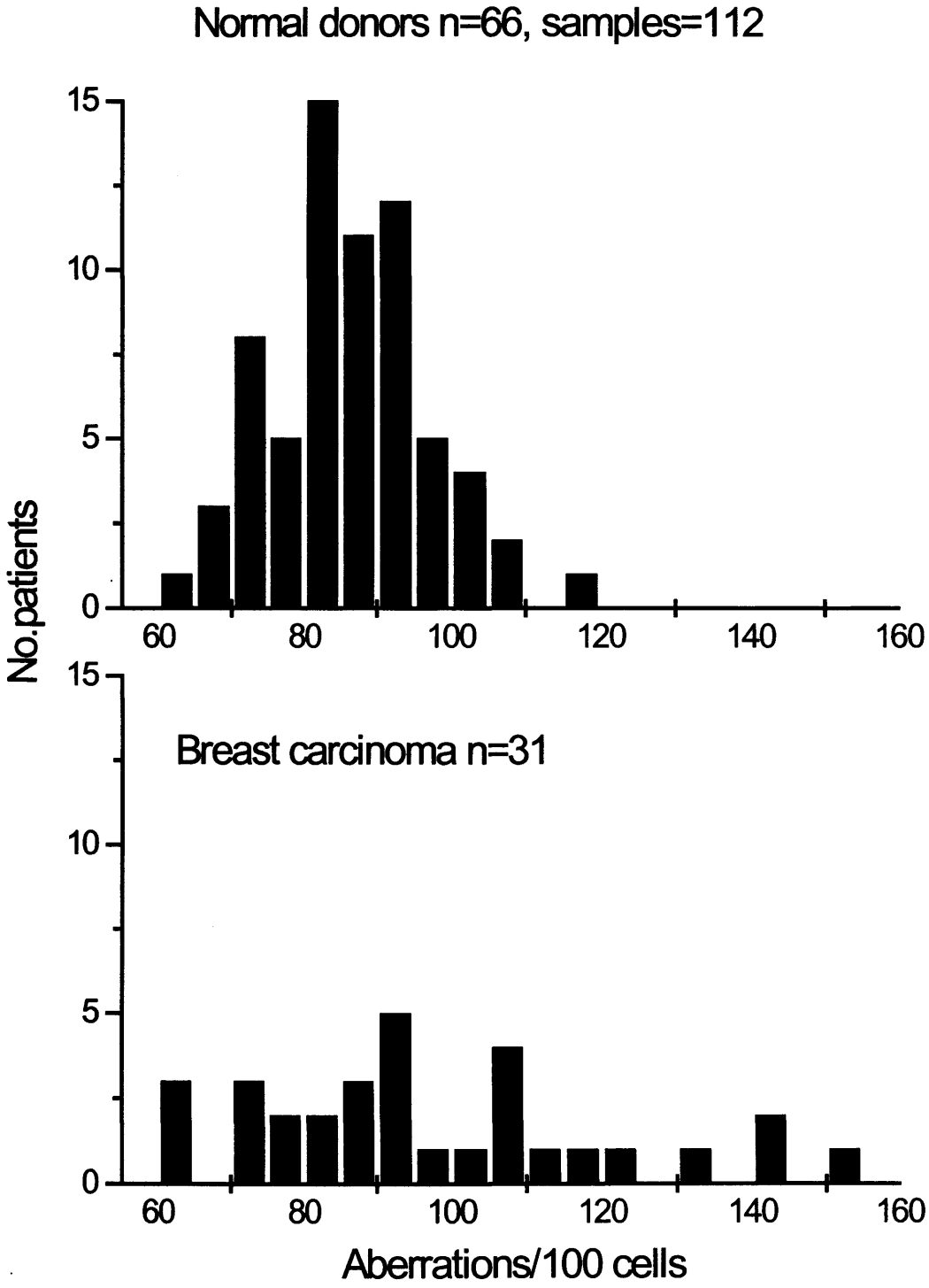

Figure 3.2 Number of patients versus aberrations/ 100 cells. Normal donors top panel, breast cancer patients lower panel.

Table 3.8 Characteristics of the BRCA screening subjects.

	Mean± SD	Median	Range
Age (years)	41.2± 7.0	42	29-52
Induced aberrations/ 100 cells	87.6± 15.9	84	66-126
Spontaneous aberrations	0.53± 0.92	0.0	0-2
Mitotic inhibition (%)	94.5± 2.6	0.2	90-98.3

3.5 Cervical carcinoma group

Results were obtained for 27 patients (Table 3.9, Figure 3.3). There was a significant positive correlation between induced aberration score and spontaneous aberration score ($r=0.48$, $p=0.01$). There was a borderline inverse correlation between score and percentage mitotic inhibition ($r=-0.33$, $p=0.08$) and no correlation between patient age and induced aberration score ($r=0.07$, $p=0.97$). Using the Kruskall-Wallis test, there was no significant difference in induced aberration score for the different stages of disease ($p=0.36$) and histological grades ($p=0.84$). Using Spearman's correlation, there was no correlation between induced aberration score and either disease stage ($r=0.03$, $p=0.90$) or histological grade ($r=0.03$, $p=0.87$). Using the cut-off point, 3/27 cases (11%) were radiosensitive (95% CI=2.3-29.2).

Table 3.9 Characteristics of the cervical carcinoma group.

	Mean± SD	Median	Range
Age (years)	59.3± 15.5	60	33-83
Induced aberrations/ 100 cells	84.6± 13.9	88	62-108
Spontaneous aberrations	1.41± 1.45	2	0-4
Mitotic inhibition (%)	93.9± 3.58	95	84-99

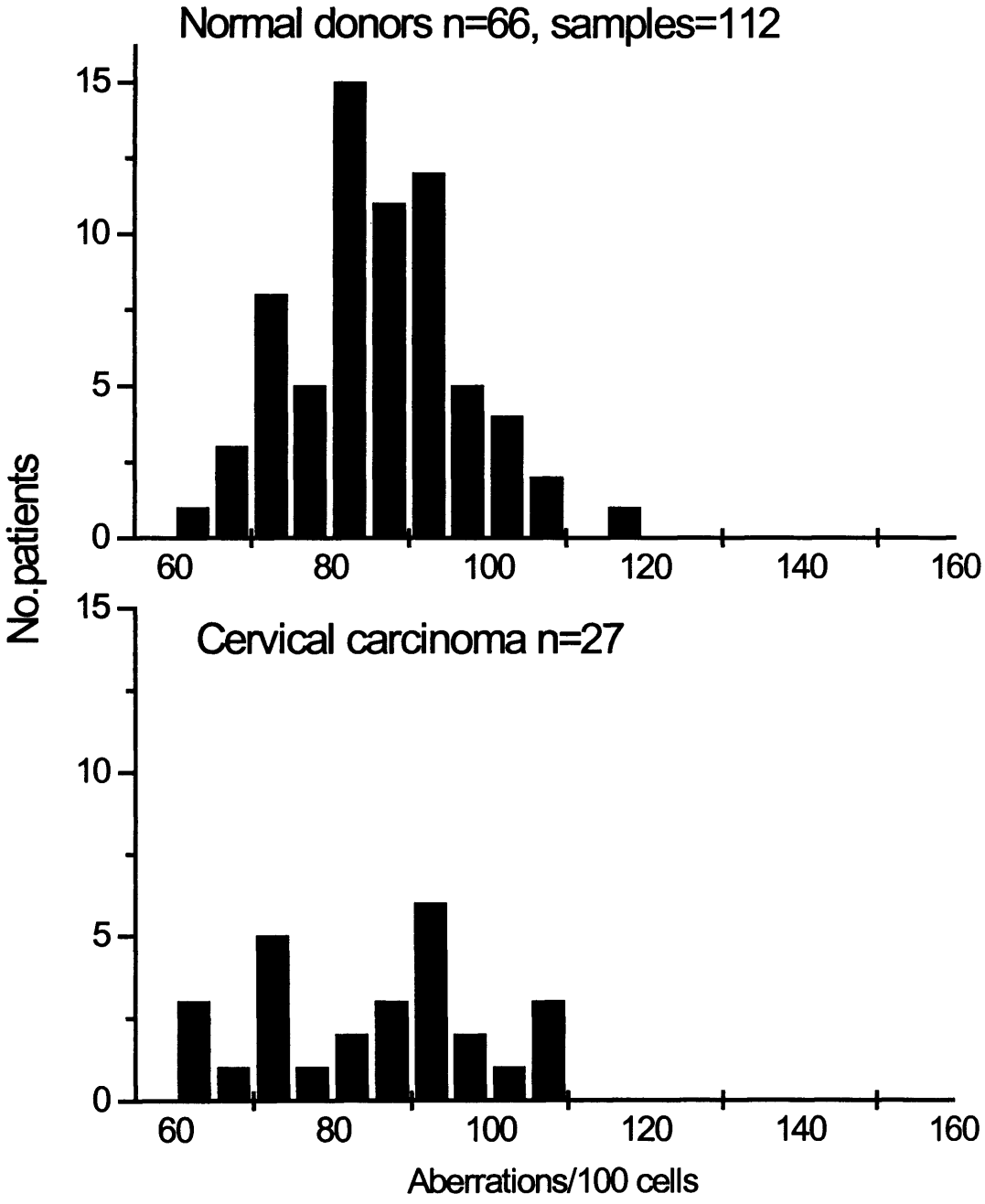

Figure 3.3 Numbers of patients versus aberrations/ 100 cells. Normal donors top panel, cervical carcinoma patients lower panel.

3.6 Colorectal carcinoma group

Results were obtained from 37 patients with colorectal tumours (Figure 3.4, Table 3.10). Twenty-two samples (59.5%) were from males and 15 samples (40.5%) were from females. There was no correlation, between induced aberration score and either spontaneous aberration score (r=-0.03, p=0.87), percentage mitotic inhibition (r=-0.09 p=0.60) or donor age (r=0.11, p=0.51). Using the cut-off point of 100 aberrations/ 100 cells, 11/37 cases (30%) were sensitive (95% CI=15.9-47.0). No significant differences were found in the induced aberration scores for the different Duke's stages of disease (p=0.33, Kruskall-Wallis test; r=-0.04, p=0.83, Spearman's correlation). There was also no relationship between induced score and histological grade (p=0.88, Kruskall-Wallis test).

Table 3.10 Characteristics of the colorectal tumour group.

	Mean± SD	Median	Range
Age (years)	63.1± 11.3	64	32-86
Induced aberrations/ 100 cells	91.5± 18.4	88	64-126
Spontaneous aberrations	1.3± 1.51	0.0	0-4
Mitotic inhibition (%)	92.1± 4.97	93	73-98

Table 3.11 Comparison of metaphase aberration yields in male and female colorectal patients.

Sex	No. donors	Means± SD	Median	p
F	15	91.5± 18.4	88.0	-
M	22	91.6± 18.0	94.0	0.99

p value, level of significance of Mann-Whitney U test.

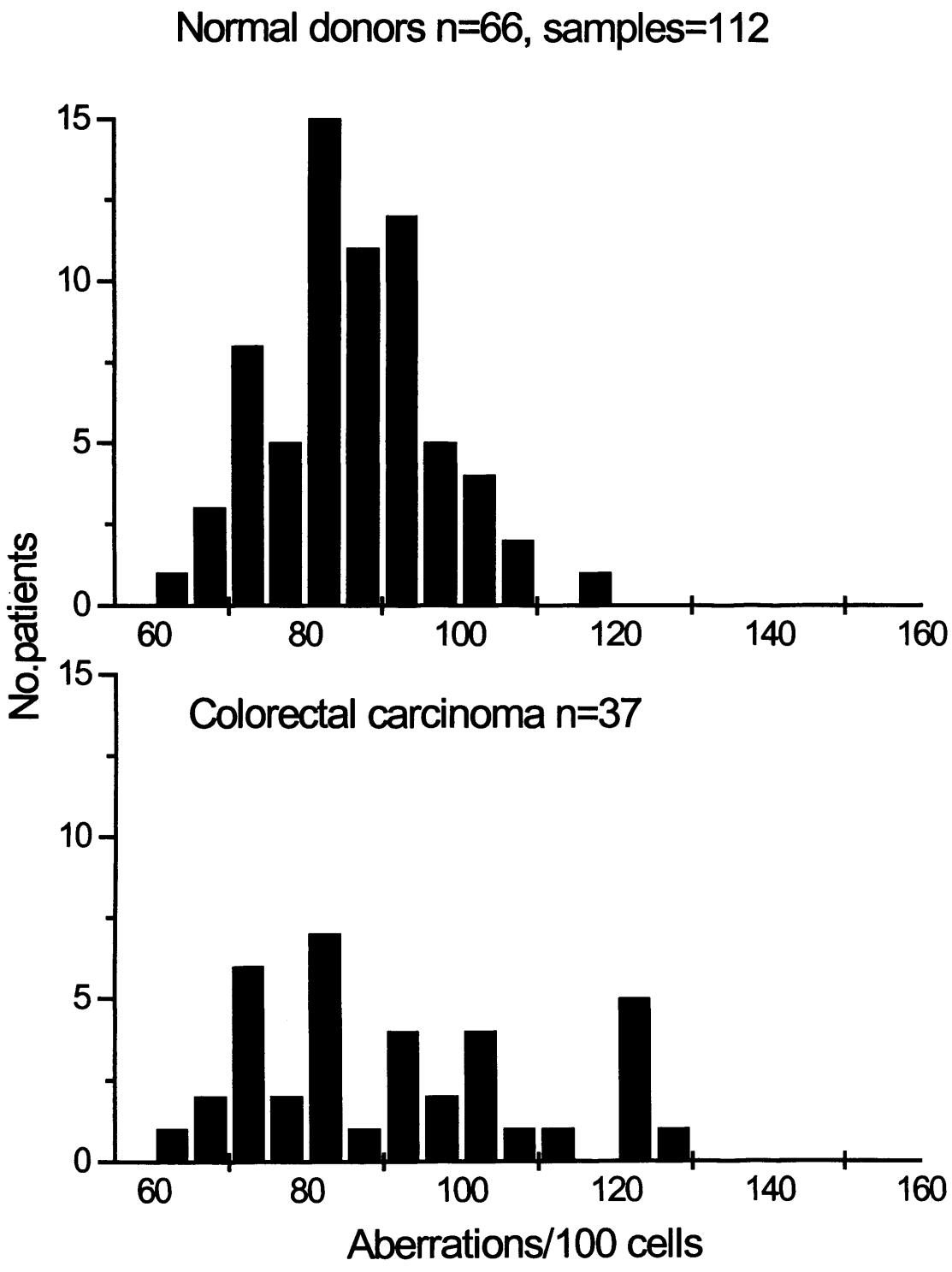

Figure 3.4 Number of patients versus number of aberrations/ 100 cells. Normal donors top panel, colorectal carcinoma patients lower panel.

3.7 Lung carcinoma group

Results were obtained on 35 patients (Figure 3.5, Table 3.12). Twenty-four samples (69%) were from males and 11 samples (31%) from females. Using Spearman's correlation there was a significant correlation between the induced aberration score and the spontaneous aberration yield (r=0.41, p=0.01). There was also a weak inverse association between induced score and percentage mitotic inhibition (r=-0.29, p=0.09). There was no correlation between patient age and induced score (r=0.20, p=0.26). There was also no influence of gender on the results of the assay (Table 3.13). No significant differences were found in the induced aberration scores for the different T stages of disease using either the Kruskall-Wallis test (p=0.27) or Spearman's correlation (r=-0.13, p=0.47). Using the cut-off point of 100 aberrations/ 100 cells, 8/35 cases (23%) were sensitive in the assay (95% CI= 10.4-40.1).

Table 3.12 Characteristics of the lung carcinoma group.

	Mean±SD	Median	Range
Age (years)	68.5± 8.5	70	50-85
Induced aberrations/ 100 cells	92.0± 14.6	92	70-124
Spontaneous aberrations	1.54± 1.54	2.0	0-4.0
Mitotic inhibition (%)	92.3± 3.4	91.8	82-98

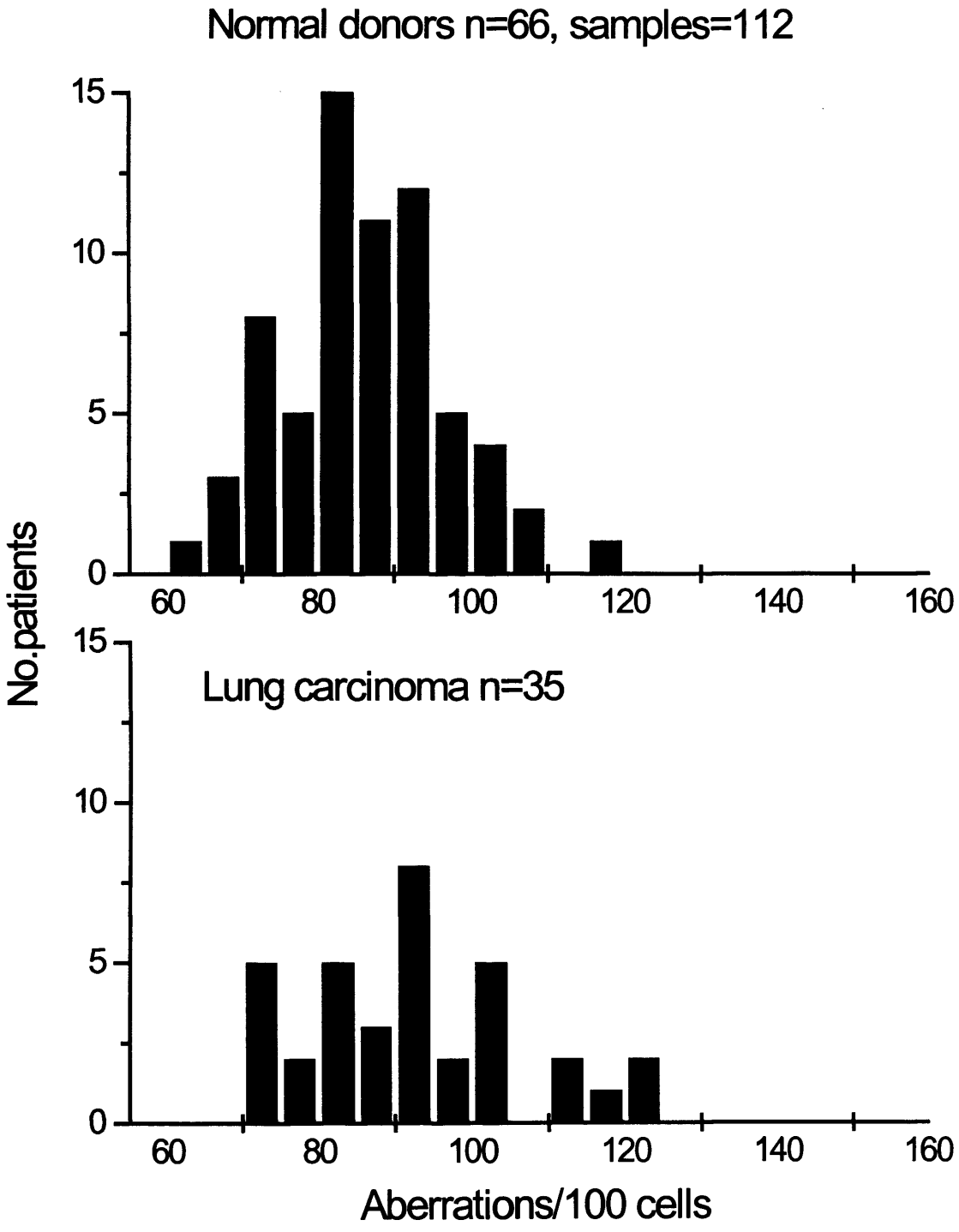

Figure 3.5 Number of patients versus aberrations/ 100 cells. Normal donors top panel, lung carcinoma patients lower panel.

Table 3.13 Comparison of metaphase aberrations in male and female lung patients.

Sex	No.donors	Means± SD	Median	p
F	11	86± 11.3	84.0	-
M	24	94.8± 15.3	94.0	0.14

p, level of significance of the Mann-Whitney U test.

3.8 Comparisons of donor groups

In general, similar yields of spontaneous aberrations were seen for the different groups (Table 3.14). However, in comparison with lymphocytes from normal donors, samples from both the benign and breast disease groups had significantly lower numbers of spontaneous aberrations. Using the Mann-Whitney U-test there were no significant differences in mean induced aberration yields between any of the groups (Table 3.15). The data were also studied using Fisher's exact test to compare the proportion of sensitive cases with that of normal donors. A highly significant percentage of breast carcinoma patients had sensitive lymphocytes (Figure 3.6). Colorectal carcinomas also had a significantly elevated frequency of radiosensitive individuals. The lung cancer group had an increased proportion of sensitive cases but this was only of borderline significance. Neither the benign disease group nor the cervix carcinoma patients were associated with an increased level of lymphocyte radiosensitivity.

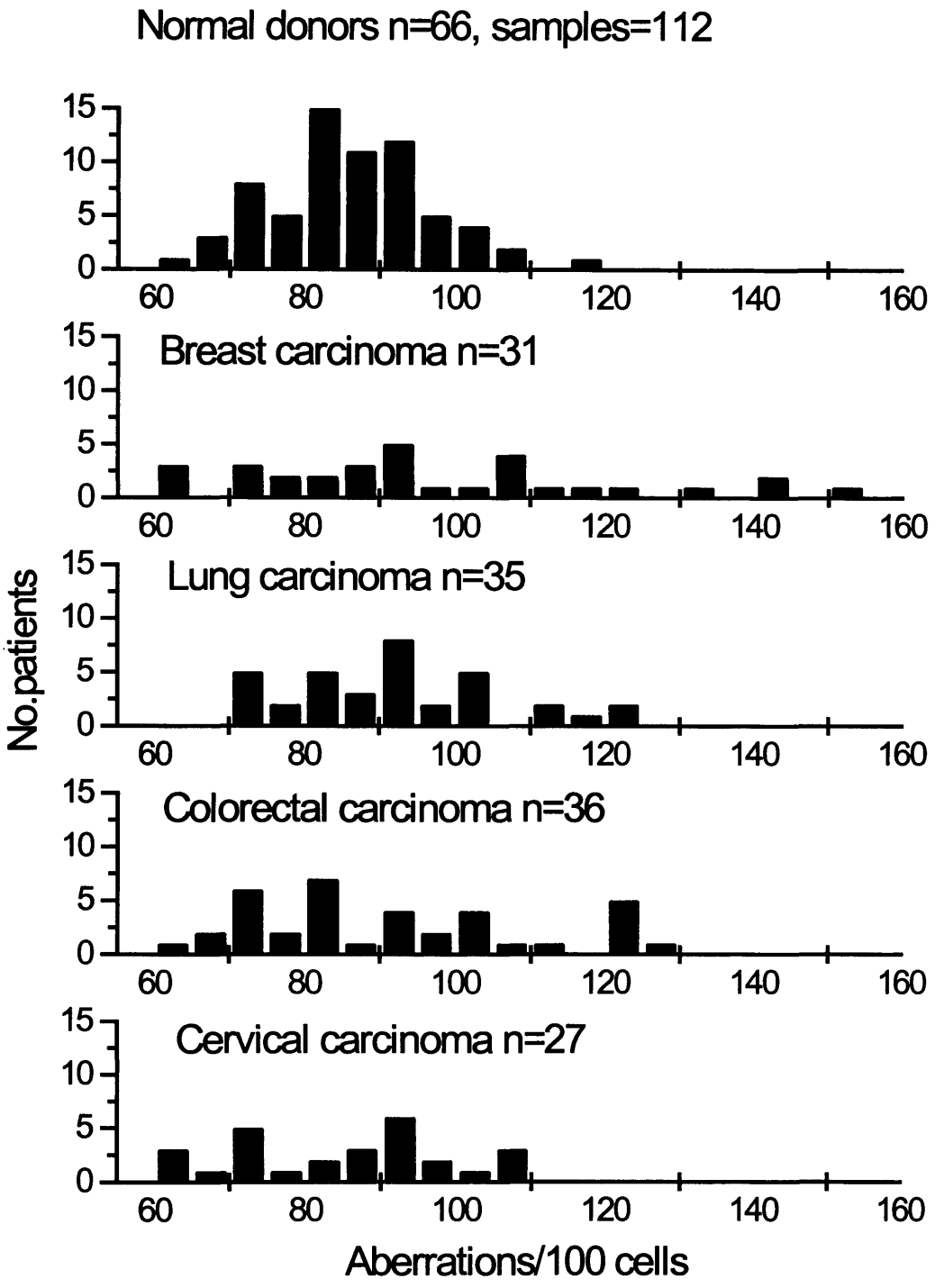

Figure 3.6 Number of patients versus aberrations/ 100 cells for various donor groups.

Table 3.14 Frequency of spontaneous chromatid aberrations/ 100 cells in different donor groups.

Donor group	No.	Mean± SD	Median	Range	p
Normals	66	1.40± 1.4	1.5	0-6	-
Benign	34	0.71± 1.47	0.0	0.6	0.001
Breast	31	0.77± 0.99	0.0	0-2	0.03
Cervix	27	1.40± 1.45	2.0	0-4	0.92
Colorectal	37	1.30± 1.51	0.0	0-4	0.50
Lung	35	1.54± 1.54	2.0	0-4	0.80

p value, the level of significance using the Mann-Whitney U test compared with normal donors.

Table 3.15 Induced aberration score/ 100 cells in normal donors and patients.

Group	No.	Mean± SD	Median	%sensitive	p	p*
Normals	66	85.8± 10.8	86.0	9.1	-	-
Benign	34	85.8± 13.7	86.0	11.8	0.90	0.73
Breast	31	96.5± 23.9	92.0	38.9	0.42	0.001
Cervix	27	84.6± 13.9	88.0	11.0	0.82	0.72
Colorectal	37	91.5± 18.4	88.0	29.7	0.26	0.01
Lung	35	92.0± 14.6	92.0	22.3	0.44	0.07

p value, the level of significance using the Mann-Whitney U test compared to normal donors. p* value, the level of significance using the Fisher's exact test.

The mean levels of radiation-induced mitotic inhibition were broadly similar for samples from all donor groups (Table 3.16). The median value of the breast carcinoma group was significantly higher and for the lung carcinoma group significantly lower compared to that of the normal donors. No correlation was seen between induced aberration scores and donor age for any of the groups studied (Table 3.17). When the data from the different groups are pooled together a significant though weak Spearman's correlation between induced score and spontaneous aberration rate is seen (r=0.16, p=0.02).

Table 3.16 Percentage mitotic inhibition in normal donors and patients.

Donor group	No.	Mean± SD	Median	Range	p
Normals	66	99.3± 3.00	93.9	83-99	-
Benign	34	90.6± 7.70	93.3	70-99.6	0.15
Breast	31	95.3± 2.17	95.8	90-99	0.04
Cervix	27	93.9± 3.58	95.0	84-99	0.83
Colorectal	37	92.1± 3.40	93.0	73-98	0.07
Lung	35	92.3± 3.40	91.8	82-98	0.01

p value, the level of significance using the Mann- Whitney U test versus normal donors.

Table 3.17 Tests for correlation of metaphase aberration yields with age.

Group	No.	Median age	Range	r	p
Normal	66	35	20-61	0.11	0.39
Benign	34	60.5	21-82	0.03	0.88
Breast	31	56	36-79	-0.004	0.98
Cervix	27	60	33-83	0.07	0.97
Colorectal	37	64	32-86	0.11	0.51
Lung	35	70	50-85	0.20	0.26

r=Spearman's correlation
p=level of significance of the correlation.

4 RESULTS: PAEDIATRIC MALIGNANCIES.

The results of the paediatric malignancies are considered separately from those of the adult malignancies as the paediatric and adult malignancy groups were analysed by two different scorers.

4.1 Success rates

A total of 148 blood samples was assayed. A proportion of samples (12%) did not yield data. The majority of failures occurred with the paediatric malignancy group (Table 4.1). None of the cultures failed because of a low mitotic index.

Table 4.1 Yields of successful samples per donor group.

Group	Total no.	Success No.	Success %	Fail No.	FC	LMI
Adult controls	61	57	93	4	4	-
Paediatric malignancy	45	32	71	13	13	-
Paediatric controls	42	41	98	1	1	-
Total	148	130	88	18	18	-

FC=failed culture
LMI=low mitotic index

4.2 Results of the G_2 assay on normal adult donors

Radiosensitivity results were obtained for lymphocyte samples from 30 normal donors (aged over 20 years, Figure 4.1 top panel). For 21 donors, a single sample was taken and assayed. For the remaining 9 donors, at least two and up to 7 blood samples were taken and assayed independently, so that the total number of samples for G_2 testing was 57. Nineteen samples (33%) were obtained from males and 38 samples (67%) were obtained from females. Table 4.2 summarises the data for the normal donors. No significant correlations were seen between the induced aberration scores and either the levels of spontaneous aberrations, the percentage mitotic inhibition or donor age. Donor gender also did not influence the results of the assay (Table 4.3).

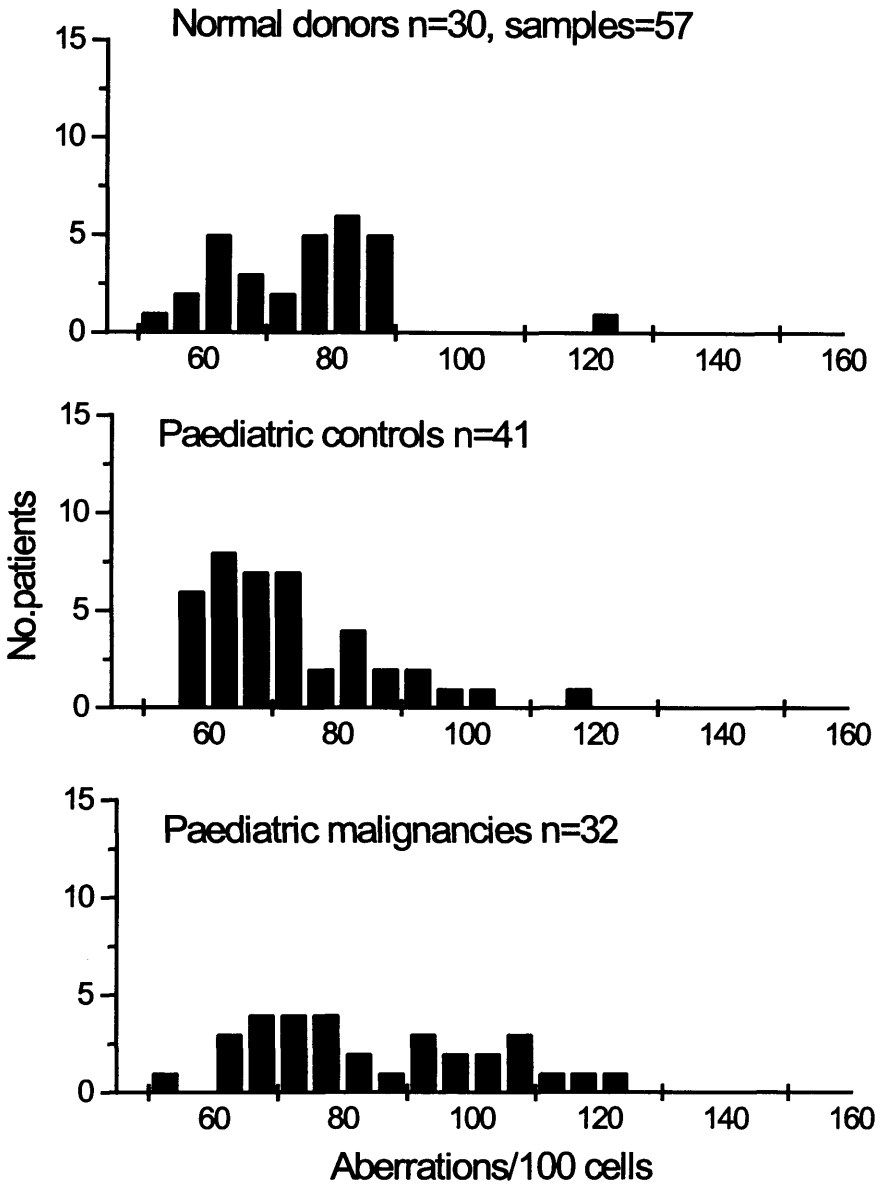

Figure 4.1 Number of patients versus aberrations/ 100 cells. Normal adult donors (top panel), paediatric controls (central panel) and paediatric malignancy group (lower panel).

Table 4.2 Characteristics of normal adult donors.

	Mean± SD	Median	Range
Age (years)	37.7± 10.7	37	20-60
Induced aberrations/100 cells	75.3± 13.4	77	54-122
Spontaneous aberrations	0.4± 0.7	0.0	0-2.0
Mitotic inhibition (%)	90.1± 3.6	90.3	80-97.1

Table 4.3 Comparison of metaphase aberration yields in male and female controls.

Sex	No. donors	Means± SD	Median	p
F	17	73.7± 9.0	76.0	-
M	13	77.3± 17.6	80.6	0.63

p value, of significance of the Mann-Whitney U test.

Assay reproducibility

The intra-individual coefficient of variation (i.e., assay reproducibility) was determined through standard one way analysis of variance of the available repeat data (a total of 23 samples from 9 individuals). For scorer 2, the coefficient of variation for induced aberrations within individuals was 18.6%. Inter-individual variability was measured for single samples from 36 donors and gave a value of 19.2%. After allowing for assay variability, it was not possible to demonstrate significant inter-donor differences in the levels of induced aberrations (Table 4.4).

Table 4.4 Coefficients of variation

	No. donors	CV	p
Inter-individual	36	19.2%	-
Intra-individual	9	18.6%	0.44

p value, the level of significance using ANOVA.

Defining a cut-off point

Scorer 2 scored on average 13% lower than scorer 1 (75.3 versus 85.8 aberrations/ 100 cells). The group of normal donors assayed by scorer 2 was too small to define a cut-off directly. The cut-off point of scorer 1, lowered by 13% was chosen, i.e., 87 aberrations/ 100 cells. Using this cut-off level 3/30 cases (10%) were found to be sensitive (95% CI= 2.1-26.5).

4.3 Paediatric and adolescent controls.

Results were obtained for 41 samples from paediatric and adolescent controls and these data are summarised in Table 4.5. Twenty-two samples (54%) were obtained from males and 19 samples (46%) were obtained from females. There were no significant correlations between the number of induced aberrations and either the level of spontaneous aberrations (r=0.001, p=0.99), the degree of mitotic inhibition (r=-0.19, p=0.23) or donor age (r=-0.20, p=0.20). Also, no influence of gender could be demonstrated on the levels of induced aberrations scored (Table 4.6).

Table 4.5 Characteristics of paediatric and adolescent controls.

	Mean± SD	Median	Range
Age (years)	8± 6.3	8	0.25-19
Induced aberrations/ 100 cells	72.9± 13.5	68	56-116
Spontaneous aberrations	0.63± 1.13	0-4	0.0
Mitotic inhibition (%)	90.7± 5.3	92.2	74.6-97

Table 4.6 Comparison of metaphase yields in male and female paediatric and adolescent controls.

Sex	No.donors	Means± SD	Median	p
F	19	73.4± 15.7	68.0	-
M	22	72.3± 10.8	74.0	0.79

p value, the level of significance using the Mann-Whitney U test.

Using a cut-off point of 87 aberrations/ 100 cells, 6/41 cases (15%) were sensitive in the assay (95% CI=5.6-29.2). A comparison was made of the induced aberration scores of normal donors with that of paediatric and adolescent controls. Using the Mann-Whitney U-test, no significant difference was seen between the median values (p=0.31). There was also no significant difference in the proportion of sensitive cases between the two donor groups (p=0.72, Fisher's exact test). Therefore, data from these two groups were pooled in order to examine further the effect of donor age on the induced aberration score (Figure 4.2). Using Spearman's correlation no significant effect of donor age was seen (r=0.04, p=0.76).

4.4 Paediatric and adolescent malignancies

Results were obtained for 32 cases of paediatric-aged and adolescent malignancies (Table 4.7, Figure 4.1 lower panel). Twenty samples (63%) were obtained from males and 12 samples (37%) from females. No significant correlations were seen between the induced aberration scores and the levels of spontaneous aberrations (r=-0.03, p=0.87), the percentage of mitotic inhibition (r=-0.22, p=0.23) and donor age (r=-0.08, p=0.66. There was also no influence of gender on the results of the assay (Table 4.8).

Table 4.7 Characteristics of paediatric and adolescent malignancies.

	Mean± SD	Median	Range
Age (years)	11.9± 5.6	13	0.5-19
Induced aberrations/ 100 cells	84.4± 18.2	81	52-122
Spontaneous aberrations	0.19± 0.78	0.0	0-4
Mitotic inhibition (%)	87.3± 8.9	90	50-98

Table 4.8 Comparison of metaphase yields in male and female paediatric malignancy patients.

Sex	No.donors	Means± SD	Median	p
F	12	80.3± 15.9	75.0	-
M	20	86.9± 19.5	89.0	0.34

p value, level of significance of Mann-Whitney U test.

Using a cut-off point of 87 aberrations /100 cells, 14/32 cases (44%; 95% CI=26.4-62.3) were sensitive. The percentage of sensitive patients was compared with the value obtained for the paediatric/adolescent control group. There was a 2.9 fold increase in the number of sensitive cases and this increase was highly significant (p=0.008). The median number of induced aberrations per 100 metaphases scored was also significantly higher for the cancer versus the paediatric control group (p=0.004).

Histology and induced aberration score

The histological types of the paediatric malignant cases were varied and included, sarcomas, Hodgkin's disease, and diverse types of brain tumours. An attempt was made to compare the mean induced aberration score for each subset of malignant disease. However, the numbers involved for a particular histological disease type were small, (Table 4.9) and it was not possible to demonstrate any differences between disease type and induced aberration score. Using the Mann-Whitney U test to compare the paediatric/ adolescent controls against the following malignancies, the following showed statistical significance; germinoma (one case only) p=0.05, Hodgkin's disease p=0.02, non-Hodgkin's lymphoma p=0.006.

Table 4.9 Induced aberration score according to histological type of disease.

Disease	No.	Mean ± SD	No. sensitive
Ependymoma	1	106	1
Ewing's sarcoma	1	112	1
Germinoma	1	52	0
Glioma	1	60	0
Hodgkin's disease	6	88± 13	3
Non-Hodgkin's lymphoma	4	102± 21	3
Medulloblastoma	1	74	0
Neuroblastoma	1	68	0
Osteosarcoma	9	83± 15	4
Ovarian teratoma	1	66	0
Rhabdomyosarcoma	5	77± 13	1
Wilm's tumour	1	100	1

4.5 Comparisons of donor groups

The data from the various donor groups were compared. In comparison with lymphocytes from paediatric/ adolescent controls, samples from the paediatric malignancy group had significantly lower numbers of spontaneous aberrations (Table 4.10). Using the Mann-Whitney U test there were no significant differences in mitotic inhibition between any of the groups (Table 4.11). The Mann-Whitney U test and Fisher's exact test were used to compare the differences of induced aberration score between the groups. In comparison to paediatric/ adolescent controls a highly significant proportion of samples from the paediatric malignancy group were sensitive (Table 4.12). There were no significant correlations between induced aberration yields and donor age (Table 4.13).

Table 4.10 Frequency of spontaneous chromatid aberrations/ 100 cells in different donor groups.

Group	No.	Mean± SD	Median	Range	p
Adult controls	30	0.4± 0.7	0.0	0-2.0	0.81
Paediatric controls	41	0.63± 1.13	0.0	0-4	-
Paediatric malignancy	32	0.19± 0.87	0.0	0-4.0	0.03

p value is the level of significance of the Mann-Whitney U test compared with the paediatric controls.

Table 4.11 Percentage mitotic inhibition in normal donors and patients.

Donor group	No.	Mean± SD	Median	Range	p
Adult controls	30	91.1±3.6	90.3	80-97.1	0.07
Paediatric controls	41	90.7±5.3	92.2	74.6-97	-
Paediatric malignancy	32	87.7±8.9	90.0	50-98	0.30

p value is the level of significance of the Mann-Whitney U test compared with the paediatric controls.

Table 4.12 Induced aberrations/ 100 cells in normal donors and patients.

Group	No.	Mean± SD	Median	% sensitive	p	p*
Adult controls	30	75.3± 13.4	77	10.0	0.31	0.72
Paediatric controls	41	72.9± 13.5	68	14.6	-	-
Paediatric malignancy	32	84.4±18.2	81	43.8	0.004	0.008

p value is the level of significance of the Mann-Whitney U test compared with the paediatric controls.
p* value is the level of significance of the Fisher's exact test.

Table 4.13 Tests for correlation of induced aberration yields with age.

Group	No.	Median age (years)	Range (years)	r	p
Adult controls	30	37	20-60	-0.02	0.91
Paediatric controls	41	8	0.3-19	-0.2	0.20
Paediatric malignancy	32	13	0.5-19	-0.08	0.66

r= Spearman's correlation
p value, level of significance of the correlation.

5 DISCUSSION

5.1 Experimental failure

There were several reasons why some experiments failed to produce results. These were the poor stimulation and proliferation of T-lymphocytes, a low mitotic index and culture infection. The ability to score 50 metaphases depends on adequate stimulation and proliferation of T-lymphocytes. However, many of the cultures failed to grow adequately especially samples from lung and colorectal carcinoma patients and those from the benign disease group. There are several possible reasons for these failures.

First, the concentrations of T-lymphocytes in the peripheral blood are variable throughout life, and tend to fall with increasing age (Smith *et al.,* 1974; Technical Report Series 260, 1986; Bradley *et al.,* 1996). The incidence of most malignancies increases with age. In order to accrue sufficient numbers of samples, it was necessary to obtain blood samples from patients who were available at the time, irrespective of age. Other conditions, which affect the numbers of circulating T-lymphocytes and which may have had an influence on the proliferative capacity of T-lymphocytes include diet, benign disease and malignancy. A poor diet and indeed cancer cachexia may affect the immune system (Bradley *et al.,* 1996). Major causes of lymphocytopenia include malignancy, collagen vascular diseases and chronic infection. Other causes of lymphocytopenia include acute infection, stressful situations and following the use of some drugs (Boldt 1998). A proportion of the patients, e.g., lung carcinoma patients are likely to have experienced states of acute and chronic infection as a consequence of the malignancy.

Second, previous work has demonstrated marked differences between healthy elderly and young people in the stimulation and proliferation of T-lymphocytes to mitogens such as PHA (Foad *et al.,* 1974; Weksler and Hutteroth 1974; Fernandez *et al.,* 1976; Beckman *et al.,* 1990; Bradley *et al.,* 1996). When the normal donor failure rate of Scorer 1 was analysed for an age effect it was found that the average age of donors for failed samples was 10 years greater than those for samples which yielded data. The mean age of samples which yielded data was 38 years (range= 20-61 years) compared to a mean of 48 years (range =34-84 years) for samples which were unsuccessful. In addition, abundant evidence has accumulated which demonstrates that lymphocyte reactivity to PHA is impaired in patients with chronic lymphocytic leukaemia, Hodgkin's disease and

non-Hodgkin's lymphoma (Sutherland *et al.,* 1971; Anderson *et al.,* 1981). Evidence also exists that the reactivity of lymphocytes from patients with non-lymphoid malignancies is also frequently impaired (Catalona *et al.,* 1973). The defect has been shown in patients with carcinoma of the breast (Keller *et al.,* 1976), lung carcinoma (Ducos *et al.,* 1970; Han and Takita 1972; Gross *et al.,* 1975) and other solid neoplasms (Garrioch *et al.,* 1970; Kaszubowski *et al.,* 1980; Dillman *et al.,* 1984).

The numbers of circulating lymphocytes decrease significantly during the months approaching death (Krant *et al.,* 1968). Many of the blood samples were obtained from patients with advanced stages of disease, who were about to undergo palliative radiotherapy for symptom control. Tancini *et al.* (1990) reported that in solid neoplasms, the mean T helper / suppressor ratio (CD4/CD8) was significantly lower in metastatic cancer patients with respect to that observed either in controls or in patients without metastases. McMillan *et al.,* (1997) also noted that the numbers of CD4+ T lymphocytes were significantly reduced in patients with recurrent colorectal carcinoma as compared to patients with early disease and normal controls. These factors provide some explanation for the failure of cultures to grow adequately. It is likely, therefore, that increasing age associated with reduced numbers of T cells, poor mitogen stimulation coupled with advanced stages of malignancy and often co-morbid disease led to poor yields of metaphase cells, suitable for chromosomal analysis. This is borne out by the high failure rate of many samples obtained from aged normal donors, benign disease group and patients with lung and colorectal carcinoma.

Third, a low mitotic index was another significant cause of experimental failure. Ionising radiation reduced the mitotic index of the irradiated lymphocytes, e.g., by 94± 3% (for the normal donors) which caused difficulties in finding 50 suitable metaphases to score.

Finally, a minor cause of experimental failure was culture infection. A small proportion of experiments (5/166, 3%) became infected and were discarded.

5.2 The effect of therapy on chromosomal radiosensitivity

Blood samples were obtained from patients prior to chemotherapy or radiotherapy. Cloos *et al.* (1994) using the G_2 bleomycin assay noted no influence of chemotherapy or radiotherapy on chromosomal sensitivity. It is possible, however, that prior

chemotherapy or radiotherapy may have an effect on G_2 sensitivity to radiation. In support of this, the cytogenetic response to *in-vitro* radiation has been shown to be modified by previous low-dose exposures to radiation (Sankaranarayan *et al.*, 1989; Cai and Liu 1990). Nevertheless, the effects described are short-lived "adaptive responses" which are unlikely to impact on chromosomal radiosensitivity after *in-vivo* irradiation of cells by therapeutic doses of ionising radiation. However, evidence exists that exposure of cells to high doses of radiation increases chromosomal radiosensitivity (Guedeney *et al.*, 1986; Rigaud *et al.*,1990). This suggests that a persistent memory of carcinogen exposure may lead to modifications in chromosomal radiosensitivity. Therefore to avoid such problems, it was decided to only accept blood samples from patients prior to treatment.

5.3 Adult malignancies

Spontaneous Aberration rates

Spontaneous chromosomal aberrations are a normal feature of cells *in-vivo* and *in-vitro*. Such changes are known to vary in frequency both between and within individuals, and the distribution of spontaneous chromosomal lesions throughout the karyotype seems to be random. Hsu (1989) reported low spontaneous aberration levels for blood cultures from numerous normal individuals (0-3% metaphases with aberrations). The levels of spontaneous aberrations found in this study (Table 3.14) were in broad agreement with a reported mean spontaneous aberration rate of 1.1±0.8/100 cells for normal donors and cancer patients (Terzoudi *et al.*, 1999). The mean spontaneous aberration frequency was lower for the benign disease group and the breast carcinoma group compared to the normal donors, cervical, colorectal and lung carcinomata groups. These differences were statistically significant (Table 3.14). It is uncertain whether these findings represent a real biological effect and there is no obvious biological explanation for this.

G_2 radiosensitivity

The normal group was used as a standard with which other donor groups were compared. The criterion defining sensitivity was the 90th percentile value was taken as the cut-off point for identifying hypersensitivity to G_2 phase irradiation. Using this arbitrary definition, 9% of the normal donors were sensitive to the effects of x-rays (possessing

chromatid aberrations above 100 breaks per 100 metaphase cells). A figure of 9% of normal donors exhibiting sensitivity is similar to the 6% found by Scott *et al.* (1999).

The specificity of the assay for malignancy as opposed to other conditions needed to be determined. It is possible that the use of drugs, immune suppression and the stress of chronic disease may influence the assay findings. Few studies have examined chromosomal radiosensitivity in subjects with benign disorders. If elevated chromosomal radiosensitivity was associated with benign conditions, its value as a marker of cancer predisposition would be diminished. Sanford *et al.,* (1993) detected levels of chromosomal radiosensitivity in patients with Alzheimer's disease similar to those in normal individuals. The work reported in this thesis found no significant difference in the chromosomal radiosensitivity of individuals with benign disease compared with normal donors. The above finding shows that chronic disease does not influence the results of the assay giving confidence that G_2 chromosomal radiosensitivity is specific for cancer predisposition.

A group of breast carcinoma patients was studied in order to see if we could reproduce the findings of Scott *et al.* (1999). Thirty-nine percent of breast carcinoma cases were sensitive in the assay, a result that compares favourably to that obtained by Scott *et al,* (1994a, 1999). As both the findings of the normal donors and the breast cancer patients are similar to that of other workers (Scott *et al.,* 1999), this gives confidence in the credibility of subsequent data. It has been suggested that low penetrant cancer predisposing genes are a cause of breast cancer (Scott *et al.,* 1994a; Scott *et al.,* 1999).The identity of the presumed low penetrant cancer predisposing genes is unknown. It is thought that they are involved in the processing of DNA damage of the type induced by ionising radiation and that mutants or variants are common within the population (Scott *et al.,* 1994a; Parshad *et al.,* 1996; Scott *et al.,* 1999) and occur at high frequencies in breast cancer patients (Scott *et al.,* 1994a; Parshad *et al.,* 1996; Patel *et al.,* 1997; Scott *et al.,* 1999). Microsatellite variants (polymorphisms) associated with XRCC DNA - repair genes are considered to be possible candidates for such genes. Polymorphisms within the XRCC genes confer cellular radiosensitivity. Price *et al.* (1997) found that the frequency of rare microsatellite polymorphisms was high (58%) amongst cancer patients and absent from healthy donors. Minisatellite variants associated with the H-ras-1 proto-oncogene have also been implicated as low-penetrance genes in about 10% of breast

cancer cases; it is believed that these polymorphisms might disrupt the expression of the H-ras-1 gene (Krontiris *et al.*, 1993). The scenario is poorly understood, however, as known mutations in H-ras -1 have little effect on cellular radiosensitivity (Su and Little 1992) or result in radioresistance (Bernhard *et al.*, 1998). It is of considerable surprise that genes that predisopse to breast cancer are involved in DNA repair, as there was no former association of a DNA repair defect in the aetiology of breast cancer.

Fifteen samples were obtained from subjects with a strong family history of breast cancer. These subjects underwent screening for the mutant genes *BRCA1* and *BRCA2*. Nine of the subjects were found to possess the mutant *BRCA1* gene. One of these cases had an induced aberration score of 108 aberrations/ 100 cells and was considered to be sensitive to the assay. Although the median induced aberration score of this small group was similar to that of the normal individuals and lower than that of the breast carcinoma group, the numbers involved were too small to allow firm conclusions to be drawn. The *BRCA1* and *BRCA2* genes are highly penetrant cancer-predisposing genes (Ford *et al.*, 1995; Brugarolas and Jacks 1997). It is of interest to investigate whether chromosomal radiosensitivity is affected by the presence of high-penetrance genes, as opposed to low-penetrance genes. To date there are no reports in the literature of studies of G_2 chromosomal radiosensitivity and carriers of mutant *BRCA1* or *BRCA2* genes. As the *BRCA1* gene product is involved in DNA repair (see Section 1.1.2.1), G_2 chromosomal radiosensitivity might be expected in carriers of the mutant *BRCA1* gene. Although this was not seen, many more cases of *BRCA1* and *BRCA2* patients need to be examined in order to obtain reliable data.

Eleven percent of cervical carcinoma cases were sensitive to the assay, a value similar to that from normal donors. This suggests that low penetrant cancer predisposing genes do not influence the aetiology of this malignancy. The observed finding could be interpreted as highlighting the importance of an environmental aetiology, e.g., HPV infection being largely responsible for its causation.

Thirty percent of colorectal carcinoma cases were found to be sensitive to the assay. It is evident that the colorectal carcinoma group exhibits a degree of chromosomal radiosensitivity that is intermediate between that of normal donors and breast cancer patients. This may represent evidence of low penetrance cancer predisposing genes being implicated in the aetiology of colorectal cancer. In support of this epidemiological

evidence exists of increased rates of colorectal carcinoma in families (Sondergaard *et al.*, 1991; St. John *et al.*, 1993; Fuchs *et al.*, 1994; Slattery and Kerber 1994) and the presumed presence of low penetrance cancer predisposing genes from this investigation adds weight to the previous findings.

Twenty-three percent of lung carcinoma cases were sensitive to the assay, a result that did not reach statistical significance. These findings suggest that a proportion of lung cancer cases may exhibit chromosomal radiosensitivity and that low penetrance genes may be implicated in the aetiology. This contrasts with the commonly held view that the aetiology of lung cancer is mainly environmental, e.g., tobacco smoking (La Vecchia *et al.*, 1991; Levi 1999). However, evidence from epidemiological studies (Ooi *et al.*, 1986; Sellars *et al.*, 1990) suggests that a familial effect is seen in the development of lung cancer and the presence of low penetrance genes could provide an explanation for this observation. It is known that some individuals possess polymorphisms of alleles involved in the metabolism of carcinogens from tobacco smoke (Bartsch *et al.*, 1991; Smith *et al.*, 1994; Fritz *et al.*, 1999) and this is associated with the development of increased rates of lung carcinoma. Polymorphisms of the xenobiotic metabolising enzymes may affect the mutation rates of "cancer genes", and inherited variability in carcinogen metabolism is important in the initial stages of neoplastic transformation (Smith *et al.*, 1994). At present there is no evidence that polymorphisms of genes involved in the metabolism of carcinogens are related to or involved with DNA-repair genes.

The effect of gender, donor age, grade and stage of disease on chromosomal radiosensitivity

Gender did not influence the induced aberration score. No statistically significant differences were observed between the medians of induced scores of males and females in any of the groups, (Tables 3.4, 3.6, 3.11 and 3.13). Attempts were made to obtain blood samples from elderly members of the general public in order to increase the mean age of the normal donor group in accordance with the advanced ages of many of the cancer patients. However, due to problems identified earlier, many of these samples proved to be unsuccessful. No significant differences existed between the age of the patient and the induced aberration score, for any of the groups (Table 3.17). The lack of

gender and age effect on the assay results suggests that these two factors pose no limitation to the assay. Also no significant differences were noted between either the stage of disease or histological grade and induced aberration score for breast, cervical, colorectal and lung carcinomata groups. However, the numbers involved in any subset were small and larger numbers may be required to detect differences.

Mitotic inhibition

The extent of cell cycle radiation-induced delay of lymphocytes irradiated in the G_2 phase was evaluated by the degree of mitotic inhibition. A similar degree of mitotic inhibition was produced in all groups by a dose of 0.5 Gy (Table 3.16). A dose of 0.5 Gy x-rays produced a mean mitotic inhibition (percentage reduction of mitotic index) of 93.9% in normal donors this compares to a figure of 88.1% recorded by Scott *et al.*, (1994b) for normal donors. The mean mitotic inhibition of the breast carcinoma group was significantly higher than that of the controls whilst the mean value for the lung carcinoma group was significantly lower than that of the normals. The differences in mean values are small and may be biologically irrelevant (Table 3.16). When the mitotic inhibition was correlated with induced aberration score a significant weak negative trend was observed for the breast carcinoma group only. Weak negative trends of reduced inhibition with increasing aberration score that were of borderline significance were seen for the cervical and lung carcinoma groups. This inverse correlation was not evident in the normal donor or benign disease groups. The inverse correlation between mitotic inhibition and chromosomal aberrations observed in the cancer groups is suggestive of a defective G_2 checkpoint, which allows less time for repair of chromosomal lesions. Scott *et al.*, (1994b) demonstrated that A-T cells irradiated in the G_2 phase of the cell cycle were less delayed in their progress to mitosis than were normal cells. Further studies (Scott *et al.*, unpublished) noted a negative correlation between chromosome damage and mitotic inhibition for Li-Fraumeni patients but not for breast cancer patients. The above findings are interesting and warrant further study in order to investigate the mechanisms of cell-cycle regulation and its influence on chromosomal radiosensitivity.

5.4 Paediatric malignancies

Spontaneous aberration rates

Similar levels of spontaneous aberrations were detected for the three groups (Table 4.10), though the mean values for normal donors were slighty lower than those seen by scorer 1 and by Terzoudi *et al.* (1999). The mean spontaneous aberration frequency was lower for the paediatric malignancy group compared to the paediatric/adolescent control group and this finding was statistically significant. The lower mean levels of spontaneous aberrations recorded by scorer 2 may be a reflection of the subjective nature of the aberration scoring between the two scorers.

G_2 radiosensitivity

The paediatric/adolescent control group was used as a standard with which the paediatric malignancy group was compared. A group of normal individuals was also studied for control purposes. As the number of individuals in the normal donor group was relatively small it was not possible to define a cut-off point directly as previously described. Using a cut-off level of 87 aberrations/ 100 cells 10% of normal donors were sensitive to the assay that compares well to that obtained by Scott *et al,* (1994a, 1999). The lymphocytes from 15% of paediatric/ adolescent controls were found to exhibit chromosomal radiosensitivity, this contrasts with 44% of lymphocytes obtained from paediatric malignancies. Although scorer 2 obtained a high CV for assay reproducibility which reduces the statistical power of a study so that larger differences in mean values between groups are required in order to detect a significant difference, the results produced for the paediatric control and malignancy groups were statistically significant. This gives one confidence in the credibility of the data and the interpretation that the differences observed are real. The above findings suggest that low penetrance genes are implicated in the aetiology of paediatric malignancies. This supports the finding from an early epidemiological study (Thompson *et al.,* 1987) which suggested that an increased cancer risk was found in the mothers of children with malignancy. However, later epidemiolodical studies (Burke *et al.,* 1991; Moutou *et al.,* 1994; Olsen *et al.,* 1995) found no increased cancer risk amongst relatives of childhood cancer cases. Finally, unlike the adult malignancies studied, no significant trend was observed between mitotic inhibition and chromosomal radiosensitivity for any of the three groups.

Gender did not appear to influence the induced aberration score for any of the three groups studied. No significant differences existed between the age of the patients and the induced aberration score, for any of the individual groups. In order to examine further the influence of donor age on induced aberration scores, the normal donors and paediatric/adolescent controls were pooled. The ages of the control subjects studied ranged from 3 months to 60 years using Spearman's correlation, no relationship was seen between donor age and chromosomal radiosensitivity (r=0.04, p=0.76, Figure 5.1).

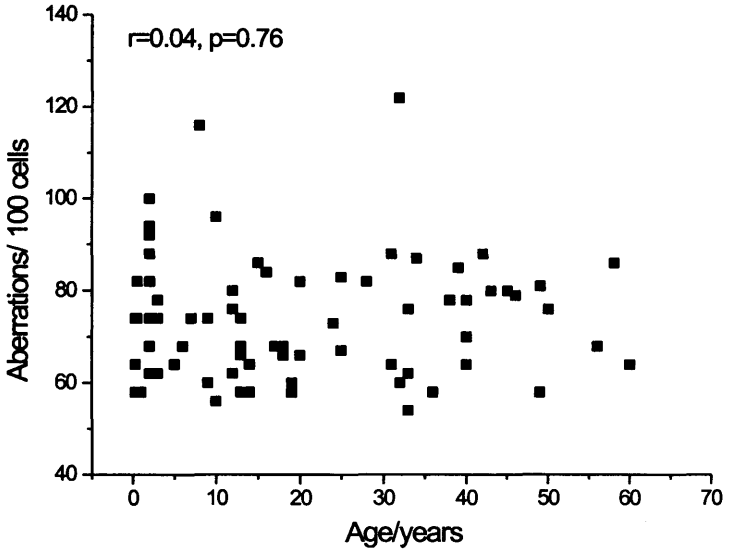

Figure 5.1 Aberrations/ 100 cells versus donor age.

5.5 Evaluation of the assay

The culturing of peripheral T-lymphocytes and subsequent scoring of metaphase chromosomes is a labour intensive and time-consuming process. Cell cultures must to be set up within 24 hours of venepuncture so much of the workload is determined by the timing of patient clinics and consultations. Considerable expertise is required to produce good, consistent results and the culturing of blood samples from poor performance status groups, e.g., lung and bowel carcinoma is often difficult. The scoring of chromatid

aberrations is labour intensive, subjective in nature and poorly reproducible between experimentors and two independent scorers are unlikely to obtain identical scores for the same slide. However, despite these limitations it is possible with experience to obtain reproducible results amongst normal donors and to detect significant differences between groups of normal individuals and cancer patients. As demonstrated in this thesis, the results of the assay are not influenced by donor gender, age or chronic benign disease.

5.6 Statistical methodology

The data obtained (i.e. induced aberration scores) were numerical and continous. The distribution of the scores was not normal. In order to analyse the data adequetely one needs to employ a non-parametric test which does not make any assumptions about the distributions of the values, only their relative rank order, such as the Mann-Whitney U test. The Mann-Whitney U test is the non-parametric equivalent of the two- sample t-test, useful for comparing data from two independent groups. This test is a significance test which tests a hypothesis; e.g. that the means or distribution of values from one group of observations is the same as that of another population. The Fisher's exact test is used to analyse categorical data, such as the proportion of cases that were considered to be sensitive after using an arbitary cut-off point.

The Fisher's exact test is an exact form of the conventional Chi-squared test and can be trusted even when the observed frequencies are low. It tests the null hypothesis that the relative frequencies observed in the groups are the same. A p- value is produced which indicates the probability of observed values arising by chance; if the total probability is small (<0.05), the data are inconsistent with the null hypothesis.

Table 3.15 indicates the induced aberration score/ 100 cells in normal donors and patients. The levels of statistical significance obtained are different, when the Mann-Whitney U and Fisher's exact tests are used. Considering the breast and colorectal groups, the Fisher's exact test produced "significant" levels of statistical significance e.g. 0.001 for breast and 0.01 for colorectal carcinoma, whilst the Mann-Whitney U test did not.

An arbitrary cut-off point was chosen at the 90th percentile for individuals (100 aberrations/100 cells), 9% of normal donors were radiosensitive. Applying this cut-off point to other groups revealed that 38.9% and 29.7% of samples from breast and

colorectal malignancy patients respectively were sensitive. By analysing the "percentage or proportion" of sensitive cases by using the Fisher's exact test, the differences detected between the groups were larger for breast and colorectal malignancies compared to normals. This is reflected in the size of the p-value and thus the null hypothesis is rejected.

Therefore the difference between the two tests for the level of induced aberration scores for breast and colorectal malignancies is marked. The reason lies in the broad distribution of induced aberration scores. There were a high proportion of "sensitive" individuals, but also a large number of individuals with sensitivities below the mean for normal donors. These low scores bring down the overall group median and thus the Mann-Whitney U test is not significant.

Interpretation of hypothesis tests and p-values

The p-value is the probability of obtaining the observed effect (or a more unlikely one) when the null hypothesis is true. The p-value assesses how likely it is to observe such an effect in a sample when there is no such difference in the population. Statistical significance should not be used as the *sole* basis of the interpretation. One must not consider that any significant effect is real and any non-significant effect is taken as having " no difference."

Spearman's correlation coefficient was used to measure the degree of association between continous variables such as induced aberration score and donor age. The standard method leads to a quantity called "r" which can take any value from -1.0 to +1.0. The correlation coefficient r measures the degree of "straight-line" association between the values of the two variables. A value of +1.0 or -1.0 is obtained if all the points in a scatter diagram lie on a perfect straight line. The correlation between two variables is positive if higher values of one variable are associated with higher values of the other and negative if one variable tends to be lower as the other gets higher. A correlation of zero indicates no linear relation of the two variables.

5.7 Future studies

In view of the above comments, the G_2 chromosomal assay is unlikely to have a role in routine clinical use, as a predictor of cancer predisposition. However, applications do exist in a research setting. It would be prudent to confirm the study findings especially

for the colorectal, lung and paediatric malignancy groups using larger numbers of patients. This would provide a greater degree of confidence in the interpretation of subsequent data. It would be interesting to extend the study to include other malignancies such as endometrial, prostate and testicular carcinoma. Endometrial carcinoma has similar hormonal risk factors as breast carcinoma and relatively large numbers of patients are readily available in the clinic. Evidence is accumulating that some cases of prostatic carcinoma have an inherited basis (Goldgar *et al.,* 1994; Eeles 1998; Hemminki and Vaittinen 1999; Wadelius *et al.,* 1999). Whilst the aetiology of testicular malignancy is to date uncertain, the incidence of the disease is increasing (Horwich *et al.,* 1995) and it would be possible to obtain relatively large numbers of samples from good performance status patients with considerable ease.

Another avenue to develop would be that of heritability studies. Roberts *et al.* (1999) has demonstrated that chromosomal radiosensitivity is an inherited phenotype amongst breast cancer patients. It would be interesting to investigate the presence of heritability amongst the relatives of "sensitive" colorectal and paediatric malignancy patients, especially as evidence exists for an increased risk of colorectal cancer development amongst relatives of colorectal cancer patients (St. John *et al.,* 1993; Fuchs *et al.,* 1994; Slattery and Kerber 1994).

It would also be interesting to investigate whether chromosomal radiosensitivity is associated with pre-invasive malignant states as opposed to frank invasive disease. To date there has been only one small study addressing this area of research. Parshad *et al,* (1996) studied the chromomal radiosensitivity of lymphocytes from 8 patients with preinvasive breast lesions, one of whom exhibited frequencies of chromatid breaks 2-3 fold higher than of normal donors. Groups of patients with benign rectal polyps and *in-situ* breast cancer would be suitable candidates for study as large numbers of such patients are accessible in the clinic and the majority of patients are in reasonably good health. Should evidence of chromosomal radiosensitivity be found in these groups this could help in the understanding of the carcinogenesis of such diseases.

In theory, prospective cohort studies are a means of validating whether chromosomal radiosensitivity is a biomarker of cancer predisposition. Considerable time and resources would be needed to carry out such a longterm study, however, the findings could provide convincing evidence that chromosomal radiosensitivity is associated with

the causation of malignancy. The ultimate aim is to identify the underlying gene defects. Linkage analysis has been used to locate genes in which the incidence of disease is high. It is possible that linkage analysis could reveal the chromosomal location of low-penetrance cancer-predisposing genes by identifying polymorphic genetic markers of known location that are co-inherited with the disease in families. Another possibility is to investigate how chromosomal radiosensitivity is related to the processing of DNA damage repair and its involvement with cell cycle checkpoints.

5.8 Conclusion

In conclusion, the hypothesis has been confirmed that malignancies associated with a genetic aetiology for instance breast, colorectal and paediatric/ adolescent malignancies are assocociated with chromosomal radiosensitivity, whilst those linked to an environmental aetiology such as cervical carcinoma are not. It was not possible to confirm the hypothesis for lung carcinoma. Benign disease did not appear to influence chromosomal radiosensitivity and therefore chromosomal radiosensitivity was considered to be specific for cancer predisposition. A weak inverse correlation was observed for mitotic inhibition and induced aberration score for breast cancer patients, suggestive of a defective G_2 checkpoint.

6 APPENDICIES

APPENDIX 1. RESULTS OF G2 ASSAY.

Expt.	Sample	Sex	Age	Diagnosis	Score/ 100cells
DHY/1	98/25	F	40	Normal donor	100
DHZ/2	98/31	F	49	Normal donor	78
DHZ/2	98/33	M	15	Ewing's sarcoma	112
DHZ/2	98/35	M	18	Osteosarcoma	88
DIB/2	98/45	F	1	Rhabdomyosarcoma	76
DIB/2	98/47	M	28	Normal donor	76
DIC/1	98/50	F	34	Normal donor	failed culture
DIE/1	98/56	F	83	Cervical carcinoma	88
DIE/1	98/57	F	49	Cervical carcinoma	76
DIE/1	98/60	F	60	Normal donor	low mitotic index
DIF/1	98/58	F	78	Colorectal carcinoma	failed culture
DIF/1	98/59	M	15	Osteosarcoma	failed culture
DIF/1	98/60	F	49	Normal donor	failed culture
DIG/1	98/71	F	45	Normal donor	culture infected
DIG/1	98/72	F	72	Cervical carcinoma	culture infected
DIG/1	98/73	F	75	Colorectal carcinoma	culture infected
DIH/2	98/83	F	45	Normal donor	80
DIH/2	98/84	F	13	Ovarian teratoma	66
DII/1	98/86	M	45	Colorectal carcinoma	low mitotic index
DII/1	98/87	F	46	Normal donor	86
DII/1	98/88	F	77	Cervical carcinoma	96
DII/1	98/99	M	75	Colorectal carcinoma	low mitotic index

DIK/1	98/101	M	58	Normal donor	82
DIK/1	98/102	M	88	Colorectal carcinoma	low mitotic index
DIK/1	98/103	M	75	Colorectal carcinoma	80
DIK/1	98/104	M	60	Colorectal carcinoma	120
DIK/2	98/101	M	58	Normal donor	86
DIK/2	98/105	F	9	Medulloblastoma	74
DIL/1	98/109	F	25	Normal donor	culture infected
DIL/1	98/110	M	60	Lung carcinoma	culture infected
DIM/1	98/115	M	55	Lung carcinoma	118
DIM/1	98/116	M	75	Lung carcinoma	low mitotic index
DIM/1	98/117	M	75	Lung carcinoma	100
DIM/1	98/118	F	49	Normal donor	94
DIM/2	98/114	M	18	Osteosarcoma	66
DIM/2	98/118	F	49	Normal donor	98
DIN/2	98/119	F	18	Osteosarcoma	72
DIN/2	98/120	M	28	Normal donor	104
DIO/1	98/123	M	60	Normal donor	84
DIP/1	98/145	F	52	Benign disease	86
DIP/1	98/146	F	75	Diabetic	74
DIP/1	98/147	M	71	Diabetic	62
DIP/1	98/148	F	52	Diabetic	92
DIP/1	98/149	F	39	Diabetic	78
DIP/2	98/144	M	19	Pineal germinoma	52
DIQ/1	98/150	F	48	Diabetic	86
DIQ/1	98/151	M	46	Diabetic	low mitotic index
DIQ/1	98/152	F	76	Diabetic	low mitotic index

DIQ/1	98/153	M	61	Diabetic	68
DIQ/1	98/154	M	62	Diabetic	76
DIQ/1	98/155	F	57	Diabetic	76
DIR/1	98/157	M	46	Colorectal carcinoma	68
DIR/1	98/158	F	49	Normal donor	80
DIS/1	98/160	F	56	Benign disease	90
DIS/1	98/161	M	81	Lung carcinoma	failed culture
DIS/1	98/162	M	78	Lung carcinoma	failed culture
DIS/1	98/163	F	72	Lung carcinoma	failed culture
DIS/1	98/164	M	65	Benign disease	failed culture
DIT/2	98/166	F	25	Normal donor	72
DIT/2	98/167	M	55	Colorectal carcinoma	low mitotic index
DIT/2	98/168	F	13	Hodgkin's disease	100
DIU/1	98/173	M	81	Lung carcinoma	failed culture
DIU/1	98/174	F	61	Benign disease	106
DIU/1	98/175	M	65	Lung carcinoma	failed culture
DIU/1	98/176	F	77	Benign disease	98
DIU/1	98/180	F	80	Diabetic	failed culture
DIU/1	98/185	M	58	Normal donor	84
DIV/2	98/188	M	28	Normal donor	86
DIV/2	98/189	F	17	Osteosarcoma	failed culture
DIV/2	98/190	M	15	Osteosarcoma	84
DIV/2	98/191	F	13	Hodgkin's disease	74
DIW/1	98/192	F	54	Diabetic	66
DIW/1	98/194	M	71	Diabetic	failed culture
DIW/1	98/195	M	65	Diabetic	failed culture

DIW/1	98/196	F	55	Benign disease	60
DIX/1	98/197	M	71	Lung carcinoma	low mitotic index
DIX/1	98/198	F	52	Lung carcinoma	failed culture
DIX/1	98/199	M	74	Lung carcinoma	failed culture
DIX/1	98/200	M	71	Lung carcinoma	failed culture
DIX/1	98/201	F	45	Normal donor	106
DIZ/2	98/207	F	24	Normal donor	76
DIZ/2	98/208	M	19	Osteosarcoma	98
DJB/1	98/214	F	78	Diabetic	100
DJB/1	98/215	F	41	Diabetic	78
DJB/1	98/216	M	61	Diabetic	92
DJB/1	98/218	M	72	Diabetic	98
DJB/1	98/219	M	61	Diabetic	92
DJC/2	98/229	F	17	Osteosarcoma	108
DJC/2	98/230	F	17	Hodgkin's disease	failed culture
DJC/2	98/231	F	25	Normal donor	68
DJC/1	98/231	F	25	Normal donor	80
DJC/1	98/232	F	57	Cervical carcinoma	98
DJD/2	98/234	F	11	Rhabdomyosarcoma	68
DJD/2	98/235	M	15	Hodgkin's disease	failed culture
DJD/2	98/239	M	28	Normal donor	74
DJE/1	98/252	M	73	Colorectal carcinoma	88
DJE/1	98/253	M	28	Normal donor	86
DJG/1	98/254	F	40	Normal donor	96
DJG/1	98/256	M	71	Diabetic	72
DJG/1	98/257	M	?	Lung carcinoma	failed culture

DJG/1	98/258	M	50	Benign disease	failed culture
DJG/1	98/259	F	54	Lung carcinoma	failed culture
DJI/1	98/266	M	82	Diabetic	70
DJI/1	98/267	F	60	Diabetic	84
DJI/1	98/268	F	82	Diabetic	82
DJI/1	98/269	M	63	Lung carcinoma	failed culture
DJI/1	98/270	M	82	Benign disease	70
DJI/1	98/271	F	46	Normal donor	76
DJK/1	98/279	F	49	Normal donor	86
DJK/1	98/280	M	81	Benign disease	100
DJK/1	98/281	F	48	Benign disease	102
DJM/1	98/284	M	28	Normal donor	84
DJM/1	98/291	M	67	Benign disease	82
DJM/1	98/292	M	41	Benign disease	90
DJM/1	98/293	F	70	Lung carcinoma	100
DJN/1	98/284	M	28	Normal donor	114
DJN/1	98/294	F	69	Diabetic	failed culture
DJN/1	98/295	M	64	Diabetic	failed culture
DJN/1	98/296	F	59	Diabetic	failed culture
DJN/1	98/297	M	67	Diabetic	failed culture
DJN/1	98/298	M	51	Diabetic	failed culture
DJR/1	98/307	M	21	Benign disease	72
DJR/1	98/308	M	60	Benign disease	low mitotic index
DJR/1	98/312	M	27	Normal donor	78
DJS/1	98/309	M	32	Diabetic	failed culture
DJS/1	98/310	M	36	Diabetic	100

DJS/1	98/312	M	27	Normal donor	110
DJW/1	98/327	M	60	Benign disease	failed culture
DJW/1	98/328	M	95	Lung carcinoma	failed culture
DJW/1	98/329	F	45	Normal donor	failed culture
DJZ/2	98/335	F	40	Normal donor	82
DJZ/2	98/336	M	16	High grade lymphoma	122
DJZ/2	98/337	M	15	Osteosarcoma	90
DKA/1	98/335	F	40	Normal donor	low mitotic index
DKA/1	98/339	F	64	Normal donor	low mitotic index
DKA/1	98/340	M	67	Normal donor	low mitotic index
DKA/1	98/342	F	34	Normal donor	low mitotic index
DKB/1	98/347	M	28	Normal donor	102
DKB/1	98/348	F	66	Colorectal carcinoma	126
DKB/1	98/349	F	82	Colorectal carcinoma	122
DKC/1	98/350	F	31	Normal donor	96
DKC/1	98/351	M	68	Lung carcinoma	failed culture
DKC/1	98/352	F	55	Benign disease	failed culture
DKC/1	98/354	F	68	Benign disease	failed culture
DKC/1	98/355	F	51	Normal donor	failed culture
DKC/1	98/356	F	69	Normal donor	failed culture
DKC/1	98/357	F	84	Normal donor	failed culture
DKD/1	98/350	F	31	Normal donor	low mitotic index
DKD/1	98/358	M	62	Normal donor	low mitotic index
DKD/1	98/359	F	62	Normal donor	failed culture
DKD/1	98/360	F	46	Normal donor	low mitotic index
DKD/1	98/361	F	53	Normal donor	72

DKD/1	98/362	F	49	Normal donor	low mitotic index
DKD/1	98/363	M	68	Normal donor	failed culture
DKE/1	98/366	F	39	Normal donor	low mitotic index
DKE/1	98/367	M	39	Normal donor	90
DKE/1	98/368	M	67	Normal donor	low mitotic index
DKE/1	98/369	M	22	Normal donor	72
DKE/1	98/370	F	45	Normal donor	low mitotic index
DKG/1	98/377	M	30	Normal donor	low mitotic index
DKG/1	98/381	M	56	Normal donor	failed culture
DKG/1	98/383	F	36	Normal donor	84
DKG/1	98/384	F	57	Normal donor	failed culture
DKG/1	98/385	F	50	Normal donor	low mitotic index
DKG/1	98/386	F	43	Normal donor	low mitotic index
DKH/1	98/377	M	30	Normal donor	80
DKH/1	98/378	F	68	Benign disease	failed culture
DKH/1	98/379	M	45	Benign disease	failed culture
DKH/1	98/380	M	37	Normal donor	78
DKH/1	98/382	F	78	Normal donor	failed culture
DKI/1	98/388	M	30	Normal donor	low mitotic index
DKI/1	98/389	M	65	Colorectal carcinoma	low mitotic index
DKJ/1	98/392	M	35	Normal donor	low mitotic index
DKJ/1	98/395	M	35	Normal donor	low mitotic index
DKJ/1	98/396	F	37	Normal donor	low mitotic index
DKJ/1	98/397	M	39	Normal donor	low mitotic index
DKJ/1	98/398	F	36	Normal donor	low mitotic index
DKK/2	98/391	M	20	Intracranial tumour	failed culture

DKK/2	98/392	M	35	Normal donor	failed culture
DKK/2	98/393	M	54	Normal donor	failed culture
DKK/2	98/394	F	33	Normal donor	failed culture
DKL/1	98/399	F	46	Normal donor	76
DKl/1	98/407	M	16	Rhabdomyosarcoma	failed culture
DKM/1	98/413	M	25	Normal donor	86
DKM/1	98/414	F	50	Normal donor	106
DKM/1	98/415	F	34	Normal donor	80
DKN/2	98/426	M	11	Rhabdomyosarcoma	98
DKN/2	98/427	M	28	Normal donor	68
DKO/2	98/432	F	15	Hodgkin's disease	106
DKO/2	98/431	M	34	Normal donor	86
DKP/1	98/434	F	24	Normal donor	low mitotic index
DKP/1	98/435	F	59	Lung carcinoma	failed culture
DKP/1	98/436	M	78	Lung carcinoma	failed culture
DKP/1	98/437	M	51	Benign disease	failed culture
DKQ/1	98/455	M	72	Benign disease	106
DKQ/1	98/456	F	72	Lung carcinoma	failed culture
DKQ/1	98/457	M	28	Normal donor	74
DKU/1	98/463	F	32	Normal donor	86
DKU/1	98/464	M	47	Benign disease	86
DKU/1	98/465	M	77	Benign disease	100
DKU/1	98/466	M	60	Lung carcinoma	122
DKS/2	98/458	F	40	Normal donor	82
DKS/2	98/459	M	34	Normal donor	90
DKS/2	98/460	F	49	Normal donor	88

DKX/1	98/470	M	58	Normal donor	100
DKX/1	98/471	M	25	Normal donor	108
DKX/1	98/472	F	30	Normal donor	100
DKY/1	98/482	M	60	Normal donor	82
DKY/1	98/483	F	51	Benign disease	low mitotic index
DKY/1	98/484	F	74	Lung carcinoma	low mitotic index
DKY/1	98/485	M	60	Lung carcinoma	low mitotic index
DKZ/1	98/487	F	49	Normal donor	82
DKZ/1	98/489	F	80	Colorectal carcinoma	failed culture
DLA/1	98/493	M	58	Normal donor	low mitotic index
DLA/1	98/494	M	65	Lung carcinoma	failed culture
DLA/1	98/495	M	63	Lung carcinoma	low mitotic index
DLA/1	98/496	M	67	Lung carcinoma	94
DLA/1	98/497	M	60	Benign disease	112
DLD/1	98/508	F	24	Normal donor	88
DLD/1	98/509	M	48	Benign disease	88
DLD/1	98/510	M	78	Lung carcinoma	low mitotic index
DLD/1	98/511	F	68	Benign disease	failed culture
DLE/2	98/508	F	24	Normal donor	70
DLE/2	98/512	M	40	Benign disease	failed culture
DLE/2	98/513	M	1.5	Ependymoma	106
DLE/2	98/514	M	5mth	Rhabdomyosarcoma	78
DLF/1	98/515	F	17	Hodgkin's disease	failed culture
DLF/1	98/516	F	39	Normal donor	88
DLH/2	98/517	F	46	Normal donor	68
DLH/2	98/518	F	8	Osteosarcoma	76

DLI/1	98/521	M	33	Normal donor	82
DLI/1	98/522	M	42	Normal donor	88
DLI/1	98/523	F	31	Normal donor	88
DLJ/2	98/521	M	33	Normal donor	62
DLJ/2	98/522	M	42	Normal donor	88
DLJ/2	98/523	F	31	Normal donor	88
DLM/2	98/536	F	44	Normal donor	70
DLM/2	98/537	F	40	Normal donor	86
DLN/2	98/539	F	13	Hodgkin's disease	84
DLN/2	98/540	F	25	Normal donor	94
DLO/2	98/543	M	49	Normal donor	58
DLO/2	98/544	F	33	Normal donor	54
DLP/2	98/547	F	24	Normal donor	72
DLP/2	98/548	M	72	Rhabdomyosarcoma	64
DLQ/1	98/549	F	40	Normal donor	96
DLQ/1	98/550	F	83	Cervical carcinoma	failed culture
DLQ/1	98/551	F	65	Cervical carcinoma	108
DLR/2	98/555	F	68	Cervical carcinoma	failed culture
DLR/2	98/556	F	49	Normal donor	70
DLS/2	98/556	F	49	Normal donor	84
DLS/2	98/557	F	9	Paediatric non-cancer	74
DLS/2	98/558	F	2	Paediatric non-cancer	94
DLT/1	98/559	M	25	Normal donor	82
DLT/1	98/560	M	13	PNET	failed culture
DLU/1	98/561	F	3	Neuroblastoma	failed culture
DLU/1	98/562	F	25	Normal donor	70

DLU/1	98/563	F	64	Cervical carcinoma	failed culture
DLV/1	98/562	F	25	Normal donor	82
DLV/1	98/566	F	7	Rhabdomyosarcoma	clotted sample
DLV/1	98/567	F	47	Cervical carcinoma	64
DLX/2	98/573	M	17	Hodgkin's disease	74
DLX/2	98/575	F	31	Normal donor	68
DLY/2	98/576	M	34	Normal donor	84
DLY/2	98/580	M	1	Paediatric non-cancer	58
DLY/2	98/581	M	4mth	Paediatric non-cancer	64
DLY/2	98/582	M	1	B Cell lymphoma	76
DLY/2	98/583	M	9	Large cell lymphoma	118
DLZ/1	98/568	F	48	Breast carcinoma	92
DLZ/1	98/569	F	36	Breast carcinoma	64
DLZ/1	98/570	M	60	Normal donor	86
DLZ/1	98/571	F	49	Normal donor	86
DMA/1	98/585	M	58	Normal donor	90
DMA/1	98/586	F	64	Breast carcinoma	132
DMA/1	98/587	F	54	Breast carcinoma	154
DMA/1	98/588	F	40	Cervical carcinoma	108
DMA/1	98/589	F	62	Cervical carcinoma	94
DMB/1	98/591	F	39	Normal donor	80
DMB/1	98/592	M	54	Colorectal carcinoma	94
DMB/2	98/590	M	16	Osteosarcoma	62
DMB/2	98/591	F	39	Normal donor	60
DMC/2	98/591	F	39	Normal donor	72
DMC/2	98/594	M	11	Paediatric non-cancer	failed culture

DMF/1	98/596	F	55	Breast carcinoma	112
DMF/1	98/597	F	75	Breast carcinoma	failed culture
DMF/1	98/598	F	57	Breast carcinoma	92
DMF/1	98/599	F	65	Breast carcinoma	140
DMF/1	98/600	F	68	Breast carcinoma	failed culture
DMF/1	98/601	F	34	Normal donor	82
DMG/1	98/595	F	40	Cervical carcinoma	low mitotic index
DMG/1	98/601	F	34	Normal donor	low mitotic index
DMG/1	98/606	F	57	Cervical carcinoma	106
DMG/1	98/607	M	61	Normal donor	64
DMH/1	98/613	M	5	Neuroblastoma	failed culture
DMH/1	98/616	M	52	Normal donor	82
DML/1	98/619	F	54	Breast carcinoma	106
DML/1	98/620	F	50	Breast carcinoma	104
DML/1	98/621	F	72	Breast carcinoma	low mitotic index
DML/1	98/622	F	68	Breast carcinoma	low mitotic index
DML/1	98/623	F	41	Breast carcinoma	88
DML/1	98/628	F	61	Normal donor	84
DMM/1	98/628	F	61	Normal donor	88
DMM/1	98/624	F	45	Cervical carcinoma	80
DMM/1	98/625	F	38	Cervical carcinoma	90
DMM/1	98/632	F	44	Cervical carcinoma	62
DMM/1	98/633	F	65	Lung carcinoma	failed culture
DMN/1	98/637	M	51	Normal donor	72
DMN/1	98/639	F	73	Colorectal carcinoma	failed culture
DMN/1	98/640	F	75	Breast carcinoma failed	failed culture

DMN/1	98/641	F	54	Breast carcinoma	106
DMN/1	98/642	F	60	Breast carcinoma	failed culture
DMN/2	98/643	F	12	Paediatric non-cancer	76
DMO/1	98/644	F	61	Breast carcinoma	culture infected
DMO/1	98/645	F	53	Cervical carcinoma	culture infected
DMO/1	98/646	F	48	Breast carcinoma	culture infected
DMO/1	98/647	F	52	Breast carcinoma	culture infected
DMO/1	98/648	F	22	Normal donor	culture infected
DMP/1	98/650	F	63	Cervical carcinoma	74
DMP/1	98/651	F	40	Normal donor	78
DMQ/1	99/1	M	34	Normal donor	102
DMQ/1	99/2	M	53	Colorectal carcinoma	122
DMR/2	99/3	F	49	Normal donor	80
DMR/2	99/5	M	7mth	Paediatric non-cancer	82
DMS/1	99/6	F	52	Breast carcinoma	80
DMS/1	99/7	F	59	Breast carcinoma	76
DMS/1	99/8	F	57	Breast carcinoma	96
DMS/1	99/9	F	53	Breast carcinoma	78
DMS/1	99/10	F	52	Cervical carcinoma	failed culture
DMS/1	99/11	M	32	Normal donor	72
DMT/2	99/11	M	32	Normal donor	122
DMT/2	99/12	M	15	Lymphoma (NHL)	92
DMU/1	99/13	F	39	Normal donor	86
DMU/1	99/14	F	78	Cervical carcinoma	70
DMV/2	99/43	F	46	Normal donor	84
DMV/2	99/44	M	2	Paediatric non-cancer	88

DMV/2	99/45	M	2	Paediatric non-cancer	100
DMV/2	99/46	M	2mth	Paediatric non-cancer	sample clotted
DMW2	99/43	F	46	Normal donor	84
DMW2	99/48	M	9	Clear cell sarcoma	failed culture
DMW	99/49	F	15	Paediatric non-cancer	86
DMX/1	99/57	F	47	Breast carcinoma	72
DMX/1	99/58	F	38	Breast carcinoma	122
DMX/1	99/59	F	56	Breast carcinoma	64
DMX/1	99/60	F	24	Normal donor	low mitotic index
DMY/1	99/60	F	24	Normal donor	76
DMY/1	99/62	F	?	BRCA genes absent	84
DMY/1	99/63	F	44	BRCA genes absent	100
DNA/1	99/64	F	60	Normal donor	86
DNA/1	99/66	M	65	Normal donor	low mitotic index
DNA/1	99/67	F	32	BRCA Predictive test	failed culture
DNB/2	99/64	F	60	Normal donor	64
DNB/2	99/68	M	2	Paediatric non-cancer	92
DNB/2	99/69	M	8	Paediatric non-cancer	116
DNB/2	99/70	M	3mth	Paediatric non-cancer	58
DNB/2	99/71	M	3	Paediatric non-cancer	62
DNB/2	99/73	M	20	Osteosarcoma	sample clotted
DNC/1	99/77	F	52	Breast carcinoma	culture infected
DNC/1	99/78	F	67	Breast carcinoma	culture infected
DNC/1	99/79	F	47	Breast carcinoma	culture infected
DNC/1	99/80	F	32	Normal donor	culture infected
DND/1	99/80	F	32	Normal donor	82

DND/1	99/82	F	75	Cervical carcinoma	failed culture
DND/1	99/80	F	32	Normal donor	82
DND/1	99/82	F	75	Cervical carcinoma	failed culture
DND/1	99/83	F	33	Cervical carcinoma	62
DND/1	99/84	F	68	Cervical carcinoma	72
DNE/2	99/87	F	25	Normal donor	78
DNE/2	99/90	F	10	Paediatric non-cancer	96
DNF/2	99/91	F	39	Normal donor	124
DNF/2	99/92	F	3	Wilm's tumour	failed culture
DNH/1	99/93	F	40	Cervical carcinoma	72
DNH/1	99/94	F	44	Normal donor	82
DNH/1	99/95	M	37	BRCA 1 gene carrier	82
DNJ/2	99/99	M	40	Normal donor	64
DNJ/2	99/100	F	3	Paediatric non-cancer	74
DNK/1	99/101	F	51	Breast carcinoma	88
DNK/1	99/102	F	43	Normal donor	94
DNK/2	99/102	F	43	Normal donor	80
DNK/2	99/103	F	10	Paediatric non-cancer	56
DNK/2	99/104	M	12	Paediatric non-cancer	80
DNK/2	99/105	F	13	Paediatric non-cancer	58
DNK/2	99/106	F	13	Paediatric non-cancer	68
DNL/2	99/113	M	14	Paediatric non-cancer	58
DNL/2	99/114	F	12	Paediatric non-cancer	62
DNL/2	99/115	F	25	Normal donor	62
DNL/1	99/115	F	25	Normal donor	88
DNL/1	99/116	F	57	Cervical carcinoma	88

DNM/2	99/115	F	25	Normal donor	72
DNM/2	99/118	M	10	Hodgkin's disease	90
DNN/1	99/119	F	72	Breast carcinoma	92
DNN/1	99/120	F	51	Breast carcinoma	144
DNN/1	99/121	F	27	Normal donor	98
DNO/1	99/121	F	27	Normal donor	90
DNO/1	99/122	F	34	Cervical carcinoma	100
DNO/1	99/123	F	73	Breast carcinoma	70
DNO/1	99/124	F	82	Cervical carcinoma	74
DNP/1	99/125	M	14	Paediatric non-cancer	64
DNP/2	99/126	M	9	Paediatric non-cancer	60
DNP/2	99/127	F	40	Normal donor	64
DNQ/2	99/127	F	40	Normal donor	76
DNQ/2	99/128	M	16	Paediatric non-cancer	84
DNR/1	99/131	F	55	Cervical carcinoma	92
DNR/1	99/132	F	39	Normal donor	76
DNS/1	99/136	M	25	Normal donor	92
DNS/1	99/137	F	78	Cervical carcinoma	failed culture
DNT/1	99/139	M	32	Normal donor	98
DNT/1	99/140	F	56	Breast carcinoma	74
DNT/1	99/141	F	60	Breast carcinoma	90
DNT/1	99/142	F	57	Breast carcinoma	108
DNU/1	99/150	M	54	Normal donor	low mitotic index
DNU/1	99/151	F	56	Breast carcinoma	84
DNU/1	99/152	F	79	Breast carcinoma	62
DNV/2	99/155	F	20	Normal donor	66

DNV/2	99/156	F	17	Paediatric non-cancer	68
DNV/2	99/157	M	19	Young adult, normal	60
DNV/2	99/158	F	38	Normal donor	78
DNV/2	99/159	M	19	Young adult, normal	66
DNV/2	99/160	F	18	Young adult, normal	58
DNX/1	99/164	F	48	BRCA genes absent	126
DNX/1	99/165	F	62	Breast carcinoma	88
DNX/1	99/166	F	63	Breast carcinoma	116
DNX/1	99/167	F	45	Normal donor	98
DNY/1	99/167	F	45	Normal donor	98
DNY/1	99/169	M	70	Lung carcinoma	failed culture
DNY/1	99/170	M	87	Lung carcinoma	failed culture
DNY/1	99/171	F	68	Lung carcinoma	failed culture
DNY/1	99/172	F	70	Lung carcinoma	failed culture
DNZ/2	99/178	M	20	Normal donor	82
DNZ/2	99/179	M	18	Young adult, normal	68
DNZ/2	99/180	M	36	Normal donor	58
DOA/1	99/181	F	40	Normal donor	72
DOA/1	99/182	F	62	Breast carcinoma	low mitotic index
DOA/1	99/183	F	66	Breast carcinoma	90
DOA/1	99/184	F	57	Breast carcinoma	106
DOA/1	99/185	F	66	Breast carcinoma	low mitotic index
DOA/1	99/186	M	56	Breast carcinoma	low mitotic index
DOB/1	99/181	F	40	Normal donor	86
DOB/2	99/187	M	75	Lung carcinoma	failed culture
DOB/2	99/188	M	66	Lung carcinoma	70

DOD/1	99/190	F	82	Colorectal carcinoma	80
DOD/1	99/192	F	33	Normal donor	98
DOE/2	99/192	F	33	Normal donor	76
DOE/2	99/193	F	6	Paediatric non-cancer	68
DOE/2	99/194	F	5mth	Paediatric non-cancer	74
DOF/1	99/195	F	52	BRCA genes absent	68
DOF/1	99/196	F	22	Normal donor	82
DOG/1	99/197	F	53	Normal donor	82
DOG/1	99/198	F	84	Lung carcinoma	low mitotic index
DOG/1	99/199	M	70	Lung carcinoma	70
DOG/2	99/197	F	53	Normal donor	failed culture
DOG/2	99/200	M	13	Paediatric non-cancer	74
DOG/2	99/201	M	2	Paediatric non-cancer	82
DOH/1	99/197	F	53	Normal donor	86
DOH/1	99/202	F	?	BRCA genes absent	low mitotic index
DOH/1	99/203	F	41	BRCA 1 gene carrier	72
DOH/1	99/204	F	35	BRCA 1 gene carrier	66
DOI/2	99/209	F	49	Normal donor	66
DOI/2	99/210	F	7	Paediatric non-cancer	74
DOI/2	99/211	F	13	Paediatric non-cancer	66
DOJ/2	99/212	F	25	Normal donor	
DOJ/2	99/213	M	3	Wilm's tumour	100
DOK/1	99/214	F	40	Normal donor	96
DOK/1	99/217	F	73	Lung carcinoma	low mitotic index
DOK/1	99/218	M	67	Lung carcinoma	80
DOK/1	99/219	F	71	Lung carcinoma	low mitotic index

DOL/1	99/214	F	40	Normal donor	88
DOL/1	99/220	F	39	BRCA 1 gene carrier	96
DOM/2	99/221	M	32	Normal donor	60
DOM/2	99/222	F	22mt	Paediatric non-cancer	62
DOM/2	99/223	M	23mt	Paediatric non-cancer	68
DOM/2	99/224	M	3mth	Paediatric non-cancer	74
DOP/1	99/225	F	56	Normal donor	84
DOP/1	99/226	F	77	Colorectal carcinoma	failed culture
DOP/2	99/225	F	56	Normal donor	68
DOP/2	99/229	F	2	Paediatric non-cancer	74
DOP/2	99/230	M	5	Paediatric non-cancer	64
DOP/2	99/231	F	3	Paediatric non-cancer	78
DOQ/1	99/232	F	22	Normal donor	90
DOQ/1	99/233	F	52	Lung carcinoma	failed culture
DOQ/1	99/234	M	63	Lung carcinoma	failed culture
DOQ/1	99/235	M	81	Lung carcinoma	failed culture
DOR/1	99/232	F	22	Normal donor	86
DOR/1	99/236	M	75	Lung carcinoma	failed culture
DOR/1	99/237	M	79	Lung carcinoma	124
DOR/1	99/238	M	54	Lung carcinoma	88
DOR/1	99/239	F	53	Lung carcinoma	98
DOR/1	99/240	F	75	Lung carcinoma	74
DOT/1	99/241	F	40	Normal donor	100
DOT/1	99/242	M	67	Colorectal carcinoma	84
DOT/1	99/243	M	71	Colorectal carcinoma	66
DOT/1	99/244	M	86	Colorectal carcinoma	76

DOU/1	99/241	F	40	Normal donor	106
DOU/1	99/245	F	82	Colorectal carcinoma	low mitotic index
DOU/1	99/246	M	58	Colorectal carcinoma	110
DOU/1	99/247	F	58	Colorectal carcinoma	64
DOU/1	99/248	M	75	Colorectal carcinoma	98
DOU/1	99/251	M	70	Colorectal carcinoma	low mitotic index
DOW1	99/278	F	34	Normal donor	78
DOW1	99/287	M	50	Colorectal carcinoma	failed culture
DOW1	99/288	F	69	Lung carcinoma	84
DOW1	99/289	M	78	Lung carcinoma	failed culture
DOW1	99/290	F	71	Lung carcinoma	94
DOW1	99/291	M	73	Lung carcinoma	low mitotic index
DOX/1	99/278	F	34	Normal donor	62
DOX/1	99/292	M	55	Lung carcinoma	102
DOX/1	99/293	F	61	Lung carcinoma	failed culture
DOY/1	99/294	F	21	Normal donor	72
DOY/1	99/295	F	72	Colorectal carcinoma	74
DOZ/1	99/294	F	21	Normal donor	76
DOZ/1	99/296	F	56	Colorectal carcinoma	80
DOZ/1	99/297	M	52	Colorectal carcinoma	74
DOZ/2	99/298	F	14	Glioma	60
DPA/1	99/299	F	45	Normal donor	118
DPA/1	99/300	M	61	Lung carcinoma	80
DPA/1	99/301	F	60	Colorectal carcinoma	104
DPA/1	99/302	M	70	Lung carcinoma	84
DPA/1	99/303	F	64	Colorectal carcinoma	84

DPA/1	99/304	M	78	Colorectal carcinoma	failed culture
DPB/1	99/299	F	45	Normal donor	116
DPB/1	99/307	F	74	Lung carcinoma	failed culture
DPB/1	99/308	F	85	Lung carcinoma	102
DPC/1	99/309	F	20	Normal donor	74
DPC/1	99/310	M	58	Colorectal carcinoma	74
DPC/1	99/311	M	64	Colorectal carcinoma	108
DPD/1	99/309	F	20	Normal donor	62
DPD/1	99/313	F	32	Colorectal carcinoma	82
DPE/1	99/317	M	32	Normal donor	90
DPE/1	99/318	F	43	BRCA1 gene carrier	108
DPF/1	99/319	M	51	Normal donor	66
DPF/1	99/320	F	56	Colorectal carcinoma	84
DPF/1	99/321	M	78	Lung carcinoma	112
DPF/1	99/322	M	77	Lung carcinoma	94
DPF/1	99/323	M	65	Colorectal carcinoma	failed culture
DPF/1	99/324	M	61	Colorectal carcinoma	124
DPG/1	99/325	F	32	Normal donor	90
DPG/1	99/326	F	55	Colorectal carcinoma	74
DPG/1	99/327	F	54	Colorectal carcinoma	failed culture
DPH/1	99/329	M	75	Lung carcinoma	failed culture
DPH/1	99/330	M	71	Lung carcinoma	failed culture
DPH/1	99/331	M	51	Lung carcinoma	failed culture
DPH/1	99/332	F	46	Normal donor	78
DPI/1	99/337	F	42	Normal donor	68
DPI/1	99/338	M	51	Colorectal carcinoma	low mitotic index

DPI/1	99/339	M	49	Colorectal carcinoma	70
DPI/1	99/340	M	65	Colorectal carcinoma	72
DPI/1	99/341	F	79	Colorectal carcinoma	76
DPK/1	99/343	F	30	Normal donor	94
DPK/1	99/347	M	73	Lung carcinoma	failed culture
DPK/1	99/348	M	67	Lung carcinoma	failed culture
DPK/1	99/349	M	53	Colorectal carcinoma	94
DPK/1	99/350	M	43	Colorectal carcinoma	low mitotic index
DPK/1	99/351	M	45	Colorectal carcinoma	failed culture
DPM/1	99/352	M	39	Normal donor	90
DPM/1	99/353	M	69	Colorectal carcinoma	94
DPM/1	99/354	M	74	Colorectal carcinoma	100
DPM/1	99/355	F	68	Colorectal carcinoma	120
DPN/1	99/358	F	39	Normal donor	74
DPN/1	99/362	M	71	Lung carcinoma	94
DPN/1	99/363	F	71	Colorectal carcinoma	102
DPN/1	99/364	M	57	Colorectal carcinoma	104
DPN/1	99/365	F	56	Lung carcinoma	failed culture
DPN/1	99/366	F	73	Lung carcinoma	80
DPO/1	99/358	F	39	Normal donor	70
DPO/1	99/367	F	83	Colorectal carcinoma	low mitotic index
DPO/1	99/368	F	67	Cervical carcinoma	80
DPO/2	99/369	M	15	Hodgkin's disease	failed culture
DPP/1	99/370	M	22	Normal donor	104
DPP/1	99/371	M	71	Colorectal carcinoma	low mitotic index
DPP/1	99/372	M	60	Colorectal carcinoma	94

DPP/1	99/373	F	64	Colorectal carcinoma	98
DPQ/2	99/376	M	50	Normal donor	76
DPQ/2	99/377	M	4.5	Neuroblastoma	68
DPR/1	99/378	F	32	Normal donor	76
DPR/1	99/381	M	74	Lung carcinoma	98
DPR/1	99/382	M	52	Lung carcinoma	failed culture
DPR/1	99/383	M	69	Lung carcinoma	failed culture
DPR/1	99/384	M	62	Lung carcinoma	88
DPR/1	99/385	F	79	Cervical carcinoma	90
DPT/1	99/428	F	44	Normal donor	88
DPT/1	99/429	M	65	Lung carcinoma	failed culture
DPT/1	99/430	M	79	Lung carcinoma	114
DPT/1	99/431	M	53	Lung carcinoma	failed culture
DPT/1	99/432	F	76	Lung carcinoma	90
DPU/1	99/428	F	44	Normal donor	82
DPU/1	99/433	F	63	Lung carcinoma	failed culture
DPU/1	99/434	M	69	Lung carcinoma	failed culture
DPW/1	99/449	M	20	Normal donor	98
DPW/1	99/450	M	75	Lung carcinoma	90
DPW/1	99/451	F	73	Lung carcinoma	failed culture
DPX/1	99/449	M	20	Normal donor	98
DPX/1	99/452	F	35	BRCA genes absent	82
DPX/1	99/453	F	60	Cervical carcinoma	88
DPX/1	99/454	F	73	Cervical carcinoma	90
DPX/1	99/455	M	78	Lung carcinoma	88
DPX/1	99/456	M	63	Lung carcinoma	74

DPZ/1	99/458	F	24	Normal donor	88
DPZ/1	99/467	F	70	Lung carcinoma	76
DPZ/1	99/468	F	69	Lung carcinoma	70
DPZ/1	99/469	F	65	Lung carcinoma	78
DPZ/1	99/470	M	74	Lung carcinoma	104
DPZ/1	99/471	M	50	Lung carcinoma	94
DQA/1	99/459	F	26	Normal donor	76
DQA/1	99/472	M	62	Lung carcinoma	92
DQA/1	99/473	F	68	Lung carcinoma	failed culture
DQA/1	99/474	M	71	Lung carcinoma	low mitotic index
DQC/1	99/481	F	40	Normal donor	76
DQC/1	99/482	M	80	Lung carcinoma	failed culture
DQC/1	99/483	F	72	Cervical carcinoma	94
DQC/1	99/484	M	75	Lung carcinoma	failed culture
DQC/1	99/485	F	29	BRCA 1 gene carrier	80
DQD/1	99/491	F	45	BRCA 1gene carrier	90
DQD/1	99/492	F	30	BRCA1gene carrier	78
DQD/1	99/493	M	51	BRCA1gene carrier	84
DQD/1	99/494	F	49	Normal donor	92
DQD/1	99/495	F	77	Cervical carcinoma	68
DQD/1	99/496	F	42	BRCA genes absent	98

score= induced aberrations/ 100 cells
1=scorer 1
2=scorer 2

APPENDIX 2. NORMAL CONTROLS BY SCORER 1.

Sample	spont.ab	MI irrad	MIunirrad	% MI	Sample	Spont.ab.	MI irrad	MIunirrad	%
98/25	0	0.8	6.2	87.0	98/87	0	0.8	4.4	82.0
98/101	0	0.1	0.5	98.0	98/118	2	0.5	4.5	89.0
98/123	0	1.1	4.2	74.0	98/158	0	0.7	4.8	85.0
98/185	0	0.4	2.9	86.0	98/201	4	0.5	3.8	87.0
98/231	0	0.4	5.1	92.2	98/253	0	1.1	3.5	69.0
98/254	0	0.2	3.6	94.0	98/271	0	0.3	4.6	93.4
98/279	4	0.3	4.7	93.6	98/284	2	0.4	5.2	92.3
98/284	4	0.2	5.7	96.0	98/312	0	0.1	8.6	99.0
98/312	0	0.2	7.8	97.5	98/347	0	0.1	3.5	97.0
98/350	2	0.2	6.6	97.0	98/361	0	0.9	5.4	83.0
98/367	2	0.1	4.0	97.5	98/369	0	0.1	8.5	99.0
98/383	2	0.2	5.6	94.4	98/377	0	0.2	5.0	96.0
98/380	0	0.4	5.3	92.4	98/399	0	0.6	6.0	90.0
98/413	0	0.3	6.8	94.0	98/414	4	0.3	4.8	94.7
98/415	0	0.5	5.8	91.0	98/457	8	0.4	5.0	92.0
98/463	0	0.1	3.0	97.0	98/470	2	0.4	2.4	98.0
98/471	0	0.6	4.3	86.0	98/472	0	0.3	4.2	92.8
98/482	0	0.4	4.0	90.0	98/487	0	0.1	4.2	97.6
98/508	2	0.4	3.8	89.5	98/516	0	0.1	3.6	97.0
98/521	2	0.1	5.0	98.0	98/522	2	0.1	3.0	97.0
98/523	0	0.1	5.2	98.0	98/549	4	0.4	3.6	89.0
98/559	2	0.1	6.4	98.5	98/562	0	0.1	3.4	97.0
98/562	6	0.3	5.0	94.0	98/570	2	0.2	3.6	94.5
98/571	6	0.3	3.6	91.7	98/585	6	0.3	3.9	90.0

98/591	0	0.3	3.6	92.0	98/601	2	0.1	3.0	97.0
98/607	0	0.4	5.6	93.0	98/616	0	0.3	4.0	92.5
98/628	0	0.2	4.8	96.0	98/628	4	0.3	5.2	94.0
98/637	2	0.3	4.0	92.5	98/651	0	0.5	6.8	93.0
99/1	2	0.4	6.0	93.0	99/11	0	0.1	6.4	98.5
99/13	0	0.3	6.8	96.0	99/60	0	0.3	5.6	94.0
99/64	2	0.3	4.8	94.0	99/80	2	0.2	5.0	96.0
99/94	2	0.1	5.4	98.0	99/102	6	0.3	4.8	93.8
99/115	2	0.4	5.6	92.8	99/121	4	0.4	6.8	94.0
99/121	0	0.4	6.4	94.0	99/132	0	0.1	5.0	98.0
99/136	0	0.1	3.6	97.3	99/139	2	0.2	5.6	96.0
99/167	2	0.3	4.0	92.5	99/167	2	0.3	4.2	93.0
99/181	0	0.1	4.0	97.5	99/181	0	0.2	5.2	96.0
99/192	4	0.4	5.0	92.0	99/196	0	0.2	3.0	93.0
99/197	2	0.1	3.6	97.0	99/197	0	0.2	4.6	95.7
99/214	2	0.4	6.0	93.0	99/214	0	0.3	3.5	91.0
99/225	2	0.2	3.0	93.0	99/232	2	0.2	5.0	96.0
99/232	0	0.5	5.0	90.0	99/241	2	0.3	4.5	93.0
99/241	6	0.3	4.5	93.0	99/278	4	0.1	3.2	97.0
99/278	2	0.1	4.0	97.5	99/294	4	0.3	3.7	92.0
99/294	4	0.2	3.0	93.0	99/299	0	0.3	4.0	92.5
99/299	4	0.1	3.9	97.5	99/309	2	0.5	5.2	90.0
99/309	0	0.1	3.0	97.0	99/317	0	0.4	4.9	92.0
99/319	0	0.1	3.3	97.0	99/325	2	0.2	3.7	95.0
99/332	0	0.1	3.6	97.2	99/337	0	0.1	3.0	97.2
99/343	0	0.1	4.6	97.8	99/352	0	0.4	4.5	91.0

99/358	2	0.2	3.6	95.0	99/358	0	0.2	3.9	95.0
99/370	6	0.2	4.5	96.0	99/378	0	0.4	4.2	95.0
99/428	4	0.5	4.0	87.5	99/428	0	0.1	3.1	97.0
99/449	2	0.3	4.6	93.5	99/449	2	0.2	4.6	96.0
99/458	0	0.5	6.2	92.0	99/459	2	0.3	3.2	91.0
99/481	0	0.2	4.2	95.3	99/494	0	0.2	4.2	95.2

spont. ab=spontaneous aberrations/ 100 cells
MI irrad= Mitotic index of irradiated cells
MI unirrad=Mitotic index of unirradiated cells
%MI= Percentage mitotic inhibition

APPENDIX 3. BENIGN DISEASE GROUP BY SCORER 1.

Sample	spont aberrations	MI irrradiated	MI unirradiated	% MI
98/145	0	0.9	5.5	94.0
98/146	0	0.7	4.8	75.0
98/147	0	0.6	4.1	75.0
98/148	6	0.3	3.8	92.0
98/149	2	0.7	3.8	82.0
98/150	0	0.5	4.4	89.0
98/154	0	1.7	5.6	70.0
98/155	0	1.0	4.0	75.0
98/160	0	0.1	6.2	98.0
98/174	0	0.3	3.0	90.0
98/176	0	0.2	2.0	90.0
98/192	0	0.2	4.3	95.0
98/196	0	0.4	3.5	89.0
98/214	0	0.1	3.2	96.0
98/215	0	0.1	3.9	97.4
98/216	0	0.2	4.8	95.8
98/218	0	0.2	2.9	93.1
98/219	0	0.1	4.0	97.5
98/256	0	0.3	4.4	93.0
98/266	0	0.2	3.6	94.4
98/267	0	0.2	3.7	94.6
98/268	0	0.1	3.0	99.6
98/270	0	0.3	4.2	92.9
98/280	2	0.3	5.0	94.0
98/281	0	0.2	4.8	95.8
98/291	0	0.2	4.4	95.4
98/292	2	0.3	4.6	93.5
98/307	4	0.1	3.5	97.0
98/310	0	0.4	2.0	80.0
98/455	2	0.1	1.9	90.0
98/464	2	0.5	4.8	90.0
98/465	4	0.1	1.8	94.0
98/497	0	0.1	2.4	96.0
98/509	0	0.1	5.2	98.0

spont. abs=spontaneous aberrations/ 100 cells
MI unirrad=Mitotic index of unirradiated cells
MI irrad= Mitotic index of irradiated cells
%MI= percentage mitotic inhibition

APPENDIX 4. BREAST CARCINOMA GROUP BY SCORER 1.

Sample	spont ab	MI irrad	MI unirrad	% MI	Stage	Histology	Grade
98/568	2	0.1	6.8	98.5	T1N0M0	ductal	2
98/569	2	0.2	3.6	94.4	T1N0M0	ductal	3
98/586	0	0.6	7.8	92.3	T1N0M0	ductal	2
98/587	2	0.4	4.0	90.0	T1N0M0	ductal	2
98/596	2	0.3	6.8	95.6	T1N0M0	ductal	1
98/598	2	0.2	4.8	95.8	T2N0M0	ductal	1
98/599	0	0.3	3.8	92.1	T2N0M0	ductal	2
98/619	0	0.1	2.0	95.0	T2N1M0	ductal	1
98/620	2	0.2	4.0	95.0	T1N0M0	ductal	1
98/623	0	0.2	5.0	96.0	T1N0M0	ductal	2
98/641	0	0.3	4.8	93.8	T1N0M0	ductal	2
99/6	2	0.1	4.6	97.8	T1N0M0	ductal	1
99/7	0	0.2	3.2	94.0	T1N0M0	lobular	-
99/8	2	0.3	5.6	94.6	T1N0M0	ductal	2
99/9	0	0.1	3.6	97.2	T1N0M0	ductal	1
99/57	2	0.2	4.8	95.8	T1N0M0	ductal	1
99/58	2	0.2	5.0	96.0	T1N0M0	ductal	1
99/59	0	0.1	5.0	98.0	T2N0M0	lobular	2
99/101	0	0.3	3.0	90.0	T1N0M0	ductal	1
99/119	0	0.3	5.0	94.0	T2N0M0	ductal	3
99/120	0	0.2	5.0	96.0	T2N1M0	ductal	2
99/123	0	0.1	3.8	97.4	T4N3M1	ductal	3
99/140	0	0.1	4.6	97.8	T1N0M0	lobular	1

99/141	0	0.2	4.8	95.8	T1N0M0	ductal	1
99/142	2	0.2	2.6	92.3	T2N0M0	tubular	1
99/151	2	0.1	3.6	97.2	T1N0M0	tubular	1
99/152	0	0.1	2.8	96.4	T2N0M0	ductal	1
99/165	0	0.2	4.4	99.5	T1N0M0	mucinous	1
99/166	0	0.1	3.8	97.3	T1N0M0	ductal	2
99/183	0	0.2	4.0	95.0	T1N0M0	ductal	2
99/184	2	0.1	2.6	96.1	T1N0M0	ductal	1

grade 1= well differentiated
grade 2= moderately differentiated
grade 3=poorly differentiated
spont. abs= spontaneous aberrations/ 100 cells
MI unirrad= mitotic index of unirradiated cells
MI irrad= mitotic index of irradiated cells
%MI-percentage mitotic inhibition
stage =TNM stage

APPENDIX 5 SUBJECTS UNDERGOING SCREENING FOR BRCA1/2 GENE MUTATIONS BY SCORER 1.

Sample	Spont. ab.	MI irrad	MIunirrad	%MI	Gene status
99/62	0	0.2	5.0	96.0	Normal
99/63	0	0.1	6.0	97.4	Normal
99/95	2	0.4	5.2	92.3	BRCA 1
99/164	0	0.2	4.6	95.6	Normal
99/195	0	0.3	3.8	92.0	Normal
99/203	0	0.2	4.2	95.0	BRCA 1
99/204	0	0.1	6.0	98.3	BRCA 1
99/220	0	0.2	5.6	96.5	BRCA 1
99/318	2	0.3	4.2	92.8	BRCA 1
99/452	0	0.3	4.9	93.9	Normal
99/491	0	0.3	3.8	92.1	BRCA 1
99/492	2	0.1	2.9	96.6	BRCA 1
99/493	0	0.1	4.0	97.5	BRCA 1
99/496	0	0.5	6.0	91.7	Normal
99/485	2	0.5	4.9	90.0	BRCA 1

spont. abs=spontaneous aberrations/ 100 cells
MI irrad= mitotic index of irradiated cells
MI unirrad= mitotic index of unirradiated cells
%MI= percentage mitotic inhibition

APPENDIX 6. CERVICAL CARCINIMA GROUP BY SCORER 1.

Sample	spont.ab.	MI irrad	MIunirrad	% MI	stage	histology	grade
98/56	2	0.4	4.3	90.6	IIB	squamous	2
98/57	2	0.4	3.1	87.0	IIIB	squamous	2
98/88	0	0.3	5.0	94.0	IIB	squamous	2
98/232	0	0.2	3.6	94.4	IIIB	squamous	2
98/551	2	0.3	3.2	90.6	II B	adenocarcinoma	1
98/567	0	0.7	7.8	91.0	IVA	adenocarcinoma	3
98588	4	0.4	3.6	88.9	IIB	squamous	2
98/589	4	0.5	3.2	84.4	IIB	squamous	3
98/606	4	0.1	4.0	97.5	IVA	squamous	2
98/624	0	0.1	3.0	96.7	IVA	squamous	3
98/625	2	0.1	6.8	98.5	IIIB	squamous	2
98/632	0	0.1	4.0	97.5	IVA	squamous	2
98/650	0	0.2	4.4	95.5	IIB	squamous	2
99/14	0	0.3	5.9	95	IIIB	squamous	2
99/83	0	0.1	3.0	97.0	IIB	adenocarcinoma	1
99/84	2	0.2	6.8	97.0	IB	squamous	1
99/93	0	0.2	6.4	97.0	IIIB	squamous	2
99/116	0	0.3	4.2	92.8	IIIB	squamous	2
99/122	2	0.3	6	95.0	IVA	squamous	2
99/124	0	0.3	5.6	94.6	IIA	squamous	2
99/131	2	0.3	4.6	93.5	IIB	squamous	3
99/368	2	0.1	3.6	97.0	IIB	squamous	2
99/385	2	0.2	2.0	90.0	IIIB	squamous	3

99/453	0	0.1	3.2	97.0	IIIB	squamous	1
99/454	2	0.4	4.6	91.3	IIB	squamous	2
99/483	2	0.1	2.7	96.3	IIIB	squamous	3
99/495	4	0.1	2.7	96.3	IB	squamous	3

grade 1= well differentiated
grade 2= moderately differentiated
grade 3= poorly differentiated
spont.abs.=spontaneous aberrations/100 cells
MI irrad= mitotic index of irradiated cells
MI unirrad= mitotic index of unirradiated cells
%MI= percentage mitotic inhibition
stage= FIGO stage

APPENDIX 7. COLORECTAL CARCINOMA GROUP BY SCORER 1.

Sample	spont.ab.	MI irrad	MIunirrad	% MI	stage	histology	grade
98/103	0	0.8	4.4	81.8	B	adenocarcinoma	1
98/104	4	0.9	6.0	85.0	C	adenocarcinoma	1
98/157	0	1.1	6.6	83.3	C	adenocarcinoma	1
98/252	2	0.8	3.0	73.3	C	adenocarcinoma	2
98/348	0	0.2	4.6	95.6	B	adenocarcinoma	2
98/349	4	0.1	4.5	97.7	B	adenocarcinoma	3
98/592	2	0.7	6.0	88.3	A	adenocarcinoma	1
99/2	0	0.5	5.4	90.7	B	adenocarcinoma	1
99/190	2	0.2	3.2	93.7	B	adenocarcinoma	2
99/242	4	0.3	3.9	92.3	B	adenocarcinoma	2
99/243	0	0.2	2.5	92.0	B	adenocarcinoma	2
99/244	4	0.1	3.4	97.0	B	adenocarcinoma	2
99/246	0	0.2	3.9	95.0	B	adenocarcinoma	2
99/247	0	0.1	3.3	96.9	C	adenocarcinoma	2
99/248	2	0.1	2.1	95.2	C	adenocarcinoma	3
99/295	0	0.1	2.0	95.0	C	adenocarcinoma	unknown
99/296	0	0.3	3.8	92.1	C	adenocarcinoma	2
99/297	0	0.4	3.1	87.0	A	adenocarcinoma	1
99/301	0	0.5	5.2	90.3	C	adenocarcinoma	2
99/303	2	0.3	4.3	93.0	B	adenocarcinoma	2
99/310	4	0.3	4.2	92.8	A	adenocarcinoma	1
99/311	0	0.1	2.6	96.8	B	adenocarcinoma	2
99/313	2	0.3	3.9	92.3	C	adenocarcinoma	3
99/320	4	0.1	5.0	98.0	B	adenocarcinoma	1
99/324	0	0.3	3.6	91.6	B	adenocarcinoma	2
99/326	2	0.1	2.1	95.2	C	adenocarcinoma	2
99/339	0	0.1	2.6	96.1	C	adenocarcinoma	2
99/340	2	0.1	2.3	95.6	B	adenocarcinoma	3

99/341	2	0.1	2.1	95.2	A	adenocarcinoma	2
99/349	0	0.1	2.1	95.2	C	adenocarcinoma	3
99/353	0	0.3	5.0	94.0	A	adenocarcinoma	1
99/354	2	0.4	4.2	90.5	B	adenocarcinoma	2
99/355	2	0.3	4.4	93.2	C	adenocarcinoma	2
99/363	0	0.3	4.0	92.5	C	adenocarcinoma	2
99/364	0	0.3	3.8	92.1	B	adenocarcinoma	2
99/372	2	0.2	3.0	93.3	A	adenocarcinoma	2
99/373	0	0.4	3.6	88.8	B	adenocarcinoma	2

grade 1= well differentiated
grade 2= moderately differentiated
grade 3= poorly differentiated
spont abs=spontaneous aberrations/ 100 cells
MI irrad=mitotic index of irradiated cells
MI unirrad=mitotic index of unirradiated cells
%MI= percentage mitotic inhibition
Stage =Duke's stage

APPENDIX 8. LUNG CARCINONMA GROUP BY SCORER 1.

Sample	spont.ab.	MI irrad	MIunirrad	% MI	Stage	Histology	grade
98/115	2	0.8	4.4	81.8	T1N1M0	squamous	1
98/117	0	0.4	3.1	87.0	T1N2M0	NSCLC	U
98/293	0	0.3	4.8	93.7	T3N1M0	squamous	2
98/466	4	0.4	3.9	89.7	T3N1M0	NSCLC	U
98/496	0	0.2	4.1	95.0	T2N1M0	NSCLC	U
99/188	0	0.1	2.2	95.5	T2N2M0	squamous	3
99/199	0	0.1	4.4	97.7	T4N3M1	squamous	U
99/218	4	0.2	3.8	94.7	T3N2M0	NSCLC	U
99/237	4	0.2	3.8	94.7	T2N0M0	not available	-
99/238	0	0.4	4.9	91.8	T3N0M0	NSCLC	U
99/239	2	0.4	4.6	91.3	T3N2M0	adenocarcinoma	U
99/240	2	0.1	3.3	97.0	T2N0M0	not available	-
99/288	2	0.3	4.5	93.3	T2N0M0	adenocarcinoma	U
99/290	0	0.2	5.0	96.0	T3N1M0	not available	-
99/292	2	0.4	4.6	91.3	T4N2M0	squamous	U
99/300	2	0.2	5.0	96.0	T3N1M0	not available	-
99/302	2	0.4	4.6	91.3	T3N1M0	not available	-
99/308	2	0.3	3.0	90.0	T3N1M0	not available	-
99/321	4	0.5	5.5	90.9	T3N2M0	NSCLC	U
99/322	2	0.2	4	95.0	T3N1M0	adenocarcinoma	U
99/362	0	0.1	3	97.0	T2N0M0	squamous	U
99/366	2	0.5	5.0	90.0	T3N3M0	squamous	U
99/381	2	0.3	3	90.0	T2N0M0	adenocarcinoma	U

99/384	0	0.1	2.0	95.0	T4N1M0	NSCLC	U
99/430	4	0.2	3.7	94.5	T4N2M0	squamous	2
99/432	0	0.3	3.6	91.6	T2N0M0	not available	-
99/450	4	0.2	3.0	94.5	T2N0M0	not available	-
99/455	2	0.3	2.7	88.9	T3N0M0	NSCLC	U
99/456	0	0.2	3.6	94.5	T4N2M1	NSCLC	U
99/467	0	0.3	2.9	90.0	T2N0M0	squamous	3
99/468	0	0.3	3.0	90.0	T4N3M0	NSCLC	3
99/469	2	0.5	3.8	86.8	T3N0M0	not available	-
99/470	4	0.4	3.6	88.8	T3N0M0	squamous	U
99/471	0	0.4	4.9	91.8	T4N0M0	NSCLC	3
99/472	0	0.5	6.2	91.9	T2N2M0	squamous	U

spont abs.=spontaneous aberrations/ 100 cells
MI irrad= mitotic index of irradiated cells
MI unirrad=mitotic index of unirradiated cells
%MI=percentage mitotic index
stage=TNM stage
grade 1= well differentiated
grade 2= moderately differentiated
grade 3= poorly differentiated
U =histological grade undetermined
NSCLC= Non small cell lung carcinoma

APPENDIX 9. NORMAL ADULT DONORS BY SCORER 2.

sample	spont.ab	MI irrad	MIunirrad	%MI	sample	spont.ab	MI irrad	MIunirrad	%MI
98/31	0	0.5	5.0	90.0	98/47	0	0.6	6.0	90.0
98/83	0	0.5	4.5	88.8	98/101	0	0.2	5.0	96.0
98/118	0	0.5	4.5	88.8	98/120	0	0.6	6.2	90.3
98/166	0	0.2	5.0	96.0	98/188	0	0.2	5.0	96.0
98/207	0	0.3	4.8	93.7	98/231	0	0.6	6.0	90.0
98/239	0	0.4	3.2	87.5	98/335	0	0.3	3.0	90.0
98/427	0	0.4	6.2	93.5	98/431	0	0.3	5.0	94.0
98/458	0	0.2	4.0	95.0	98/459	0	0.2	5.0	96.0
98/460	0	0.2	3.0	93.3	98/508	0	0.1	3.1	96.7
98/517	2	0.7	5.7	87.7	98/521	2	0.6	7.2	91.7
98/522	0	0.8	5.8	86.2	98/523	0	0.5	5.2	90.4
98/536	2	0.9	7.6	88.1	98/537	0	0.8	6.5	87.7
98/540	0	0.4	5.1	92.2	98/556	2	0.3	4.6	93.5
98/556	0	0.3	5.3	94.3	98/575	0	0.3	3.8	92.1
98/576	0	1.1	5.2	78.8	98/591	0	0.2	4.2	95.2
98/591	2	1.2	7.7	84.4	99/3	0	0.5	4.0	87.5
99/11	0	0.8	7.4	89.2	99/43	0	0.8	7.4	89.2
99/43	2	0.8	9.0	91.1	99/64	0	0.9	7.5	88.0
99/87	0	0.2	7.1	97.2	99/91	0	0.3	5.6	94.6
99/99	0	0.7	7.8	91.0	99/102	0	0.2	4.1	95.1
99/115	0	0.6	6.0	90.0	99/115	0	0.8	7.3	89.0
99/127	2	1.0	7.5	86.6	99/127	2	0.7	7.6	90.7
99/155	0	0.6	6.2	90.3	99/158	0	0.6	6.7	91.0

99/178	0	0.8	7.0	89.0	99/180	0	1.0	5.9	83.0
99/192	0	0.6	6.3	90.4	99/209	0	1.1	8.4	86.9
99/212	4	0.9	5.4	83.3	99/221	0	1.1	7.4	85.2
99/225	0	0.7	5.7	87.7	99/376	0	1.2	6.0	80.0
98/547	0	0.9	6.2	85.5	98/543	0	0.1	3.1	96.7
98/544	2	0.1	3.5	97.1					

spont abs=spontaneous aberrations/ 100 cells
MI irrad=mitotic index of irradiated cells
Mi unirrad= mitotic index of unirradiated cells
%MI= percentage mitotic inhibition

APPENDIX 10. PAEDIATRIC/ ADOLESCENT MALIGNANCY GROUP BY SCORER 2.

Sample	spont.ab.	MI irradiated	MI unirradiated	% MI	Malignancy
98/33	0	0.6	5.0	82.0	Ewing's sarcoma
98/35	0	0.8	6.8	82.0	Osteosarcoma
98/45	0	0.3	6.2	95.0	Rhabdomyosarcoma
98/84	0	0.2	3.0	93.0	Ovarian teratoma
98/105	0	1.1	5.0	78.0	Medulloblastoma
98/114	0	0.4	4.3	89.0	Osteosarcoma
98/119	0	0.6	5.0	88.0	Osteosarcoma
98/144	0	0.7	9.2	92.0	Germinoma
98/168	0	0.9	5.5	84.0	Hodgkin's disease
98/190	0	0.9	4.6	80.0	Osteosarcoma
98/191	0	0.1	6.4	98.0	Hodgkin's disease
98/208	0	0.2	2.0	90.0	Osteosarcoma
98/229	0	0.3	4.5	93.3	Osteosarcoma
98/234	0	0.4	5.2	92.0	Rhabdomyosarcoma
98/336	0	0.4	4.0	90.0	Lymphoma
98/337	0	0.3	4.4	93.0	Osteosarcoma
98/426	0	0.4	5.6	92.8	Rhabdomyosarcoma
98/432	2	1.4	6.5	79.0	Hodgkin's disease
98/513	0	0.6	5.3	89.0	Ependymoma
98/514	0	0.5	5.0	90.0	Rhabdomyosarcoma
98/518	0	0.6	5.8	90.0	Osteosarcoma
98/539	0	0.5	3.3	85.0	Hodgkin's disease

98/548	4	0.5	5.6	91.1	Rhabdomyosarcoma
98/573	0	0.2	3.0	93.0	Hodgkin's disease
98/582	0	0.2	4.5	95.5	Lymphoma
98/583	0	0.2	5.3	96.3	Lymphoma
98/590	0	0.3	3.6	92.0	Osteosarcoma
99/12	0	1.0	6.1	84.0	Lymphoma
99/118	0	0.9	1.8	50.0	Hodgkin's disease
99/213	0	1.1	4.0	72.5	Wilm's tumour
99/298	0	0.3	4.0	92.5	Glioma
99/377	0	1.2	8.8	86.0	Neuroblastoma

spont abs.=spontaneous aberrations/ 100 cells
MI irrad=mitotic index of irradiated cells
MI unirrad=mitotic index of unirradiated cells
%MI=percentage mitotic inhibition

APPENDIX 11. PAEDIATRIC/ ADOLESCENT CONTROL GROUP BY SCORER 2.

Sample	spont. ab.	MI irrad	MI unirradiated	% MI
98/557	0	0.3	6.3	95.2
98/558	2	0.3	4.6	93.2
98/580	0	0.2	6.9	97.0
98/581	0	0.5	7.5	93.0
98/643	2	0.4	4.5	91.1
99/5	0	0.5	4.5	89.1
99/44	0	0.6	8.2	93.0
99/45	0	1.3	7.0	82.0
99/49	0	1.6	7.0	78.2
99/68	0	1.0	10.0	90.0
99/69	0	0.6	9.0	94.0
99/70	0	0.7	12.5	94.4
99/71	4	1.0	6.2	75.8
99/90	0	0.8	8.7	91.8
99/100	0	0.5	9.6	94.8
99/103	0	0.3	6.1	95.1
99/104	0	0.9	5.5	74.6
99/105	0	0.8	7.3	90.0
99/106	4	0.4	5.1	92.2
99/113	0	0.6	6.0	90.0
99/114	0	0.6	7.7	92.2
99/125	0	0.8	6.0	87.0

99/126	2	0.5	6.6	92.5
99/128	2	0.7	6.2	89.0
99/156	0	0.7	7.0	90.0
99/157	0	0.4	6.0	93.4
99/159	0	0.7	7.8	91.0
99/160	2	0.5	8.0	93.7
99/179	0	0.9	6.7	87.0
99/193	2	0.4	6.9	94.2
99/194	2	0.5	6.0	91.6
99/200	0	0.7	14.1	95.0
99/201	0	0.5	15.6	96.8
99/210	0	0.5	11.1	95.5
99/211	0	0.4	6.9	94.2
99/222	0	0.5	8.1	93.8
99/223	0	0.8	9.4	91.5
99/224	0	1.2	7.0	82.9
99/229	2	0.6	10.8	94.4
99/230	2	0.6	8.1	92.6
99/231	0	0.7	4.7	85.1

spont abs.=spontaneous aberrations/ 100 cells
MI irrad=Mitotic index of irradiated cells
MI unirrad= Mitotic index of unirradiated cells
%MI= percentage mitotic inhibition

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