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RISK FACTORS FOR PROSTATE CANCER: A CASE-CONTROL STUDY INVESTIGATING SELECTED KEY EXPOSURES AND THEIR INTERACTIONS WITH PREDISPOSITION GENES

DR ANEELA ATTA UR RAHMAN

Thesis submitted to the

University of Nottingham for the degree of

Doctor of Philosophy

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In loving memory of my beloved parents

Huzoor and ***Siddique***

who made the bad times bearable and the good times magical

Abstract

Background

Prostate cancer is the UK number one male cancer. Evidence from epidemiological studies suggests only age, race and family history as established risk factors. Other factors such as low dose diagnostic radiations and surrogate hormone markers such as baldness, finger length pattern and acne are hypothesized to have a potential role in the aetiology of prostate cancer. It is evident that genetics plays an important role in prostate cancer aetiology.

This thesis focuses both environmental and genetic factors. The environmental factors include selected surrogate hormone markers, medical diagnostic radiation procedures and family history of prostate cancer. The genetic part explores genetic polymorphisms that could have implications for interactions with exposures studied. Single nucleotide polymorphisms (SNPs) involved in mechanistic pathways related to DNA repair genes and potential hormone marker genes were the main targets.

Aims

1. To extensively review and analyses of some important environmental factors such as family history, diagnostic radiations and hormone markers such as male pattern baldness, right hand pattern and acne.

2. To evaluate the role of DNA repair genes involved in diagnostic radiation process in the aetiology of prostate cancer and the hormone genes such as male pattern baldness in prostate cancer risk.

3. To assess gene environment interactions of selected genes and studied exposures.

Methods

The study was a nationwide population based case- control study, 1112 cases and 1872 controls were included. Data collection spanned over a ten year period from 1999-2009. The data collection tool was self completed postal questionnaire with ten sections on lifestyle and a separate section on diet. Biological samples including toenail clippings and 18ml blood samples were collected. Data on family history , diagnostic radiations and hormone markers such as baldness, right hand pattern and acne were analysed using multivariate logistic regression to obtain odds ratios (OR) and 95% confidence intervals (CI). Blood samples were processed and DNA was extracted for further genotyping. Sixteen selected SNPs from two groups of genes including DNA repair and balding genes were analysed to obtain their estimated risks. Gene and environment interaction analysis was carried out to assess the interactions between selected SNPs and environmental factors.

Results

Family history

Family history of prostate cancer in first degree relatives (father and brother) was a strong risk factor for prostate cancer (OR 7.93, 95% C.I. 6.17-10.20). Within subjects age <60 years with positive prostate cancer family history ,a highly significant association was observed as compared to risk seen in all ages (OR 12.55 compared to 7.93).

A modest risk was observed in a group of subjects with the positive family history of breast cancer in their first degree female relatives (mother, sister and daughter) (OR 1.39, 95% C.I. 1.07-1.79).

Diagnostic radiation

Hip / pelvic X-ray procedure increased the risk of developing prostate cancer in subjects who reported expose to procedure one time regardless time of the exposure (OR 3.15, 95% C.I. 1.81-5.47). Furthermore when time of exposure was censored at > 5, 10 or 15 years prior to case diagnoses or to control receiving questionnaire, all estimated risks were statistically significant (>5years OR 3.42, 95% C.I. 1.56-7.50, >10years OR 4.18, 95% C.I. 1.69-1.30 and >15 years OR 4.69, 95% C.I. 1.77-12.47) as compared to those who were

unexposed. All other procedures such as barium meal, barium enema, IVP and Upper leg/thigh X-ray were non significant.

Hormone markers

Baldness:

Prevalence of baldness increased with age in both case and control group, however there was no association between balding and prostate cancer risk at any age (20s, 30s and 40s) (all confidence intervals include 1). Subjects with positive family history of prostate cancer in their first degree relatives and who reported having had hair recession either frontal or vertex balding at age 30s show a positive association with prostate cancer risk (OR 2.06, 95% C.I. 1.01-3.83 and OR 1.85, 95% C.I. 1.03-3.31) respectively.

Right hand pattern:

Index longer than ring finger showed a borderline statistically significant risk reduction (OR 0.63 95% C.I 0.37-1.07) as compared to index finger shorter than ring (reference group). While Index finger equal to ring appeared to be non significant when compared with reference group (OR 1.01, 95% C.I. 0.83-1.22).

Acne:

Acne was not associated with prostate cancer risk at puberty, age 20s, teens through 20s. However subjects who reported having had acne at age 30s and who reported having had acne from their teen through to age 30s are at greater risk as compared to subjects who never had acne (OR 1.59, 95% C.I. 1.06-2.39 and OR 1.60, 95% C.I. 1.04-2.45 respectively).

DNA repair genes

The prevalence of all eleven polymorphism is very similar in both cases and controls. None of the analysed SNPs appeared to be a risk factor for prostate cancer.

Baldness genes

There was no associations between five SNPs and prostate cancer risk (all confidence interval include 1).

Gene-environment interaction

Hip/pelvic X-ray, DNA repair genes and prostate cancer risk:

Gene and environment interaction analysis using multiplicative model suggested a modest increased risk with (of Xeroderma Pigmentosum Group C (XPC Lys939Gln gene, rs2228001) (OR 1.66, 95% C.I. 1.02-2.71).

Universal X-ray exposure (expose to any of the 5 studied radiological procedures), DNA repair genes and prostate cancer risk:

The negative interaction was suggested with SNP rs7003908 (XRCC7G6721T) (OR 0.60, 95% C.I. 0.39-0.93).

Baldness genes:

There was no multiplicative interaction between "balding genes", baldness and prostate cancer.

Conclusion

In summary, family history was a strong risk factor for prostate cancer. The findings confirm the importance of low dose ionizing radiations in prostate cancer aetiology. Risk reduction seen in subjects with female phenotypic hand pattern and risk increased seen in subjects who reported appearance of acne at age 30s and from their teens to their 30s and also subjects with positive prostate cancer in their family who reported hair recession at age 30s supported the probable role of androgens in prostate carcinogenesis either linking with pre and post natal life exposure.

Polymorphisms in DNA repair genes along with exposures to universal hip/pelvic and universal hip X-ray suggested some interactions between the genetic and environment exposure while polymorphisms in balding genes and balding phenotype did not support any interactions. The interaction analysis between gene and environment may help identifying genetically predisposed individuals who are more sensitive to environmental exposures compared to non genetically predisposed.

Key words: non screen detected prostate cancer; family history; diagnostic radiation, male pattern baldness, right hand pattern, acne, gene and environmental interaction, prostate cancer risk.

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Abstracts accepted and conferences

1. **Hand pattern and prostate cancer risk**, AACR 101st Annual meeting 2010, April 17-21 at Walter E. Washington Convention Centre, Washington DC, USA.

2. **Omega 3 polyunsaturated fatty acids (PUFAs) and risk of early onset prostate cancer**, AICR Annual Research Conference on food, nutrition, physical activity & cancer, from 21-22 October 2010, Washington DC, USA.

3. **Hand pattern and early-onset prostate cancer risk**, EuroEpi Conference 2010, to be held on 6th-9th November, 2010 in Florence, Italy.

Papers related to the study

1. *Paper published:* **Dietary fat and early-onset prostate cancer risk,** British Journal of Nutrition (2010), 103(9):1375-80

2. *Paper accepted:* **Hand pattern indicates prostate cancer risk**, British Journal of Cancer.

3. *Paper submitted:* **Association of common variation in Kallikrein genes KLK5, KLK6, KLK12 and KLK13 with risk of prostate cancer and tumour aggressiveness**, Cancer Epidemiology, Biomarkers & Prevention.

4. *Paper in write-up:* **Omega 3 polyunsaturated fatty acids (PUFAs) and risk of early onset prostate cancer.**

Abbreviation

ACS	American Cancer Society
AGA	Androgentic Alopecia
AR	Androgen Receptor
BAUS	British Association of Urological Surgeon's
BEIR	Biological Effects of Ionizing Radiation
BER	Base Exision Repair
BPC3	Breast and Prostate Cancer Consortium
BPH	Benign Prostatic Hyperplasia
CAG	Cytosine, Adenine and Guanine
CR UK	Cancer Research United Kingdom
DHEAS	Dehydroepiandrosterone Sulphate
DHT	Dihydrotestosterone
DNA	Deoxyribonuleic Acid
DRE	Digital Rectal Examination
DSBs	Double Strand Breaks
GCSE	General Certificate of Secondary Education
GP	General Practice
GWAs	Genome wide Association study
HRR	Homologous Recombination Repair
htSNP	Haploytype-Tagging Single Nucleotide Polymorphism
ICR,UK	Institute of Cancer Research, United Kingdom
LET	Linear Energy Transfer
LH	Luteinizing Hormone
MMR	Mismatch Repair
MPB	Male Pattern Baldness
NER	Nucleotide Exision Repair
NHEJ	Non-homologous End Joining
NHS	National Health Service
PAP	Prostatic Acid Phosphatase
PCRF	Prostate Cancer Research Foundation
PIN	Prostatic Intraepithelial Neoplasia
PS	Sample Size Programme
PSA	Prostate Specific Antigen
RMH	Royal Marsden Hospital

SI Units	International System of Units
SIRs	Standardized Incidence Ratios
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical Package for the Social Science
TNM	Tumour, Nodes, Matastasis (TNM) Staging System
TRUS	Transrectal Ultrasound
TURP	Trans Urethral Resection of the Prostate
UK	United Kingdom
UKAEA	United Kingdom Atomic Energy Authority
UKGPCS	UK Genetic Prostate Cancer Study
UTIs	Urinary Tract Infections

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Chapter 1 Prostate and it's cancer and general demographic features of study population

This chapter aims to describe basic structure and function of prostate gland along with its pathology. The review of the literature includes both clinical as well as epidemiological aspects of prostate cancer. Clinical aspects include symptoms and different diagnostic procedures including a brief review on prostate specific antigen (PSA). It also covers staging and grading of prostate cancer. The epidemiological part presents the recent incidence, prevalence, survival and mortality rates of prostate cancer.

There is also overview of general demographic features of the study population. General demographic features are also discussed by comparing data with previous evidence from different epidemiological studies.

1 Literature review

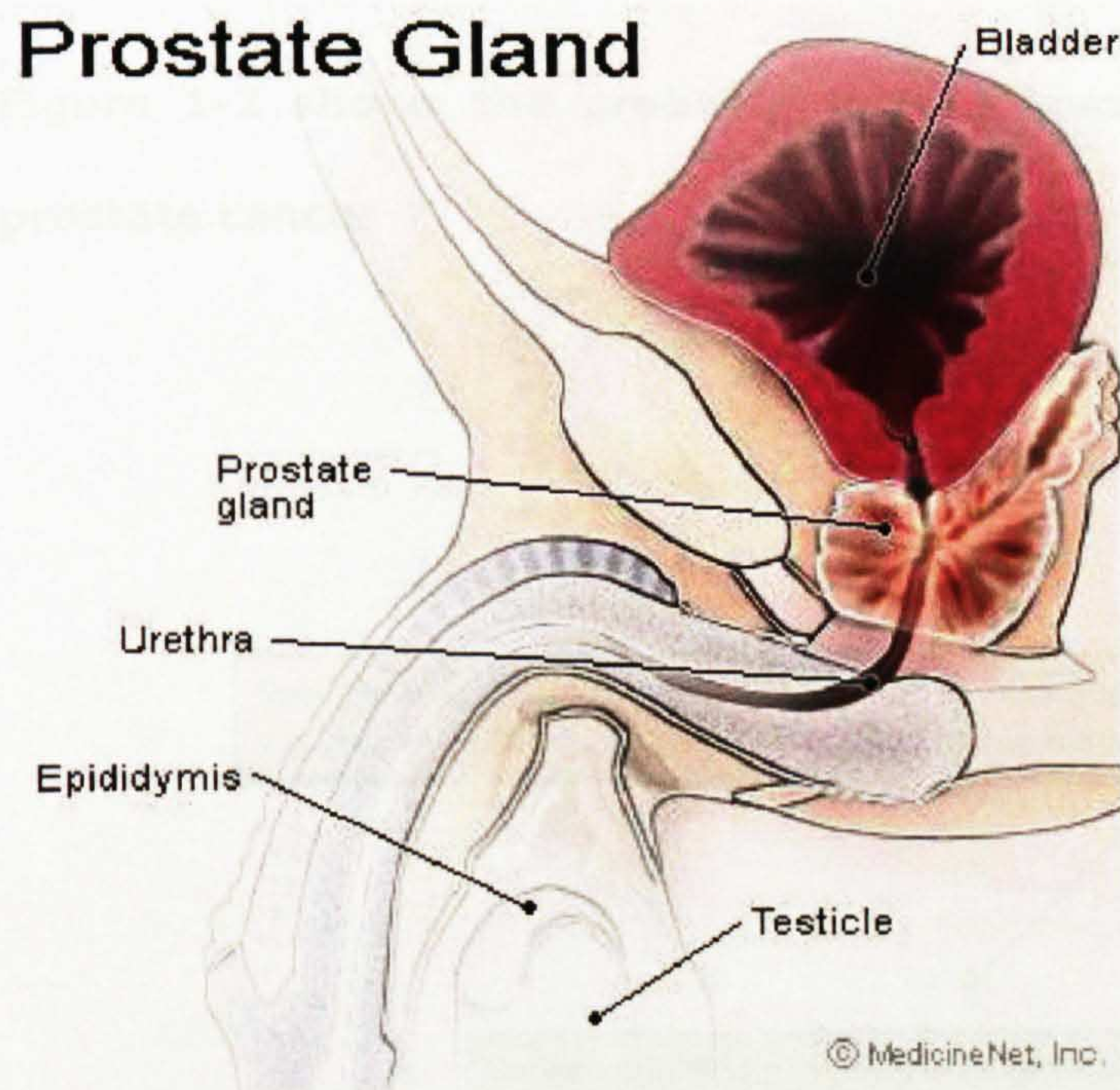
1.1 Anatomy and physiology of the prostate

The prostate is an encapsulated epithelial gland located under the urinary bladder and it surrounds the upper part of the urethra (see Figure 1-1). The prostate gland is divided into three zones, the peripheral, transition and central zones. The peripheral zone comprises the major portion of the prostate gland (Kirby *et al*, 1996).

The main function of the prostate gland is to produce prostatic secretions. These secretions contribute about 20% of the volume of seminal fluid and have neutralizing effect on acidic vaginal secretions for increased sperm viability and

contain enzymes responsible for the formation of a seminal clot to retain sperm in the female reproductive tract (Sherwood, 2004).

Figure 1-1 Anatomy of the prostate gland



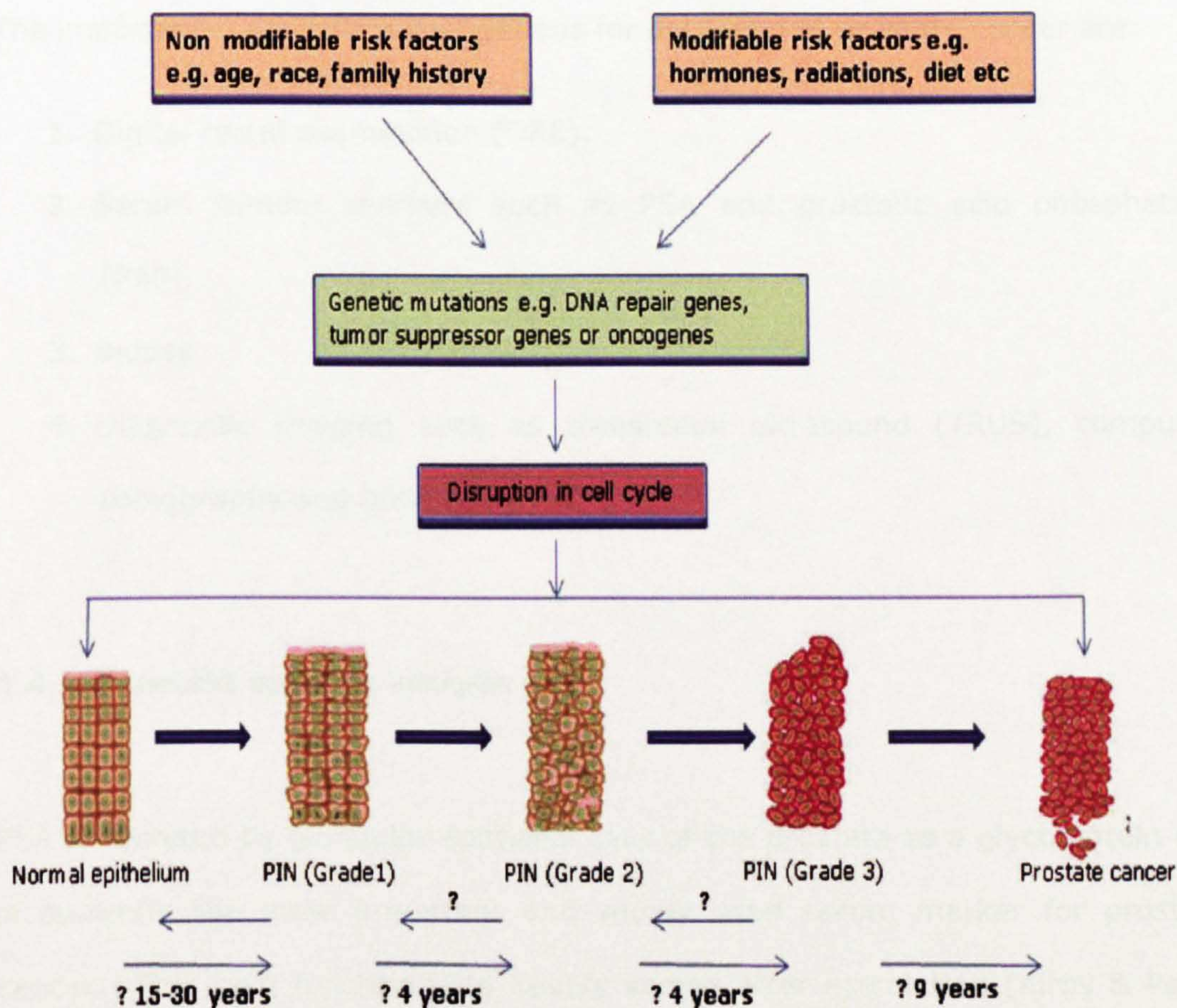
(Figure adopted from www.medicinenet.com/prostatecancer/article.htm)

1.2 Pathogenesis of prostate cancer

The majority of prostate cancers (>70%) are classified as adenocarcinoma or glandular cancer, arising from normal semen-secreting prostate gland cells near the capsule from the peripheral zone and nearly 5-10% arise from central zone and the remaining from the transition zone, which is a common site for benign prostatic hyperplasia (BPH) (Devita Jr *et al*, 2005; Kirby & Patel, 2009). The exact pathogenesis of prostate cancer is not known but it has been suggested that a condition known as carcinoma in situ or prostatic intraepithelial neoplasia (PIN) classified as low or high grade leads to the development of invasive carcinoma (Willis & Wians Jr, 2003). PIN is usually associated with cancers

arising from the peripheral zone, the most common site for prostate cancer (Kirby *et al*, 1996). Number of factors affecting the process of oncogenesis of prostate cancer such as age, race, family history, environmental factors e.g. diet, radiations etc, steroid hormones (testosterone), but the process of oncogenesis yet to be cleared (see Figure 1-2).

Figure 1-2 shows the probable factors involved and pathogenesis of prostate cancer



1.3 Clinical features of prostate cancer

Early and localised prostate cancer usually causes no symptoms and is often diagnosed during a routine investigation. The main symptoms of localised spread are increased frequency of urination, urgency, poor stream, haematuria and sometimes blood in the semen. Bone pain is often felt by patients with bone metastasis, the first and most common site of metastasis. Metastatic disease to the spinal cord produces weakness and numbness in the lower extremities due to cord compression (Tanagho & McAninch, 2004).

1.4 Diagnosis of prostate cancer

The important diagnostic investigations for detection of prostate cancer are:

1. Digital rectal examination (DRE).
2. Serum tumour markers such as PSA and prostatic acid phosphatase (PAP).
3. Biopsy.
4. Diagnostic imaging such as transrectal ultrasound (TRUS), computed tomography and bone scan.

1.4.1 Prostate specific antigen

PSA is secreted by glandular epithelial cells of the prostate as a glycoprotein and is currently the most important and widely used serum marker for prostate cancer. The main function is to liquefy semen after ejaculation (Kirby & Patel, 2009). There is no conventional cut off point for PSA as in the past the 4.0ng/ml was used as cut-off point, but new data showed that in men younger than 60 years a cut-off of 2.5ng/ml can double the cancer detection rate from 18-36% with minimal negative effect on specificity. As PSA is organ specific not cancer

specific, PSA level can also increase in the other conditions other than prostate cancer such as BPH, prostatitis, urinary tract infections (UTIs), perineal or prostatic trauma, recent ejaculation and even bicycle riding and can give false positive and false negative results.

Since the start of PSA screening in 1986 the detection of early prostate cancer became much easier but the PSA sensitivity and specificity remain controversial and for that several other more specific PSA derivatives are now used to improve the clinical value of the test such as PSA density, PSA velocity, Age specific reference ranges and different molecular forms such as free PSA and free: bound ratio (Berger *et al*, 2007; Kirby & Patel, 2009; Potter & Carter, 2000).

The clinical usefulness of PSA density and velocity has potential limitations such as in PSA density, volume calculation (usually measured by trans-rectal ultra sound [TRUS]), PSA variability (shows natural fluctuation) and sampling bias (Kirby & Patel, 2009) and in PSA velocity, is its too few measurements within short period of time (at least three values over one or two years) which could be inaccurate as PSA levels show natural fluctuations (Kirby & Patel, 2009).

Age specific ranges to improve cancer detection in younger men are based on the concept of by lowering the normal range of PSA level and to have more age-specific references values. Based on evidence from different studies that there are variations in PSA serum level of age-specific reference groups in different races such is lower in Japanese and higher in African-American as compared to white men. May be because of different genetic/physiological composition, it is

now crucial to have different age-specific cut-off points for different races (Morgan *et al*, 1996; Oesterling *et al*, 1995).

Ratio of free (unbound) to total PSA helps in distinguishing between BPH and prostate cancer because, due to unknown reasons free PSA is lower in prostate cancer as compared to BPH (Elabbady & Khedr, 2006; Zucchelli *et al*, 1997). However, many clinicians are still reluctant to adopt these modifications because of unclear benefits (Wilbur, 2008) and still 4.0ng/ml is used as standard cut-off point to consider biopsy.

1.4.1.1 Screening for prostate cancer

Screening of prostate cancer is based on DRE and serum PSA level (Stenman *et al*, 1994). PSA is the single most effective screening test available for early detection of prostate cancer and can detect twice the number of prostate cancer cases compared to DRE only. But its predictive value increases if combined with DRE (Kirby & Patel, 2009). In recent years, prostate cancer screening using the PSA has gained attention in many parts of the world and this may be one of the reasons for the steep rise in prostate cancer incidence (Coldman et al, 2003). Because it is evident from different reviews that the benefits of widespread use of this test are still unclear, the government policy in the UK for the National Health Service (NHS) prostate cancer programme, supports this view, with the addition of informed decision of the individual (Donovan et al, 2001).

In the UK and other European countries, PSA testing is not recommended for screening because:

- Some men with prostate cancer do not have a raised PSA level.

- 2 out of 3 men with a raised PSA do not have prostate cancer.
- Natural history of prostate cancer poorly understood.
- There is uncertainty about the best way to treat early prostate cancer.
- The treatments can cause unpleasant side effects (NHS, 2008; Schroder, 2005; Selley *et al*, 1997; Thompson *et al*, 2004).

PSA screening also results in overdetection and lead time bias (the difference in time between screen detection and clinical detection in the absence of screening). Lead-time bias is estimated to be 5-12 years, depending on men's age at screening (Draisma *et al*, 2003; Parker *et al*, 2006; Pashayan *et al*, 2006).

According to the American Urological Association and American Cancer Society (ACS) guidelines, the PSA and Digital rectal examination (DRE) should be suggested every year, starting from the age of fifty, to men who have a life expectancy of ten years. Prior to that informed choice with the help of a clinician must be obtained (Smith et al, 2003).

Recently, the findings from a large European trial suggested that unnecessary biopsies can be avoided by using individual prostate cancer risk prediction. The risk can be calculated using the risk calculators combining for example the logarithmic transformations of prostate volume and prostate-specific antigen (PSA), digital rectal examination, previous biopsy status, and age (Cavadas *et al*, 2010). Results from another recent European Randomized Study of Screening for Prostate Cancer evaluating the effect of screening with prostate-specific–antigen (PSA) testing on death rates from prostate cancer showed reduction in mortality rates by 20% but was associated with increased risk of over diagnosis (Schröder *et al*, 2009).

1.4.1.1.1 Active Surveillance

Nearly 50-80% of all prostate cancer cases detected by PSA are over diagnosed and remained asymptomatic even without treatment. On the other hand it is also responsible for >9000 deaths/year in the UK. Therefore it is important to distinguish patients who actually need treatment from those who merely need careful monitoring. Active surveillance is relatively new method of closely monitoring low risk prostate cancer cases to avoid unnecessary treatment. It involves close monitoring of PSA, with repeat biopsy. The decision for starting treatment based on evidence of disease progression (PSA doubling time or by looking at grading 'upgrading' at repeat biopsy). Active surveillance aims to reduce the burden of side effects from treatment without compromising survival. It is different approach as compare to watchful waiting, in which if treatment is required should be palliative (Hardie *et al*, 2005).

1.4.2 Staging and grading of prostate cancer

1.4.2.1 Staging

There are two staging systems to evaluate the spread of prostate cancer, (1) the Jewett-Whitmore system and (2) the Tumour, Nodes, Metastasis (TNM) staging system. TNM is commonly used staging system in UK. This system assesses the tumour size, the number of lymph nodes involved and the presence of metastasis (Schroder *et al*, 1992; Selley *et al*, 1997).

The TNM classification (Kirby & Patel, 2009; Wittekind.C *et al*, 2005)

Primary tumour

TX Primary tumour cannot be assessed

T0 No evidence of primary tumour

T1 Clinically inapparent tumour not palpable or visible by imaging

T1a Tumour incidental; histological finding in 5% or less of tissue resected

T1b Tumour incidental; histological finding is more than 5% of tissue resected

T1c Tumour identified by needle biopsy (e.g. because of elevated PSA)

T2 Tumour confined within the prostate[1]

T2a Tumour involves 50% or less of one lobe

T2b Tumour involves more than 50% of one lobe but not both lobes

T2c Tumour involves both lobes

T3 Tumour extends through the prostatic capsule[2]

T3a Extracapsular extension (unilateral or bilateral)

T3b Tumour invades seminal vesicle(s)

T4 Tumour is fixed or invades adjacent structure other than seminal vesicles, bladder neck, external sphincter, rectum, levator muscles and/or pelvic wall

Regional lymph nodes

Nx Regional lymph nodes cannot be assessed

N0 No regional lymph node metastasis

N1 Regional lymph node metastasis

Distant metastasis[3]

Mx Distant metastasis cannot be assesed

M0 No distant metastasis

M1 Distant metastasis

M1a non-regional lymph node(s)

M1b Bone(s)

M1c Other site(s)

[1] Tumour found in one or both lobes by needle biopsy, but not palpable or visible by imaging, is classified as T1c.

[2] Invasion into the prostatic apex or into (but not beyond) the prostatic capsule is not classified as T3, but as T2.

[3] When more than one site of metastasis is present, the most advanced category should be used.

1.4.2.2 Grading

The Gleason grading is widely recognised and frequently used to classify the tumour because it is reproducible, simple and predictable. This system has prognostic significance and value in selecting the treatment of choice. This is the most common grading system used in the UK, based on the evaluation of cyto-architectural details of individual cancer cells (Miller & Torkko, 2001).

Five distinct patterns of growth from well to poorly differentiated are described on a scale from pattern 1 to 5 (Kirby & Patel, 2009).

Table 1-1 Five stages of Gleason grading for prostate cancer and their histological features

Grade	Histological features
1	small, uniform glands with minimal nuclear changes
2	medium size acini, More closely arranged but still separated by stromal tissue
3	marked variation in glandular size and organization with infiltration of stromal and neighbouring tissues(most common finding)
4	marked atypical cells with extensive infiltration
5	sheets of undifferentiated cancer cells

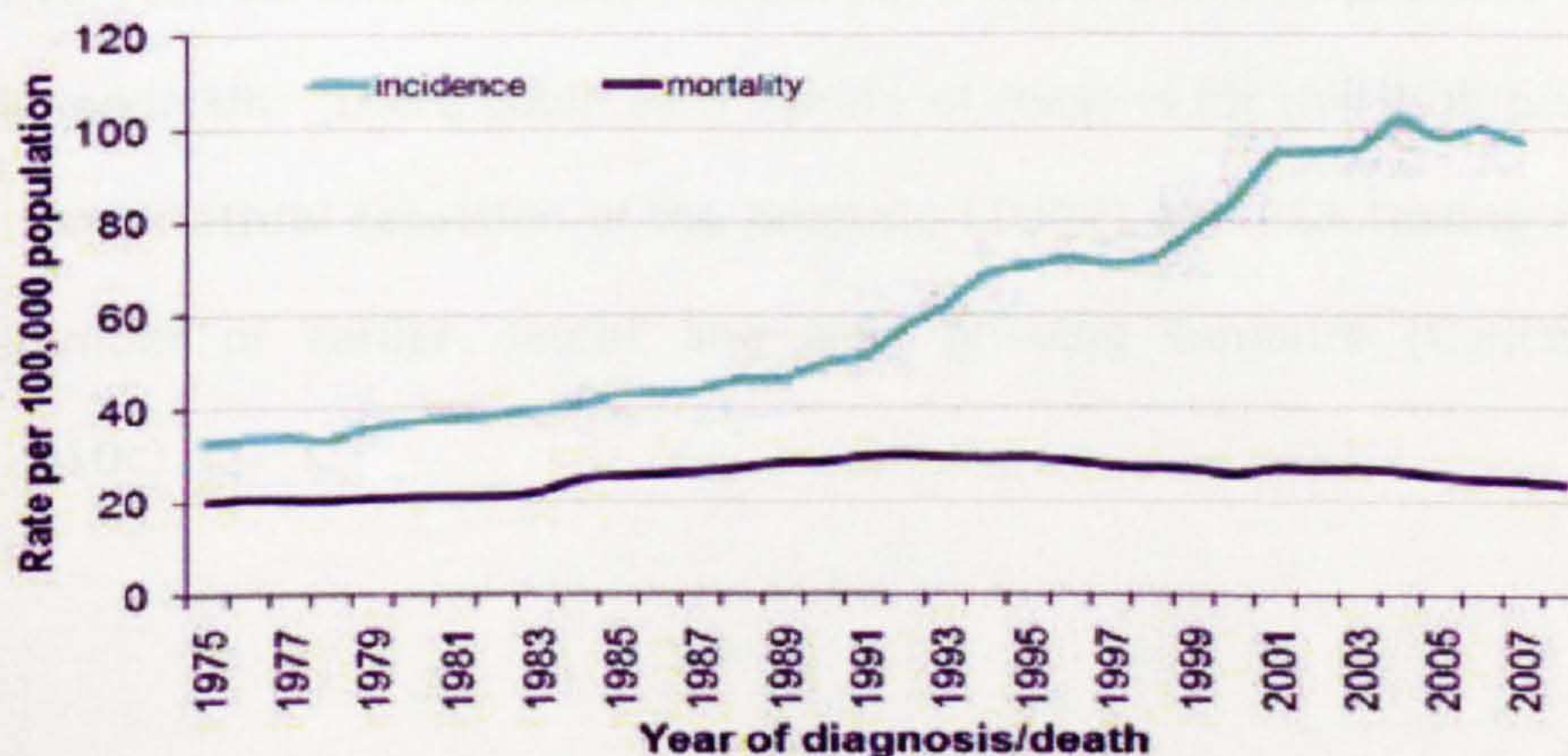
The final Gleason score is the sum of grades of the primary and secondary growth pattern. Mostly prostate tumours have a Gleason score of ≥6 (True et al, 2006).

1.5 Epidemiology

1.5.1 Incidence

According to Cancer Research UK, more than 34,000 new cases are diagnosed every year, making it the most common cancer in men in the UK. It has been reported that there is significant increases in incidence of prostate cancer in many countries, including the UK (Hsing *et al*, 2000). From 1975 to 2007 there was threefold rise in prostate cancer incidence with 33/100,000 in 1975 to 97/100,00 in 2007 (see Figure 1-3). This steep rise in the incidence may be due to widespread use of PSA in UK, it was estimated that around 5-6% of men over the age 40 have PSA test each year (Cancer Research UK, 2010c; N Ireland Cancer Registry, 2010; Office for National Statistics, 2010).

Figure 1-3 Age standardized incidence rate, Prostate cancer GB, 1975-2008



Taken from the webpage of Cancer Research UK (2010)

There are wide international and inter-ethnic differences in prostate cancer incidence (Gronberg, 2003; Hass & Sakr, 1997). In 2008, an estimated 913,000 men were diagnosed with prostate cancer worldwide and more than two third were in developed countries (Andriole *et al*, 2009; Ferlay. J *et al*, 2010). These differences may be due to genetic variations, exposure to external risk factors or may be due to different diagnostic modalities and variations in cancer registration and differences in health care provision. Another important reason for these variations in incidence rate may be the differences in life expectancy, as the prostate cancer is an age related disease (Kirby *et al*, 1996). Though geographical variations in prostate cancer in England and Wales were not clear but the incidence rate is slightly higher in north England than in the south (Quinn & Babb, 2000).

1.5.2 Prevalence

In UK, the prevalence of prostate cancer is high because of high incidence and five-year survival rate after diagnosis. Nearly 215,000 prostate cancer cases are living in UK. There could be a variety of reasons for this high prevalence such as trans urethral resection of the prostate (TURP) and PSA testing revealing greater number of earlier, latent and slow growing tumours (Cancer Research UK, 2010c).

1.5.3 Survival rate

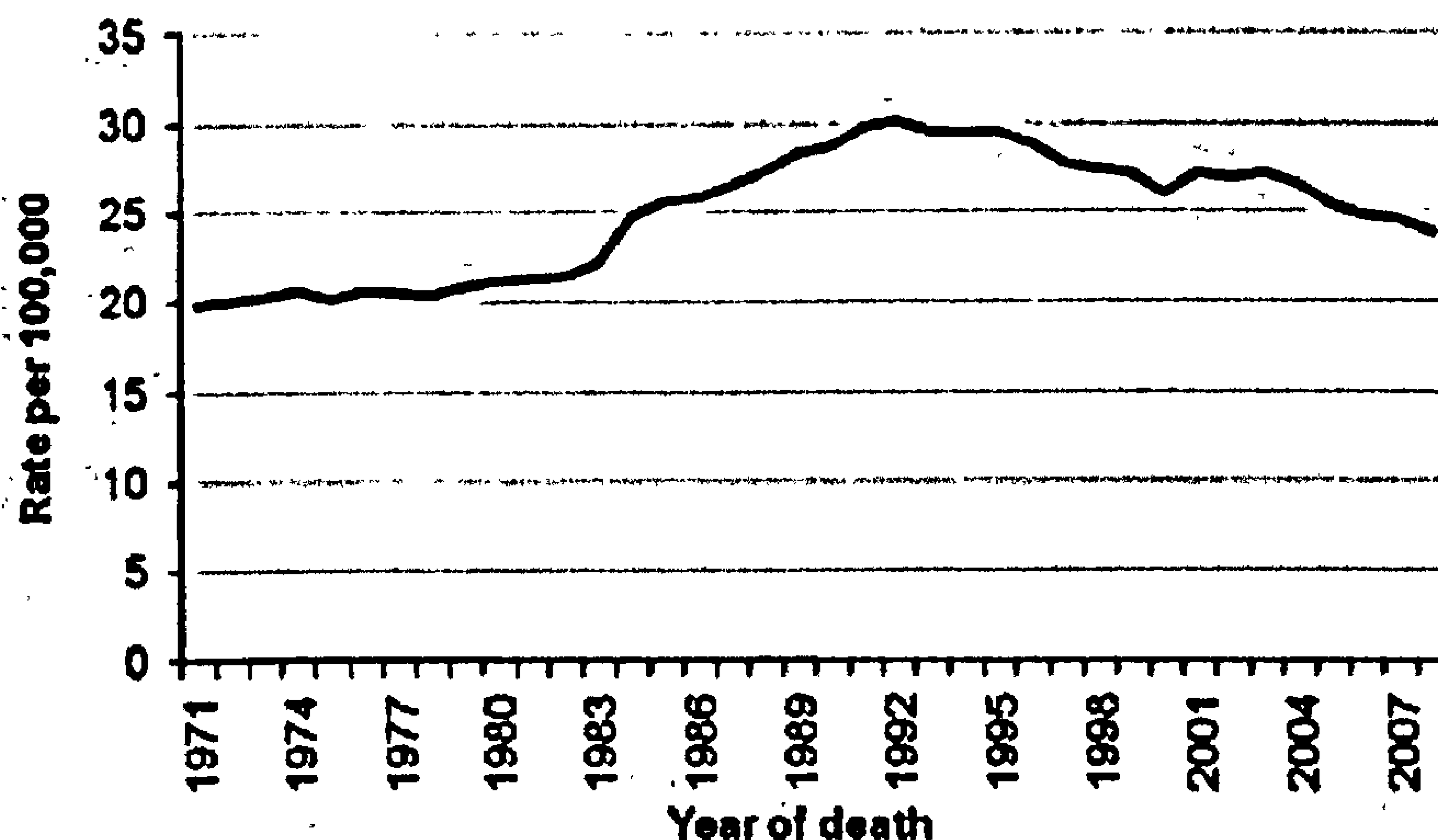
Although the incidence of the disease has been on the rise during last 20 years, the survival rates have improved during this period with a relative five year survival rate of 77% during 2001-2006, compared with 31% during 1971-75.

The survival rate depends upon the stage of disease at the time of diagnosis , if it is metastatic by that time five-year survival rate become much lower (30%) (Cancer Research UK, 2010c; Office for National Statistics (ONS), 2007).

1.5.4 Mortality rate

There were about 10,168 deaths occur in UK from prostate cancer in 2008. Nearly 12% men died in UK from prostate cancer, making it most common cause of death after lung cancer. Mortality for prostate cancer in UK is 23.9/100,000 in 2008 (Cancer Research UK, 2010d; Etzioni *et al*, 1999; ISD Online, 2010; Office for National Statistics, 2009). As the graph shows, mortality is on decline as compare to 1990's. This may be due early detection and improved treatment.

Figure 1-4 Age standardized mortality rate, Prostate cancer GB, 1975-2008



Taken from the webpage of Cancer Research UK (2010)

1.6 Review of demographic factors of prostate cancer

1.6.1 Age

Age is an established risk factor for prostate cancer. It is common in the elderly population and with an increasing aging population, prostate cancer is becoming one of the major public health problems (Hass & Sakr, 1997). In 65% of men aged >80 years, prostate cancer remains symptomless and only revealed at the time of post mortem examination (Pienta & Esper, 1993). There is evidence from epidemiological studies that despite prostate cancer being an age related disease, its onset might start at a young age due to substantial increase in androgens at the time of puberty (Diamandis & Yu, 1996). Delongchamps et al, 2006, however, stated age as a controversial risk factor, they suggested that it is not age directly that is responsible, but aging probably provided the time necessary for the cumulative effects of environmental exposures and cellular changes essential for the development of a carcinogenic lesion (Delongchamps *et al*, 2006).

1.6.2 Ethnicity

International variations in prostate cancer incidence have suggested the crucial role of a variety of environmental risk factors, including ethnicity. According to previous studies, three-quarters of all incident cases occur in developed countries with the highest incidence in North America and lowest rates in Asian countries. In the UK, black Caribbean and black Africans have 2-3 times more risk of developing prostate cancer than the white men (Ben-Shlomo *et al*, 2008; Cancer Research UK, 2007; Jack *et al*, 2007; Wild *et al*, 2006). Morton reported

racial differences in adenocarcinoma of the prostate in North American men. The disease is more frequently developed in black Americans and who also have a worse prognosis as compared to white Americans (Hass & Sakr, 1997). There could be a variety of explanations for these racial differences like genetic factors, dietary patterns, diagnostic approaches and access to care (Bostwick *et al*, 2004).

1.6.3 Social class

In UK social class is determined by considering the employment status and occupation. There are 6 categories of social class, class I-Professional, class II-Managerial and technical occupations, class III-(N) skilled non-manual and class III-(M) skilled manual, IV-Partly skilled occupations, V- Unskilled occupation (HMSO, 1991). Evidence that social class influences prostate cancer risk remains inconclusive. Evidence from previous studies suggested that prostate cancer incidence is higher in high social class but poorer outcome is associated with low social class (Gilligan & Gilligan, 2005).

Occupation is of key importance in determining social class. There is evidence from epidemiological studies that few occupations have 7-12% increased risk of prostate cancer such as farmers and other agricultural workers and this may be due to high consumption of meat and fatty diet and exposure to certain chemicals used in agriculture. There is also high risk of prostate cancer in workers in heavy industry, news paper printing and rubber manufacturing. Possible explanation for high incidence may be chemical exposure or other hidden factors present in the working environment (Hsing & Chokkalingam, 2006).

1.6.4 Education

Evidence is very limited for education as a risk factor of prostate cancer and discussed in the discussion part of this chapter.

1.6.5 Marital status

Evidence from several previous epidemiological studies for marital status as a risk factor for prostate cancer is inconsistent (Newell *et al*, 1989; Newell *et al*, 1987; Talaminil *et al*, 1986). But as a social indicator, marital status may help in alleviating different stresses of life by leading to more sober and disciplined lifestyle and help protecting against various social disparities, which may protect from prostate cancer risk (Nielsen *et al*, 2007).

1.7 The study hypotheses and aims

After close examination of literature presented in this thesis, this study proposes to meet following study hypotheses and aims:

The study hypotheses

1. Prostate cancer risk is increased with positive family history of prostate and breast cancer.
2. Prostate cancer risk increased after an exposure to low-dose ionising radiations.
3. There is an association between surrogate markers of male hormones (hand pattern, acne, balding) and prostate cancer risk.

4. There are possible interactions between environmental exposures including low dose diagnostic radiation, balding, acne and selected single nucleotide polymorphisms.

The study aims

This study has 2 main aims. The first aim is to examine associations of selected environmental exposures such as family history of prostate cancer in the first degree relatives, low dose diagnostic radiation, surrogate markers of male hormones (hand pattern, acne, balding) and prostate cancer risk. The second aim is to start to explore possible interactions between environmental exposures including low dose diagnostic radiation, balding, acne and selected single nucleotide polymorphisms that are suggested to relate with disease aetiology/pathways.

More specific hypotheses and aims for each of these investigations are described in each chapter.

1.8 Demographic features of study population

In this chapter, the following basic demographic features were analysed and discussed.

1.8.1 Response rate

Case and control response rate was assessed individually and was calculated on the bases of returned questionnaires as compared to sent number of questionnaires.

1.8.2 Age

Data on age for cases was received from GPs and from Royal Marsden Hospital and for controls from different GP practices from where they had been selected. If age at diagnosis was missing due to any reason, age at diagnosis was calculated by subtracting diagnosis date (available with the case downloads) from date of birth.

1.8.3 Ethnicity

The questionnaire used in study has ten different ethnic groups white, Black-Caribbean, Black-African, Indian, Pakistani, Jewish, Sephardic, Ashkenazi, Chinese and Other. Due to very small number of subjects in last three groups (Sephardic, Ashkenazi and Chinese), they was merged in group "other".

1.8.4 Social class

Social class was determined using individual's longest held job and classified into 6 classes class I-Professional, class II-Managerial and technical occupations, class III-(N) skilled non-manual and class III-(M) skilled manual, class IV-Partly skilled occupations, class V- Unskilled occupation (HMSO, 1991). For analysing

the risk of prostate cancer social class was used as trichotomous variable based on similarities of class. Social class 1=I-II, 2= III-IV, 3=V-VI.

1.8.5 Education

Education level was classified into four groups. First group with no formal education, second GCSE, O level or equivalent, third group with A levels or equivalent and fourth group was higher and professional qualifications, this group also includes other with higher education.

1.8.6 Marital status

In marital status, married and common law partners were merged in one group and widowed, divorced or separated were merged in other group. Third group was single.

1.9 Results

1.9.1 Response rate

The response rates of study participants is shown in table 1-2

Table 1-2 Response rate of cases and controls

Study phase	Case (%)	Control (%)
First phase	78.9	61.8
Second phase	91.0	87.0
Total	85.0	74.4

The overall response rate for cases was 85.0% and for controls 74.4%.

1.9.2 Age

Table 1- 3 shows age statistics

Table 1-3 Age at diagnosis

Group	Number	Median	Age range
Case	1112	60	36-85
Control	1872	59	36-76

Cases on average are slightly older than control

1.9.3 Ethnicity

Table 1-4 below illustrated distribution values of ethnic groups

Table 1-4 Distribution of ethnic groups

Ethnic Group	Case (%)	Control (%)
White	1055(96.4)	1829(98.7)
Black-Caribbean	13(1.2)	2(0.1)
Black-African	5(0.5)	3(0.2)
Indian	7(0.6)	7(0.4)
Pakistani	2(0.2)	0(0.0)
Jewish	4(0.4)	5(0.3)
Other	8(0.7)	7(0.4)
Total	1094(100.0)	1853(100.0)
Missing	*18*	*19*

Chi-square test, p-value 0.000

The majority of study subjects are Caucasian (over 90%).

1.9.4 Social class

Table1- 5 shows the distribution and estimated risk values of social class

Table 1-5 Distribution and risk estimates of social class

Social class	Case (%)	Control (%)	OR†	95%CI Lower-upper
I&II	577(53.9)	978(54.9)	1.00	
III & IV	401(37.4)	673(37.8)	1.04	0.88-1.22
V & VI	93(8.7)	130(7.3)	1.26	0.94-1.67
Total	1071(100.0)	1781(100.0)	*P for trend*	*0.23*
Missing	*41*	*91*		

†Adjusted for age
Class I-Professional, Class II-Managerial and technical occupations, Class III-(N) skilled non-manual and class III-(M) skilled manual, Class IV-Partly skilled occupations, Class V- Unskilled occupation

Results indicate that social class distribution is similar between case and control group and is not associated with prostate cancer risk (p for trend 0.23).

1.9.5 Education

Table 1-6 shows distribution values and odds ratio of education

Table 1-6 Education and prostate cancer risk

Education level	Case (%)	Control (%)	OR†	95%CI Lower-upper	p-value
None	294 (27.1)	531(28.7)	1.00		
GCSE or O level	198(18.3)	308(16.7)	1.25	0.98-1.59	0.07
A level	74(6.8)	134(7.2)	1.05	0.75-1.48	0.76
Higher or Professional	518(47.8)	876(47.4)	1.16	0.94-1.43	0.18
Total	1084(100.0)	1849(100.0)			
Missing	*28*	*23*			

†adjusted for age and social class

Almost 50% of cases and controls reported having had achieved higher education or professional level. About a quarter of study subjects had no education. Odd ratios suggest that education level is not associated with prostate cancer risk.

1.9.6 Marital status

Table 1-7 shows the marital status and prostate cancer risk.

Table 1-7 Marital status and prostate cancer risk

Marital status	Case (%)	Control (%)	OR†	95%CI Lower-upper
Married or partner	906(82.6)	1572(84.8)	1.00	
widowed or divorced or separated	140(12.8)	228(12.3)	1.05	0.83-132
Single	51(4.6)	54(2.9)	1.63	1.10-2.41
Total	1097(100.0)	1854(100.0)		
Missing	*15*	*18*		

†adjusted for age and social class

About 80% of both case and control were still married or living with their partner. Being Single shows a moderate increase risk with (OR 1.63 and 95% C.I. 1.10-2.41).

1.10 Discussion

1.10.1 Response rate

Response rate is one of the essential elements of epidemiological studies as it provides information on the proportion of the targeted population who has participated (Slattery *et al*, 1995). In this study, the response rate for the first phase was 78.9% for cases and 61.8% for controls and for the second phase response rate for cases 91.0% and 87.0% for controls. The overall response rate for cases was 85.0% and for controls 74.4%. Cases responded more than controls. This may be due to their illness and therefore are more receptive. Achieving high response rate is vital in case-control studies as it decreases the chance that selection bias have any impact on the results (Colt *et al*, 2005).

In summary, the study has yielded a good response rate from both groups.

1.10.2 Age

Median age for cases was 60 years and for controls it was 59 years. The findings of most of the previous studies support increasing age as a strong risk factor for prostate cancer, though it can also occur at a young age (Veldhuizen *et al*, 2006). Clinical prostate cancer is rare under fifty years of age as compare to higher incidence in men age over sixty (Kirby & Patel, 2009). After fifty years of age incidence increase steadily and it is at faster rate as compare to any other malignancy (Hass & Sakr, 1997). While Bostwick et al, 2004 stated that it is oxidative stress accumulate with aging may be the one of risk factor for the prostate cancer but evidence on this theory is limited (Bostwick *et al*, 2004).

In this study case and controls were age-frequency matched thus age was not computed for estimated risk.

1.10.3 General characteristics

1.10.3.1 Ethnicity

The majority of the study population is Caucasian (>95%). There were no differences in the ethnicity in cases and controls. Globally, the incidence of prostate cancer varies with ethnicity, with highest incidence in black Americans with very poor prognosis and lowest incidence is among men in China and Japan (Whittemore *et al*, 1995).

Though the previous data suggest that ethnicity is a well established risk factor for prostate cancer, it still needs to be investigated as to whether ethnicity is a direct cause or there are other factors which are closely related with different ethnic backgrounds that affect quality of life such as socioeconomic status, education, awareness and access to health care.

1.10.3.2 Social class

The proportion of cases and controls are fairly similar in each social class although the highest percentage was found in social class I and II. There was no significant association between social class and prostate cancer risk. Similar findings have been previously reported showing no association between social class and prostate cancer risk (Severson *et al*, 1989). The higher percentages in

upper classes may be due to overall improvement in socioeconomic status of UK population over the years. Possible explanation of almost similar percentages of cases and controls in each class is may be due to successful random selection procedure of controls.

However, some studies showed that prostate cancer risk was associated with high socioeconomic status (Bouchardy *et al*, 2002; Harvei & Kravdal, 1997; Lund Nilsen *et al*, 2000). In contrast, some studies have demonstrated an increasing trend of prostate cancer with decreasing socioeconomic status (Hass & Sakr, 1997). One study revealed that incidence is higher with high socioeconomic status on the other hand mortality was higher in low socioeconomic class (Cheng *et al*, 2009).

The majority of earlier studies failed to demonstrate any association between social class and prostate cancer risk. One of the explanations for the high percentage in the upper social classes is that it may be possible that these people have more access to health care and have more positive attitudes towards health care.

1.10.3.3 Education

The results of this study indicate that there is no association between education level and prostate cancer risk (all confidence interval include 1). Reported evidence is limited and inconclusive for education and prostate cancer risk. Similar findings have been previously reported (Ewings & Bowie, 1996; Ilic *et al*, 1996; Key *et al*, 1997; Severson *et al*, 1989).

The results from the case-control study conducted by Newell et al, 1989 however, had showed significant association between higher education level and prostate cancer (OR 2.10, 95% C.I. 1.10-4.04) (Newell *et al*, 1989). Also the results of a large census-based cohort study showed positive association between higher education level and prostate cancer risk. Although the education classification they had used does not match our study, they classified education in three levels and reported standardized incidence ratios (SIRs). They found that higher education level is a potential risk factor for prostate cancer (SIR=1.17, 95% C.I. 1.05-1.30). The three educational levels used in that study was basic education include only elementary education (9-10 years), shorter courses or did not complete elementary education. Second was medium education included college graduates and with vocational training a third was academic education comprising of university graduates (Vidarsdottir *et al*, 2008). These results are also supported by another cohort study in which lower education level was negatively associated with prostate cancer risk (RR 0.79, 95% C.I. 0.74-0.85) (Mouw *et al*, 2008).

As the education is one of the important indicators of social class and higher education as a risk factor for prostate cancer might not be a direct cause but suggestive of possible differences i.e. diet or other lifestyle factors and good quality health care.

1.10.3.4 Marital status

More than 80% of study population was married or has a common law partner. Being single appeared to be a heightened risk factor for prostate cancer (OR 1.63, 95% C.I. 1.10-2.41). Similar results have been found in a case-control

study in Canada with 382 cases and 625 controls having significant association among never married as compared to married men (OR 1.93, C.I. 1.08-3.44) (Fincham *et al*, 1990). The possible explanation is that single men may have unhealthy life style such as multiple sexual partners and more prone to acquire sexually transmitted diseases, which can lead to chronic infection and have some role in causation of prostate cancer (Fincham *et al*, 1990).

Some previous studies, however, suggested negative association among the single or never married compared with married men (Harvei & Kravdal, 1997; Severson *et al*, 1989). However no association was found in several other studies (Harvei & Kravdal, 1997; Ramon *et al*, 2000; Severson *et al*, 1989).

1.11 Conclusions

The study achieved overall very good response rate for both cases and controls. Age was used as a confounding factor in the present study, as it was known priori confounding factor in prostate cancer epidemiological studies. Study population was homogenous with the majority of study population was Caucasian (>95%). Social class distribution is not associated with prostate cancer risk (p for trend 0.23). Nearly 50% of the cases and controls had achieved high education and quarter of study subjects has no education. No risk is associated with education level. Being single appeared to be a risk factor for prostate cancer (OR 1.63, 95% C.I. 1.10-2.41).

Chapter 2 Study methodology

2 Background and design of the study

The Study of Gene -Environment Interactions in Prostate Cancer is an ongoing and a large scale case-control study. The study is in collaboration between the University of Nottingham (epicentre), Warwick and the Institute of Cancer Research UK. The study began in 1999 and sets out to investigate environmental exposures associated with risk and also to explore genetic components involved in disease aetiology. The data collection was divided in to two phases, the first phase collection focussed on young onset cases (≤60 years) and began in March 1999 and in December 2004, data set was frozen for the purpose of interim analysis, review/modify questionnaire, simplify/improve data collection process. The second phase started in December 2007 and data was frozen once again in September 2009. This was done to assess new leads of both genetics and environmental exposures. The second phase extended the collection to cover subjects at all ages. It is the data collection of **age equal to or greater than 60** that the author was fully responsible for the whole processes, for the age less than 60 two other research staff was responsible. The third phase is proposed to start in year 2010.

2.1 Ethical approval and funding

The study has been ethically approved by the Trent Multi Research Ethics Committee MREC/99/4/013(Mar) and 07/MRE04/29. There were main funding streams to support all epidemiological data collection and control biological sample collections including the Prostate Cancer Research Foundation (PCRF), the Cancer Research UK (CR UK). For the genetic part of the study, the study partner, the ICR was responsible for case blood collections and further genetic analysis. The work is funded by the Cancer Research UK grant C5047/A3354.

2.2 Data collection

Data consists of epidemiological data which are collected using self administered questionnaire and biological samples including toe nail clippings and 18 ml blood samples.

Details of data collection of both phases are described below

2.2.1 Subjects identification in the first phase

2.2.1.1 Case

First phase cases were identified from the British Association of Urological Surgeon's (BAUS) database and the Royal Marsden Hospital, London. These patients registered with the UK Genetic Prostate Cancer Study (UKGPCS) (see Figure 2-1). The BAUS database is a nationwide cancer registry for urologists who have notified urological cancers to the BAUS organisation. If cases had been diagnosed with prostate cancer from January 1997 to September 2004 and were $\leq$ 60 years of age they were eligible for the study. The General practice (GP) of each of the eligible cases was then identified and approached.

Figure 2-1 UKGPCS recruitment centres

Source: Professor Ros Eeles, ICR UK

Criteria for case recruitment

Inclusion Criteria

- Age ≤60 years at diagnosis.
- Men diagnosed with primary prostate cancer (Histologically confirmed).
- Currently living in the UK.
- Able to understand the information sheet and give informed consent directly or via an interpreter.

Exclusion criteria

- Age >60 years at diagnosis.
- The consultant or GP in charge considers that it would be inadvisable, for some reason, not to contact them e.g. too ill to complete the questionnaire.
- The subject's English is inadequate to understand the information provided and no translator is available.

2.2.1.2 Controls

Men aged ≤ 60 years without any history of prostate cancer were selected as a control for the first phase of study. They were randomly selected from GP practices where cases were registered. Controls were matched by age and geography. Controls were only excluded by GPs if they are too ill or unwilling to participate.

2.2.2 Subject identification in the second phase

2.2.2.1 Case

Second phase cases were identified from The Royal Marsden Hospital, London. These patients registered with the UK Genetic Prostate Cancer Study (UKGPCS). The list of cases had been received through series of case downloads from the Royal Marsden Hospital, London. These cases are either referral cases or had been notified by their consultant to the study team at the Royal Marsden Hospital.

Inclusion criteria

- Men diagnosed with primary prostate cancer at any age.
- Histological confirmed diagnosis.
- Currently living in the UK.
- Able to understand the information sheet and give informed consent.

Exclusion criteria

- The consultant or GP in charge considers that it would be inadvisable, for some reason, not to contact them e.g. too ill to complete the questionnaire.
- The subject's English is inadequate to understand the information provided and no translator is available.

2.2.2.2 Control

Age-frequency-matched men who were randomly selected from the GP practices without any history of prostate cancer. All participants have to be able to understand the information sheet and give informed consent. **Exclusion criteria for controls were identical as for cases.** In addition, who were ineligible or who were unwilling to participate were recorded and further remove from the working database.

2.2.3 The recruitment procedure

2.2.3.1 Case recruitment for the first phase

The initial approach to GPs was made to explain the study and seek their co-operation. If they were willing to take part in the study, the study group would arrange patient information sheets and consent forms to be dispatched to practices. The invitation letter was signed by the GP and printed on practice headed paper. All documents, including the invitation letter, patient information sheet, consent form and one reminder were sent out via GP practices until the consent was given or if no reply was received within 4 weeks, no further follow-up was made. Patient consent forms were returned to the epicentre; and personal information including study ID, NHS number, name, date of birth, and contact details was then be recorded onto database. Once patients consented to fill the questionnaire and provide biological samples including blood, toe nail clippings, the questionnaires were sent to participants and blood kit and plastic vial was sent to the practice and with the arranged phlebotomist of the practice, the blood sample was taken and sent back to the research team at the Royal Marsden Hospital. Toenail samples were sent back to the epicentre.

2.2.3.2 Case recruitment for the second phase

The Royal Marsden hospital were in charge of identifying and getting consent from eligible cases, taking blood samples and notifying epicentre if the patients gave consent to provide questionnaire data. Data was sent to epicentre through secure FTP server. Personal information including study ID, NHS number, name, date of birth, date of blood collection and contact details was recorded on the epicentre database.

As the UKGPCS consent form only covers blood sample collection and permission to participate in epidemiological study, a separate invitation letter together with the patient information sheet and consent form was sent out from epicentre. One reminder was sent via epicentre, if no reply was received within 4 weeks, no further follow-up was made. Consent form includes:

1. Completing the study questionnaire
2. Giving a toenail clipping sample (optional)
3. Providing the blood sample (optional)
4. Giving the permission for the study group to access their medical records (optional).

Once the consent form was received, a written instruction to explain the procedure, a copy of the questionnaire and/or a plastic vial/bag for toenail sample collection together with a self-addressed envelope for returning questionnaire and toenail clipping sample were sent.

A telephone helpline was provided at the back of the questionnaire to help clarify any further queries regarding the study (see the appendix). If

questionnaire/toenails were not received within four weeks, one reminder was sent without further follow-up.

Blood collection for cases was carried out by Research team at the Royal Marsden Hospital, London.

2.2.3.3 Control recruitment procedure

For both phases of data collection, there were similar approaches only the second phase controls were sought locally within the Nottingham area as well as nationally. Initially, the study was designed to use individual-matched controls (matched on age within five years and GP surgery). However, due to low response rate of GP practices, an alternative approach was introduced later on by selecting GPs from ten representative areas (one GP per one area) in the country to help identify age-frequency matched controls. Practices were asked to randomly select health controls with no prostate cancer history from their patient list. Initial approach was made by GP and participants were invited to fill out the study questionnaire, to give 2 x 9 ml of blood sample (optional) or give toenail clipping samples (optional) for further analysis. All blood samples were taken at GP practice then posted to the Royal Marsden Hospital (first phase) or the epicentre (second phase) on the same day or the next working day. All samples were logged and kept at -70^{0}c secured deep freezer.

It is noted that the study had offered to cover administrative costs for each practice. As mentioned above, controls selection was expanded to cover local area in Nottinghamshire. The reason is that the study applied a newly developed

computer program aiming to help saving GP time/workloads and as it was done locally, any technical problems could be sorted out in person very quickly to make sure the program functioned well. The procedure is described below.

The Nottingham centre

Controls from city of Nottingham had been selected from GP electronic records using series of Medical" Read" codes. The Read Codes cover a wide range of clinical terms from signs and symptoms, diagnostic tests, drug appliances, treatment and therapies received to diagnosis. A list of codes was set up to identify both prostate cancer patients and healthy control based on Read Codes versions 2 and 3. A computerised programme compatible with the GP practices working system EMIS and System One had been designed to generate a list of potential control subjects.

The list was then passed onto the GP for further checking/confirmation of their well-being. After GP validation, any subjects that were not suitable were removed from the database. Invitation letters were generated automatically from the list at the practice using installed letter template that accompanied with the program. All documents were then packed and sent out from the practice to each individual. Once subject sent their consent form back to the researcher; the next steps followed the same procedures as described above.

2.2.4 Blood collection for local controls (Nottinghamshire area)

After receiving questionnaire, the letter was sent to the participant (along with a blood sample collection pack together with an instruction letter to practice nurse/phlebotomist) to book an appointment for blood collection with their GP practice.

To facilitate the phlebotomist at different GP practice in Nottingham and to help other prostate study running simultaneously by the study group such as Benign Prostatic Hyperplasia study, the author had taken phlebotomy course at King's Mill hospital, Mansfield for seven days; this was carried out to comply with the UK regulations. A separate honorary contract was obtained to work as phlebotomist.

Blood samples were sent back to study base in Nottingham, from there, samples were sent back to the Royal Marsden Hospital for DNA extraction and genetic analysis.

2.2.5 The study questionnaire

The Questionnaire covers a wide range of topics and took no longer than 45 minutes to complete. It was well received by the target population and no complaint was raised during the study period. Information under the following broad headings from cases and controls using a structured questionnaire designed for this study are collected.

- Demographic features
- Occupation
- Hormone markers
- Smoking habits
- Sexual behaviour
- Sunlight exposure
- Family history
- Physical activity
- General health and medication including diagnostic X-ray exposure
- Anthropometric measures
- Diet

The second phase questionnaire was slightly modified from the previous one particularly the radiology section (details are described in methodology section of chapter 3).

2.3 Data entry

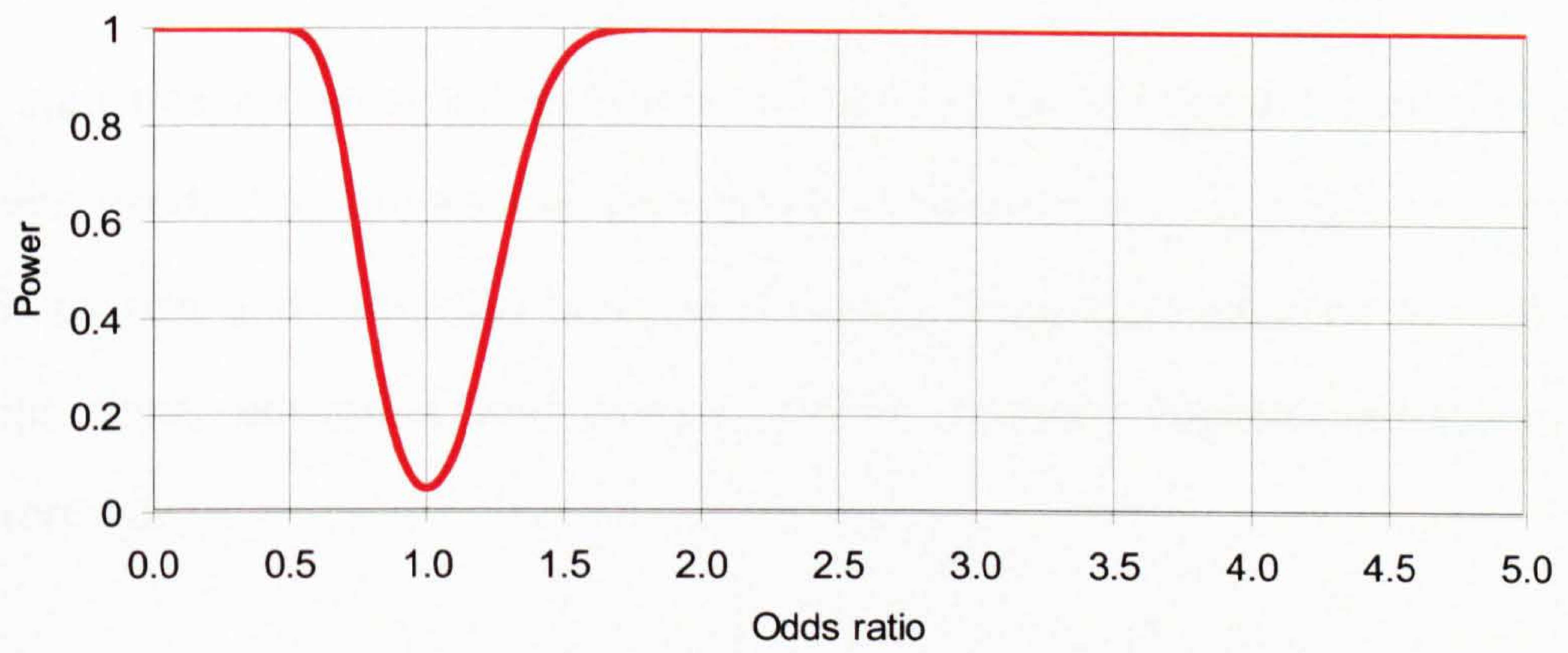
Data from the first phase was already entered and cleaned. For the second phase data entry database was created in Microsoft Access. Data was then entered in Microsoft access database and then transferred to Microsoft Excel. Data was checked thoroughly using filters in Excel. As data were entered by different people, data input was re-checked for quality control purpose. Data was checked to exclude any error using Microsoft Excel by re-entering randomly selected questionnaires and compare them with the actual data. Less than 0.5% error was found, which is negligible for large dataset like this. After that data of first and second phase was merged taking in account the difference in questionnaires of both phases. Social class coding was manually cross checked

by an expert. Recoding of variables was the next important step to make the analyses easier and flawless.

2.4 Power and sample size calculation

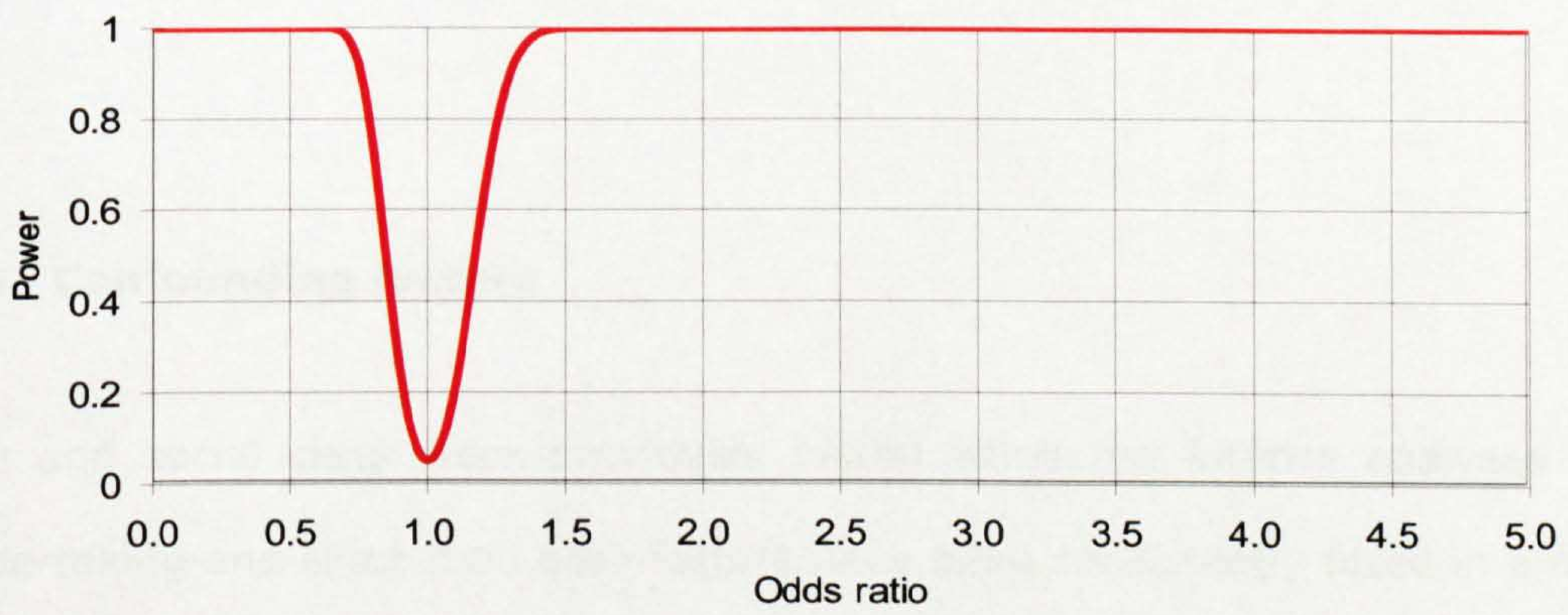
Sample size and power was calculated using power and sample size programme (PS) version 3.0.7. The total number of cases and controls in the study are 1112 and 1872 respectively. This setting will have 80% power to detect odds ratios of 1.4, 1.3, 1.2 or the same power is also able to detect risk reduction with odds ratios of 0.6, 0.7 and 0.8 when the exposure rates in controls are at 10%, 20% and 30% respectively. Alpha level was set at 0.05 for the calculations (see Figure 2-2).

Figure 2-2 Detectable Odds ratio



Prevalence of exposure rate in controls=10%

Prevalence of exposure rate in controls=20%



Prevalence of exposure rate in controls=30%

2.5 Data analysis

For statistical analysis, SPSS (Statistical Package for the Social Sciences) version 16 was used. To compare the demographic characteristics of cases and controls, such as age and ethnicity, univariate logistic regression were performed. For social class, education and marital status, multiple logistic regression was performed.

For all other environmental exposures, unconditional logistic regression was used to generate odds ratios and 95% C.Is.

To assess for a trend in prostate cancer risk across the categories test for linear trend was performed, considering selected exposures as continuous variables.

The methodology and analysis pattern for gene and environmental interaction will be mentioned in chapter five.

2.6 Confounding factors

Age and social class were previously tested when the interim analyses were undertaking and since then both factors have been consistently fitted in a model as confounding factors throughout all analyses of the study. Age was included as a continuous variable whereas social class was fitted as a categorical variable.

2.7 Literature search

Searches were done using the "Pubmed" search engine. The topics of review include prostate cancer and its potential risk and protective factors including family history, X-rays and DNA repair genes involved in the X-ray repair, hormone markers including baldness, hand pattern and acne and also gene and environmental interaction for DNA repair genes involved in the X-ray repair, acne and baldness.

Chapter 3 Family history

3 Literature review

3.1 Family history and prostate cancer risk

The majority of cases of prostate cancer are not due to an inherited predisposition to develop the condition (about 75%) and are known as sporadic cases. In these cases damage to the genes occurs due to exposure to various environmental factors after birth (NCI, 2008). These genes are known as low penetrance genes (Shields & Harris, 2000). Another group of prostate cancer is known as familial (25%), with a family having more than one person affected with prostate cancer with no definitive pattern of inheritance. The aetiology of familial cancer varies from familial exposure to different environmental and dietary factors, polygenic inheritance, low penetrance single gene and to chance alone (Walsh & Partin, 1997).

Data from previous epidemiological studies are very persuasive suggesting family history as a strong and established risk factor for developing subsequent prostate cancer and it is now considered as one of the strongest hereditary cancers (Witte, 2009). Results from several case-control and cohort studies are consistent and suggested a strong association of family history with prostate cancer. Highly significant associations were found with first degree relatives especially father and brother(s) having prostate cancer. All these studies showed two to six times increased risk of acquiring prostate cancer with positive family history. A border line risk is also evident with second degree relatives (See Table 3-1) (Cerhan *et al*, 1999; Isaacs *et al*, 1995; Kalish *et al*, 2000; Mettlin *et al*, 1995; Spitz *et al*, 1991).

According to the UK genetic prostate cancer study (UKGPCS) and several other studies, tendency towards developing prostate cancer has a genetic component, but the genes that have been shown to be involved in hereditary prostate cancers are not thought to be mutated in sporadic cancers (Gelmann & Gelmann, 2003). These genes affecting familial cases are known as high penetrance genes (Shields & Harris, 2000). Mutations in high penetrance susceptibility genes greatly increases the risk of prostate cancer as compared with low penetrance polymorphisms but as the low penetrance polymorphisms are more common they may therefore be more prevalent in the population (Porkka *et al*, 2004).

Very small numbers (about 5-10%) of the cases of prostate cancer have an obvious strong genetic predisposition, but those who are diagnosed at a younger age are more likely (Crawford & Crawford, 2003; Gibbs *et al*, 1999). A hereditary prostate cancer is subtype of familial cancer with a Mendelian inheritance pattern of distribution and has an autosomal dominant trait (Carter *et al*, 1992; Tavtigian *et al*, 2001; Walsh & Partin, 1997). It has a faulty copy of one of the cancer protective genes that usually control cell division and growth and passed through the family line (NCI, 2008; Walsh & Partin, 1997).

According to Cancer Research UK, family history of breast cancer is also an important risk factor for prostate cancer. It is mainly due to BRCA1 and BRCA2 genes both are risk related with breast and prostate cancer (Cancer Research UK, 2010b). However Kalish et al didn't find any association between prostate cancer risk and family history of breast cancer (Kalish *et al*, 2000).

Table 3-1 Summary of epidemiological studies of prostate cancer and family history

Study(reference)/site	Study design	No of subjects	Relatives affected	OR/RR	95% C.I	p-value
(Ghadirian *et al*, 1997),Canada	Case-control	640 cases, 639 controls	Father and brother	3.32	2.18-5.05	0.0001
(Lesko *et al*, 1996), Massachusetts, USA	Case-control	563 cases, 703 controls	Father and brother	2.3	1.7-3.3	-
(Hayes *et al*, 1995), USA	Case-control	479 cases/black, 502 cases/white and 594 controls/black,721 controls/white	Father and brother	3.2	2.3-12.5	-
(Isaacs *et al*, 1995)USA	Case-control	690 cases, 640 controls	Father and brother(prostate cancer probands)	1.76	1.28-2.43	-
(Mettlin *et al*, 1995) USA	Case-control	1271 cases, 1909 controls	Father and brother	6.5	1.4-30.5	-
(Whittemore *et al*, 1995), Canada	Case-control	1655 cases, 1127 controls	Positive family history	2.5	1.9-3.3	-
(Spitz *et al*, 1991)USA	Case-control	385 cases, 385 controls	Father and brother	2.41	1.30-4.47	<0.001
(Steinberg *et al*, 1990), USA	Case-control	691 cases, 640 spouse controls	Father and brother	2.7	0.5-13.2	-
(Schuurman *et al*, 1999), Netherlands	Case-cohort	704 incident cases, 1688 controls	Father and brother	5.57	1.61-19.26	
(Chen *et al*, 2008) USA	Cohort	Subcohort of the health professionals follow-up study with 3,695 cases	Father and brother	2.3	1.76-3.12	-
(Kalish *et al*, 2000)USA	Cohort	1149 Massachusetts cohort with 57 cases	Positive family history	3.29	1.82-5.94	-
(Cerhan *et al*, 1999) USA	Cohort	Population -based Iowa cohort with 101 cases	Father and brother	3.2	1.8-5.7	-

3.2 Genetics of prostate cancer

Cancer is a disease that occurs when cell division gets out of control, may be due to impairment of a DNA repair pathway, the transformation of normal gene into oncogene or due to the malfunction of tumour suppressor gene. The Majority of genetic mutations take place after exposure to environmental carcinogens, but some mutations have genetic predisposition to cancers (Porkka *et al*, 2004).

3.2.1 Genes involved in predisposition and progression of prostate cancer

According to different linkages studies several genetic loci have been found to be associated with prostate cancer predisposition, these include HPC1 at 1q24-q25 (Smith *et al*, 1996). It is evident from previous studies that chromosome 1q24-q25 is linked with the families where prostate cancer is diagnosed at an early age (65 or less), male to male transmission and at-least five or more affected family members (Singh *et al*, 2000). PCAP at 1q42.4-q43 (Berthon *et al*, 1998) has also been linked with early onset (age 60 or less) (Singh *et al*, 2000). HPCX at Xq27-q28, suggests X-liked inheritance of prostate cancer (Xu *et al*, 1998), CAPB at 1p36, showing links between both families with high risk of prostate cancer and brain cancer (Gibbs *et al*, 1999), HPC20 at 20q13, provides very strong evidence in familial lines without male to male transmission (Berry *et al*, 2000), 8p22-23 prostate cancer susceptible genes may have a possible role in the genetic inheritance of prostate cancer and in prostate cancer pathogenesis (Xu *et al*, 2001). There is strong evidence to suggest that variations ELAC2 at 17p have increased the risk of prostate cancer (Tavtigian *et al*, 2001). It is evident from results of a study involving 5q,7q,19q that there may be some role of genes present on these candidate regions in progression of prostate cancer (Witte *et al*, 2000). From these chromosomal regions only three genes have

been identified (Porkka *et al*, 2004). The First is ELAC2 (MIM 605367) from the HPC2, locus. The second is RNASEL (MIM 180435) from the HPC1, locus but is as yet to be definitively confirmed (Ikonen *et al*, 2003), and the third is MSR1 (macrophage scavenger receptor 1) gene located at 8p22-23 (Porkka *et al*, 2004). According to the results of a study conducted by Nupponen, these three genes do not appear to be mutated in sporadic prostate cancer (Nupponen *et al*, 2004) (see Table 3-2).

The genetic analysis from the UKGPCS study as one part of this present study (included subjects only age<60) suggested that seven loci are associated with prostate cancer on chromosomes 3, 6, 7, 10, 19, and x and confirmed association of common loci with prostate cancer at 8q24 and 17q. Also three new loci have been identified as having candidate susceptibility genes: MSMB, LMTK2 and KLK3 (Eeles *et al*, 2008).

Table 3-2 Genes involved in predisposition and progression of prostate cancer

Genes and loci	**Description**	**Justification/Limit OF Detection(LOD)**	**Ref**
HPC1 at 1q24-q25	Hereditary prostate cancer1	**5.43**	(Smith *et al*, 1996)
PCAP at 1q42.4-q43	Predisposing for Cancer Prostate	**40-50%** of French & German Families linked	(Berthon *et al*, 1998)
HPCX at Xq27-q28	hereditary prostate cancer ,X-linked	**4.6**	(Xu *et al*, 1998)
CAPB at 1p36	Cancer Prostate and Brain	**3.22**	(Gibbs *et al*, 1999)
HPC20 at 20q13	Hereditary prostate cancer20	**2.69**	(Berry *et al*, 2000)

Genes and loci	Description	Justification/Limit OF Detection(LOD)	Ref
ELAC2 at 17p	ElaC homolog protein 2/ Heredity prostate cancer protein 2	Maximum 2-point LOD score at **4.5** at marker D17S1289 and Maximum 3-point LOD score at **4.3** at marker D17S1289 and D17S921	(Tavtigian *et al*, 2001)
5q,7q,19q		*(P=0.0002), (P=0.0007), (P=0.0004)*	(Witte *et al*, 2000)

LOD: *Is the log of the odds of linkage and LOD >3.0(log_{10} 3.0+1000 to 1 odds of linkage). LOD of <-2.0 is evidence against linkage of 100 to one (Singh et al, 2000) .*

Several studies have reported familial association between prostate cancer risk among male relatives of female patients with breast cancer (Chen *et al*, 2008; Goldgar *et al*, 1994; Rodriguez *et al*, 1998; Tulinius *et al*, 1992). Germline mutation in BRCA1 and *BRCA2* genes are responsible for inherited predisposition of breast cancer. Families of the carriers of these genes have an increased risk of prostate cancer (Edwards *et al*, 2004; Edwards *et al*, 2003; Ford *et al*, 1994; Sigurdsson *et al*, 1997), but the risk of developing prostate cancer is more likely with BRCA2 mutations than with BRCA1 mutations (Cancer Research UK, 2010b; Edwards *et al*, 2003; Mitra *et al*, 2008).

3.3 Hypotheses and aims

Hypotheses

1. Prostate cancer risk is increased with positive family history of prostate cancer.
2. Prostate cancer risk is increased with positive family history of breast cancer.

Based on above hypotheses following are the aims:

Aims

1. To evaluate the association between first degree relatives (father and brother) of the proband with prostate cancer on prostate cancer risk.
2. To assess the association between first degree relatives (father and brother) with prostate cancer and prostate cancer risk among the young age group (<60 years).
3. To investigate the role between history of breast cancer in a family (mother, sister and daughter) and risk of prostate cancer.

3.4 Methodology

To investigate the strength of familial factors in prostate cancer and the association between family history of breast cancer, the first step was to create dichotomous variable with family history of any cancer vs. no cancer in family. However, considering first degree relatives as an important risk factor as evident from previous epidemiological studies these variables had been filtered using Microsoft Excel and "exposed" group (only those with father and brother had had prostate cancer) vs. "unexposed" group. The unexposed group is defined by

i) subjects who reported none of their family members are affected with cancer of any type

OR

ii) subjects who reported their first degree relatives affected with other cancers but not prostate cancer.

iii) subjects with 2nd or 3rd degree relatives members in their family affected with other cancers including the prostate cancer.

To evaluate the effect of family history with first degree relatives on young-onset prostate cancer, controls age <60 years were selected and analysis was done.

A new variable has been created for looking at the risk of prostate cancer with family history of breast cancer within first degree relatives, by selecting female first degree relatives such as mother, sister and daughter (exposed) vs. (unexposed) all other cancers in all other relatives including the breast cancer.

3.4.1 Definitions of variables

3.4.1.1 First degree relative (exposed)

First degree relative (exposed) was defined as biological father and brother who had developed prostate cancer.

3.4.1.2 Family history of breast cancer (exposed)

Family history of breast cancer was defined as biological mother, sister and daughter suffering from breast cancer.

3.5 Analysis

Multiple logistic regression models were used to estimate odds ratios and 95% confidence intervals. Potential confounders such as age and social class were controlled for in the multivariate analyses.

3.6 Results

3.6.1 Family history of prostate cancer in first degree relatives

Table 3-3 shows distribution values and risk estimates when first degree relatives (father and brother) affected with prostate cancer.

Table 3-3 Distribution and odds ratios for family history in the probands with prostate cancer -all ages

first degree relatives	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
unexposed	734(70.0)	1701(94.9)	1.00		
exposed	314(30.0)	91(5.1)	7.93	6.17-10.20	<0.001
Total	1048(100.0)	1792(100.0)			

†adjusted for age and social class

The results showed a highly significant increased risk (OR 7.93, 95% C.I. 6.17-10.20) among subjects whose first degree relatives are affected with prostate cancer compared to those who either don't have any family history of cancer or they have family history of other cancers including prostate cancer. The latter is only valid in the second or third degree relatives but not in the first degree relatives.

3.6.2 Family history of prostate cancer in first degree relatives age <60

Table 3-4 shows value and estimated risk of family history of prostate cancer age<60

Table 3-4 Estimated risks for family history in the probands with prostate cancer age<60

first degree relatives <60 yrs	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
unexposed	232(57.9)	553(94.4)	1.00		
exposed	169(42.1)	33(5.6)	12.55	8.35-18.86	<0.001
Total	401 (100.0)	586 (100.0)			

†adjusted for age and social class

The risk estimate shows a highly significant association between family history of prostate cancer in first degree relatives and prostate cancer risk in age <60 years as compared to risk in all ages (OR 12.55 compared to 7.93).

3.6.3 Family history of breast cancer in first degree relatives

Table 3-5 shows value and estimated risk of prostate cancer with family history of breast cancer.

Table 3-5 Estimated risks for prostate cancer with family history of breast cancer

Family History	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
No	930(88.6)	1638(91.5)	1.00		
Yes	120(11.4)	152(8.5)	1.39	1.07-1.79	<0.01
Total	1050(100.0)	1790(100.0)			

†adjusted for age and social class

The results showed a lower risk (OR 1.39, 95% C.I. 1.07-1.79) for those who have family history of breast cancer in their first degree relatives compared to those who either don't have any family history of cancer or they have family history of other cancers and breast cancer but not in the first degree relatives.

3.7 Discussion

3.7.1 Family history of prostate cancer with affected first degree relatives

The results showed a highly significant association between prostate cancer in first degree relatives and prostate cancer risk (OR 7.93, 95% C.I6.17-10.20). The results of this study are consistent with the several case-control and cohort studies. For example Australian study showed six times increased risk of prostate cancer with affected first degree relatives (Mettlin *et al*, 1995). In a Swedish study (RR 3.2, 95% C.1.2.1-5.1) was found with either father or brother affected by prostate cancer. Results from a case-control study conducted in Montreal, Toronto and Vancouver between 1989- 93 showed strong association between those who have at-least one blood relative(father or brother) affected and prostate cancer (RR 3.32, 95% C.1. 2.18-5.05). Another case-control study by Spitz et al, also reported positive association (OR 2.41, 95% C.I. 2.24-2.66) (Spitz *et al*, 1991).

Cerhan et al, in their cohort study reported increased risk of developing prostate cancer in those whose father or brother had suffered from prostate cancer(RR 3.2, 95% C.I. 1.8-5.7)(Cerhan *et al*, 1999). Our results are also consistent with the findings of Chen and colleagues, who have recently demonstrated that a family history of prostate cancer in both father and brother doubled the risk of developing prostate cancer (RR 2.3, 95%C.I. 1.76-3.12) (Chen *et al*, 2008).

In most of these studies risk was inversely proportional with age of diagnosis, such a study conducted by Lesko et al, showed a fivefold increased risk in probands younger than 60 years. Our results also showed highly significant risk (OR 12.55, 95%C.I. 8.35-18.86) among the subjects age <60 years with positive family history of prostate cancer.

Taken together, these results provide a strong body of evidence that family history is one of the important risk factor of prostate cancer.

3.7.2 Family history of breast cancer with affected first degree relatives

This study also demonstrated an association between history of breast cancer in family and prostate cancer (OR 1.39, 95% C.1.1.07-1.79). There is growing body of evidence on the role of family history of breast cancer and risk of development of prostate cancer among the male members of the family, especially when first degree relatives like mother or sister are affected (Chen *et al*, 2008; Goldgar *et al*, 1994; Rodriguez *et al*, 1998; Tulinius *et al*, 1992). For example, a case-control study conducted by Rodriguez et al, suggested similar increased risk to the present study that in the mother and sister with breast cancer (RR 1.16, 95% C.1.1.01-1.33) (Rodriguez *et al*, 1998).

Also, results from cohort studies showed increased risk of prostate cancer, if first degree female relatives such as mother or sister had breast cancer. For example, Chen et al suggested a similar increased risk (RR 1.22, 95% C.I. 1.08-1.38) (Chen *et al*, 2008). But Kalish et al in their study found no association of prostate cancer with the family history of breast cancer (RR 1.18, 95% C.I. 0.51 to 2.43) (Kalish *et al*, 2000).

One of the important limitations of case-control study is recall or memory bias (Coughlin, 1990). Especially when it is self reported and for recalling for some chronic illness like cancer, where natural history of illness is remains obscured until full blown disease is evident. However, information for first degree relatives is more reliable than second or third degree relatives. A Swedish case-control study of 356 cases and 712 controls was conducted to assess the reliability of self-reported family history of prostate cancer, results found it a

reliable method to measure the true incidence of prostate cancer in immediate family members (Bratt *et al*, 1999).

3.8 Conclusion

The findings from these two analyses suggested that family history with first degree relatives with prostate cancer is a strong risk factor in prostate cancer and a family history of breast cancer appeared to be a lower risk factor in its development.

Family history appeared to be a strong risk factor in all ages, and showed the importance of genetics in the aetiology of the disease, not only in the young but also that it can have an influence at any age.

Chapter 4 Radiation

4 Literature review

4.1 Radiation

Radiation is termed ionising when it has the capacity to penetrate and deposit its energy in the tissue such that an electron will be removed from its orbit. Ionising radiation is divided in to two major groups, *Electromagnetic radiations:* X-rays and gamma rays and Particular *radiation:* alpha, beta particles (electrons) and protons (Fajardo L-G *et al*, 2001; Park, 2005).

4.1.1 Units of radiation

There are different ways to measure radiation potency through SI units (international system of Units):

1- *Coulomb per kilogram*(C/Kg): is the unit of exposure.
2- *Gray* (Gy): is the unit of absorbed dose.
3- *Sievert* (Sv): the unit indicates the degree of potential danger to health as it is the measure of absorbed dose, for X-rays Gy=Sv (Park, 2005).

As all forms of radiation do not have the same biological effect per unit of energy absorbed, the idea of *dose equivalent* (H) has been introduced and the equivalent dose in Sv is equal to the absorbed dose in Grays multiplied by a quality factor Q which is corresponding radiation weighting factor (which depends upon the density of ionisation produced in the tissue by the radiation) 1Sv=1000 mSv=100rem (Cardis *et al*, 2005; Park, 2005).

Ionising radiation is one the important risk factor in the development of many cancers (Myles *et al*, 2008). Studies on animal models to see the effect of radiation as a carcinogen were started soon after World War II and showed strong evidence of radiation carcinogenesis followed by the evidence from various epidemiological studies in the human population. These studies proved radiation as a "universal carcinogen" (Little, 2000). High dose radiation is proven risk factor for developing many cancers and has adverse effects on genetic makeup; however we know little about the effect of low-dose ionising radiation such as diagnostic, therapeutic, occupational or natural. Low dose radiation should be more concern as these are more common than high dose exposures (Shore, 2009).

4.1.2 Biological effects of radiation

It is now evident from several epidemiological and molecular studies that radiation can cause a wide range of DNA lesions including damage to nucleotide bases, cross-linking and DNA single- and double- strand breaks (DSBs) and the latter class of damage is potentially cytotoxic (Little, 2000). Bhatti and his colleagues demonstrated in their study that even low dose radiation i.e. 50mGy and lower can cause chromosomal damage; especially "translocations" the intermediate biomarker of cancer risk and can cause harmful health effects including cancer (Bhatti *et al*, 2010; Sigurdson *et al*, 2008). The results from a collaborative cohort study conducted in fifteen countries included 407,391 nuclear industry workers to estimate the cancer risk following prolonged exposure to low doses of ionising radiation showed significant association between radiation dose and different types of cancers such as lung and multiple myeloma and all-cause mortality (Cardis *et al*, 2007).

4.2 X-rays/ Diagnostic Medical Radiography

The largest man-made source of radiation exposure in the general population is diagnostic X-rays. Worldwide diagnostic radiological exposures contribute about 14% of total exposure annually from all sources (Berrington de Gonzalez *et al*, 2004). X-rays were first discovered by Wilhelm Konrad Roentgen, a professor of Physics in his laboratory at New Physical Institute of University Of Wurzburg on 8th November 1895(Rontgen, 1972). It is one of the most important seminal discoveries in the field of medicine. X-rays have short wave lengths and hence have the ability for deep penetration (Park, 2005). They have important roles in the diagnosis of many health problems and have a wide range of applications from simple chest X-ray to forensic procedures (Brailsford, 1946; Frenz & Mee, 2005). X-rays, however, have energy capable of causing ionisation in targeted tissue leads to harmful biological effects (Armstrong & Wastie, 2001).

Figure 4-1 First ever human X-ray by Rontgen(his wife's hand also showing wedding ring) (Rontgen, 1972).

The UK population is subjected to a lower annual per capita dose of X-rays (0.38mSv) as compared to other developed countries with similar health system (see Table 4-1). This may be due to health policy which avoids unnecessary X-ray exposure and lower doses as compared to other countries (Hart & Wall, 2004).

Table 4-1 International comparison of annual per caput effective dose from medical radiology

Country	Time period	Annual per caput effective dose(mSV)
Germany	1990-1992	1.9
France	-	1.0
Switzerland	1998	1.0
Canada	-	0.94
Russia	-	0.9
Australia	1996	0.8
Norway	1993	0.8
Poland	-	0.8
Bulgaria	-	0.75
Portugal	1991	0.71
Sweden	-	0.68
Romania	-	0.61
Netherlands	1998	0.52
USA	-	0.5
Ukraine	1994	0.5
Finland	-	0.45
Spain(regional)	1990	0.4
UK	2001/2002	0.38
Denmark	-	0.36
Taiwan	1993	0.23
Brazil	-	0.09
Malaysia	1994	0.05

Taken from the article "UK population dose from medical X-ray examinations" with the permission from author (Hart & Wall, 2004).

4.3 X-rays and cancer

Although the potential carcinogenic effect was recognized soon after the discovery of X-rays by Roentgen in 1895, the first ever observed radiation induced cancer noticed was a skin lesion in the form of an ulcer to Clarence Madison Dally (assistant of Thomas Edison) in 1902 and he is the first person to die of radiation induced cancer in 1904 (Fajardo L-G *et al*, 2001; Little, 2000). Marie Curie and her daughter Irene are thought to have died of complications resulting from radiation exposure and cause of death may have been due to leukaemia (Little, 2000). Although diagnostic X-ray procedures are of high benefit to human-kind, they also show some risk of developing cancer. Berrington de Gonzalez et al, reported that the UK has the lowest annual frequency of diagnostic X-rays and that Japan has the highest. About 0.6% (700 cases of cancer/year) of the cumulative risk of cancer at age 75 years could be attributed to diagnostic X-rays in the UK (Berrington de Gonzalez *et al*, 2004).

Though there is no reliable data available proving diagnostic radiography as a cause of cancer, the National Health Institute had added X-rays to the list of carcinogens in their eleventh report on carcinogens. They reported that nearly fifty five percent of global radiation exposure is a result of exposure to diagnostic radiography. In their report, it has also been added that childhood exposure may lead to leukaemia and thyroid cancer and exposure in women during pregnancy may lead to breast cancer and congenital malformation in the foetus, if the procedure was carried out during first trimester of pregnancy (Kay & Chronicle, 2005).

Track analysis studies of X-rays and their interaction with DNA provides evidence of DNA cluster damage which can produce DSBs (Little, 2000). There is a wide range of different diagnostic radiological procedures available ranging from simple chest X-ray to highly advanced imaging procedures, however there is no standard cut-off points available for radiation dose, and there is also no suggested standard value available for the dose of diagnostic X-rays, which can induce cancer (Brenner *et al*, 2003; Kalender, 2000).

Tracy Hampton in her article "Researchers examine long-term risks of exposure to medical radiation" labelled radiation "double edge sword", as it is used for both the diagnosis and treatment, but can cause subsequent health problems including cancer. The major health risk of diagnostic radiography is the development of cancer, which might develop soon after the exposure or later in life, however, the exact mechanism is still not clear as to how these low dose ionising radiations can cause cancers. One theory " The bystander effect " is a phenomenon in which radiation-damaged cells may send mutation signals to neighbouring cells that then become malignant themselves (Hampton & Hampton, 2006). Other theories suggest sub-lethal damage to cells which then carry mutational damage.

When looking at acute dose response relation with cancer incidence, Brenner et al found that those subjects who exposed to radiation doses ranging from 5-100msv show increased risk for solid cancers (p value =0.05) as compared to dose less than 5msv. It shows a definite dose response relationship (Brenner *et al*, 2003). Many studies suggested that the earlier and frequent the exposure, the greater chance of developing cancer (Bassal *et al*, 2006; Berrington de Gonzalez *et al*, 2004 ; John *et al*, 2007; Miller *et al*, 1989).

4.4 Low dose diagnostic radiations and prostate cancer risk

Our study group was the first group to report the results of preliminary analysis on association of certain types of diagnostic radiological procedures and early onset prostate cancer risk. The study investigated five radiological procedures involving the lower trunk of human body; barium enema, barium meal, IVP, hip/pelvic and leg/thigh. Since there was no information available on possible deliverable dose to prostate gland of these procedures, the study presented the estimated average dose, as shown in Table 4-2. The dose for leg/thigh X-ray was not shown as exposure was considered to be negligible (Myles *et al*, 2008).

Table 4-2 Mean minimum and maximum estimates of the dose to the prostate gland

Examination	Mean minimum/mSv	Mean maximum/mSv
Barium enema	10	25
Barium meal	0.2	0.4
IVP	3	4
HIP/pelvic	2	5

Taken from the article "Diagnostic radiation procedures and risk of prostate cancer" with the permission from author (Myles *et al*, 2008).

The analysis included 431 cases and 409 controls. The results suggested that exposed to barium enema and hip/ pelvic X-ray at 5 years prior to diagnosis were positively associated with early onset prostate cancer (OR 2.06, 95%C.I.1.01-4.20 and OR 2.23, 95% C.I.1.42-3.49 respectively) (Myles *et al*, 2008).

The subsequent work was carried out by Hussain (2009). The analysis was based on the extended interim dataset of 831 cases and 1298 controls (age ≤60) and included the same five radiological procedures; barium meal, barium enema, IVP, hip/pelvic X-ray and leg/thigh X-ray. The purpose of the study was to confirm the previous findings with the larger dataset. The results suggested increased risk of early onset prostate cancer with hip/pelvic X-ray >5yrs before diagnosis OR 1.89, 95% C.I.1.16-3.09, >10yrs before diagnosis OR 1.92, 95% C.I.1.09-3.36, with upper leg/thigh X-ray >5yrs before diagnosis the OR 1.90, 95% C.I. 1.05-3.45, >10yrs OR 2.16, 95% C.I. 1.10-4.25 and >20yrs OR 2.46, 95% C.I. 1.10- 5.50. Barium enema was no longer associated with early onset prostate cancer risk.

In this chapter, the analyses of all these five procedures are carried out with the extensive larger dataset particularly with the inclusion of subjects with all ages together and with the different approaches to data filtering.

4.5 Hypotheses and Aims

Hypotheses

There is an association between exposure to low-dose ionising radiations (diagnostic medical radiations) and prostate cancer risk.

Based on above hypothesis following are the aims:

Aims

1. To investigate the association between diagnostic radiation procedures and prostate cancer occurring at any age.
2. To assess the independent effect of different X-ray procedures and prostate cancer risk.

4.6 Methodology

The analysis includes data from the first phase (used by Myles and Hussain) (Hussain, 2008/2009; Myles *et al*, 2008), and the second phase (the author's collection). It is noted that in both phases questionnaires differed slightly from each other. In the first phase questionnaire, it was asked whether subject had had any of the procedures including barium meal, barium enema, IVP, hip/pelvic X-ray and upper leg/thigh X-ray, how many times and if so only the first date of each procedure was recorded. In the second phase, questionnaire was modified by including more procedures such as CT scan, MRI, angiograms etc, and more detailed on date(s) of each procedure and reason for each.

Since data are slightly different, the main analyses include 5 procedures that were previously reported and this was done in view of two main reasons, firstly to have a larger number which increase power particularly with the further statistical analysis of gene and environment interaction on X-ray exposure and

DNA repair genes (see Chapter 5). Secondly, the larger sample size will allow the analysis of exposure to any individual procedure alone without confound with other procedures with enough power to detect any significant associations.

In sum, the study analyse 1112 cases and 1872 controls.

4.6.1 Coding procedures

To investigate the individual and independent effect of each procedure, the following was carried out.

1. Only subjects reported having had that procedure one time in life and not the others are included. The dichotomous variable is then created as exposed subjects (exposed to particular individual diagnostic X-ray one time and never had any other procedures) and non-exposed subjects (never had procedures). The time of having the procedure was not taken into account so it could either be before or after diagnosis in case group and either before or after receiving questionnaire in control group).
2. To measure the relevant time period of the procedure that could potentially effect prostate cancer risk, the data was filtered and new variable was created for. The exposed group consisted of subjects who reported had the procedure once and the timing of that procedure was greater than five, ten and fifteen years prior to their diagnosis date in case group and to their receiving questionnaire in control group. The non-exposed group consisted of subject who reported never had any procedures during their lifetime.

4.7 Analysis

Unconditional logistic regression was performed to obtain odds ratios and confidence intervals. All analyses were adjusted for age and social class.

4.8 Results

4.8.1 X-rays

4.8.1.1 Barium meal

Table 4-3 below shows the distribution and risk estimates of barium meal.

Table 4-3 Distribution and odds ratios of barium meal

Barium meal	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Never had any procedure	204(85.7)	852(87.1)	1.00		
Barium meal once	34(14.3)	126(12.9)	1.19	0.78-1.82	0.43
Total	238(100.0)	978(100.0)			

†adjusted for age and social class

The results showed no association between barium meal and prostate cancer risk (OR 1.19, 95% C.I. 0.78-1.82).

Result on barium meal five, ten and fifteen years prior to diagnosis and prostate cancer risk is presented in Table 4-4.

Table 4-4 Distribution values and odds ratios of barium meal >5, 10 & 15 years

Barium meal	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
>5years					
Never had any procedure	204(87.6)	852(88.6)	1.00		
Once and >5years prior to diagnosis	29(12.4)	110(11.4)	1.15	0.73-1.82	0.54
Total	233(100.0)	962(100.0)			

-Continue-

Barium meal	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
>10years					
Never had any procedure	204(87.6)	852(88.6)	1.00		
Once and >10years prior to diagnosis	29(12.4)	110(11.4)	1.15	0.73-1.82	0.54
Total	233(100.0)	962(100.0)			
>15years					
Never had any procedure	204(87.6)	852(88.6)	1.00		
Once and >15years prior to diagnosis	29(12.4)	110(11.4)	1.15	0.73-1.82	0.54
Total	233(100.0)	962(100.0)			

†adjusted for age and social class

There was no significant link between prostate cancer and having been exposed to barium meal at more than five, ten and 15 years before the diagnosis (All confidence intervals include 1).

4.8.1.2 Barium enema

Figures from the table below show distribution and risk estimates of barium enema and prostate cancer risk.

Table 4-5 Distribution and odds ratios of prostate cancer relative to barium enema exposure

Barium enema	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Never had any procedure	204(95.8)	852(96.3)	1.00		
Barium enema once	09(4.2)	33(3.7)	1.17	0.55-2.51	0.69
Total	213(100.0)	885(100.0)			

†adjusted for age and social class

Results support no association between exposure to barium enema and prostate cancer risk (OR 1.17, 95% C.I. 0.55-2.51).

Table 4-6 illustrates distribution and risk estimates of barium enema five, ten and fifteen years prior to diagnosis and prostate cancer risk.

Table 4-6 Distribution and odds ratios of barium enema >5, 10 & 15 years

Barium enema	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
>5years					
Never had any procedure	204(98.1)	852(97.6)	1.00		
Once and >5years prior to diagnosis	04(1.9)	21(2.4)	0.85	0.28-2.53	0.77
Total	208(100.0)	873(100.0)			
>10years					
Never had any procedure	204(98.6)	852(98.0)	1.00		
Once and >10 years prior to diagnosis	03(1.4)	17(2.0)	0.78	0.22-2.73	0.69
Total	207(100.0)	869(100.0)			
>15years					
Never had any procedure	204(99.5)	852(98.6)	1.00		
Once and >15years prior to diagnosis	01(0.5)	12(1.4)	0.41	0.05-3.26	0.40
Total	205(100.0)	864(100.0)			

†adjusted for age and social class

A very small percentage of both case and control group reported exposed to procedure at all periods. Barium enema was not associated with prostate cancer risk at five, ten or fifteen years prior to diagnosis.

4.8.1.3 IVP (Intravenous pyelogram)

The results on independent effect of IVP in relation to prostate cancer are shown in Table 4-7

Table 4-7 Distribution and odds ratios of IVP and prostate cancer

IVP	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Never had any procedure	204(95.3)	852(96.9)	1.00		
IVP once	10(4.7)	27(3.1)	1.49	0.70-3.17	0.31
Total	214(100.0)	879(100.0)			

†adjusted for age and social class

The proportion of cases and controls in the exposed group is similar. There is no association between IVP procedure and prostate cancer risk.

Table 4-8 shows IVP five, ten and fifteen years prior to diagnosis and prostate cancer risk.

Table 4-8 Distribution values and estimated risk of IVP >5, 10 & 15 years and prostate cancer

IVP	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
>5years					
Never had any procedure	204(97.6)	852(97.8)	1.00		
Once and >5years prior to diagnosis	05(2.4)	19(2.2)	1.11	0.40-3.06	0.84
Total	209(100.0)	871(100.0)			
>10years					
Never had any procedure	204(99.0)	852(98.2)	1.00		
Once and >10years prior to diagnosis	02(1.0)	16(1.8)	0.56	0.13-2.49	0.44
Total	206(100.0)	868(100.0)			

-continue-

IVP	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Never had any procedure	204(99.0)	852(98.8)	1.00		
Once and >15years prior to diagnosis	02(1.0)	10(1.2)	1.09	0.23-5.14	0.92
Total	206(100.0)	862(100.0)			

†adjusted for age and social class

None of the results is statistically significant (all ORs are closed to one and all confidence intervals include 1).

4.8.1.4 Hip and pelvic X-ray

Distribution values and risk estimates of hip/pelvic X-ray are presented in table 4-9

Table 4-9 Hip and pelvic X-ray exposure and prostate cancer risk

Hip/pelvic X-ray	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Never had any procedure	204(88.7)	852(96.3)	1.00		
Hip/pelvic once	26(11.3)	33(3.7)	3.15	1.81-5.47	<0.001
Total	230(100.0)	885(100.0)			

†adjusted for age and social class

The results showed a significant increased risk among subjects that reported having hip/pelvic X-ray once compared to those who never exposed to the procedure (OR 3.15, 95% C.I. 1.81-5.47).

The results on hip/pelvic X-ray exposure five, ten and fifteen years prior to diagnosis and prostate cancer risk are presented in Table 4-10

Table 4-10 Hip and pelvic X-ray >5, 10 & 15 years and prostate cancer risk

Hip/pelvic X-ray	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
>5years					
Never had any procedure	204(94.4)	852(98.2)	1.00		
Once and >5years prior to diagnosis	12(5.6)	16(1.8)	3.42	1.56-7.50	<0.001
Total	216(100.0)	868(100.0)			
>10years					
Never had any procedure	204(95.3)	852(98.7)	1.00		
Once and >10years prior to diagnosis	10(4.7)	11(1.3)	4.18	1.69-10.30	<0.001
Total	214(100.0)	863(100.0)			
>15years					
Never had any procedure	204(95.8)	852(99.1)	1.00		
Once and >15years prior to diagnosis	09(4.2)	08(0.9)	4.69	1.77-12.47	<0.001
Total	213(100.0)	860(100.0)			

†adjusted for age and social class

There was a positive association between hip/pelvic X-ray exposure five years prior to diagnosis and prostate cancer risk with the odds ratio of 3 (p-value <0.001). Highly Increased risk of four times is observed amongst the subjects with history of hip/pelvic X-ray at ten and fifteen years prior to diagnosis and prostate cancer risk (p-value <0.001).

4.8.1.5 Upper leg and thigh X-ray

Table 4-11 shows distribution and risk estimates on upper leg and thigh X-ray in relation to prostate cancer

Table 4-11 Distribution and odds ratios of upper leg and thigh X-ray and prostate cancer risk

U Leg & thigh X-ray	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Never had any procedure	204(97.6)	852(97.4)	1.00		
Leg &thigh once	05(2.4)	23(2.6)	1.05	0.39-2.83	0.93
Total	209(100.0)	875(100.0)			

†adjusted for age and social class

The overall distribution is similar between cases and controls. There is no association between single exposure of upper leg and thigh X-ray and prostate cancer risk.

Results on upper leg and thigh X-ray five, ten and fifteen years prior to diagnosis and risk of prostate cancer is illustrated in Table 4-12

Table 4-12 Distribution and risk estimates of leg and thigh X-ray >5, 10 & 15 years

U Leg & thigh	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
>5years					
Never had any procedure	204(98.1)	852(98.3)	1.00		
Once and >5years prior to diagnosis	04(1.9)	15(1.7)	1.14	0.37-3.52	0.82
Total	208(100.0)	867(100.0)			

-Continue-

U Leg & thigh	**Case (%)**	**Control (%)**	**OR†**	**95%CI Lower-upper**	**P-value**
Never had any procedure	204(98.1)	852(98.7)	1.00		
Once and >10years prior to diagnosis	04(1.9)	11(1.3)	1.58	0.49-5.09	0.44
Total	208(100.0)	863(100.0)			
>15years					
Never had any procedure	204(98.1)	852(98.7)	1.00		
Once and >15years prior to diagnosis	04(1.9)	11(1.3)	1.58	0.49-5.09	0.44
Total	208(100.0)	863(100.0)			

†adjusted for age and social class

None of the results was statistically significant and the adjusted OR ranged from 1.14-1.58.

4.9 Discussion

The study analysed 1112 prostate cancer cases and 1872 healthy controls. The main exposures were five individual diagnostic medical procedures. The results suggested that subjects who were exposed to hip/pelvic X-rays only once in their lifetime regardless of timing (OR for time non-specified 3.15, 95% C.I. 1.81-5.47) are at greater risk of developing prostate cancer. This finding supports the priori hypothesis in that any insult from the low dose radiation to the prostate gland conveys a risk. When time of exposure was filtered by 5, 10 and 15 years prior to diagnosis in the case group (or to receiving questionnaire in control group), the risks are greater (>5years OR 3.42, 95% C.I. 1.56-7.50, >10years OR 4.18, 95% C.I. 1.69-1.30 and >15 years OR 4.69, 95% C.I. 1.77-12.47). These results though suggested that being exposed to hip/pelvic X-ray only one time date back as far as more than 15 years prior to diagnosis increase risk greater than being exposed during 5 or 10 years prior to diagnosis, the increased risk could be due to recall bias particularly in case group.

To authors' knowledge the study group is the first to investigate an association between low dose ionising radiation from diagnostic radiological procedures and prostate cancer risk. Thus the discussion focuses on the results of different phases of the study including the results of first phase of study on diagnostic radiation procedure and young-onset prostate cancer risk (age≤60) published in 2008 and the subsequent analysis by Hussain (Hussain, 2008/2009).

In the article by Myles, the sample size consisted of 431 young-onset prostate cancer cases and 409 age frequency matched controls. Due to small sample size, the exposed group for each diagnostic X-ray procedure was defined by

subjects who reported ever had a procedure and date at first procedure was recorded and further filtered by 5, 10, 20 years prior to diagnosis in case group and receiving questionnaire in control groups. This was carried out to test a priori hypothesis if there was any suggested evidence between diagnostic medical X-ray procedures and early onset prostate cancer. The findings suggested positive associations with barium enema (OR at 5 years and 10 years prior to diagnosis was 2.06, 95% C.I. 1.01-4.20 and 2.49, 95% C.I. 1.07-5.78, respectively) and hip and pelvic X-ray (OR at 5 years prior to diagnosis was 2.23, 95% C.I. 1.42-3.49 and at 10 years prior to diagnosis 2.65, 95% C.I 1.60-4.39 and 20 years prior to diagnosis 2.87, 95% C.I. 1.47-5.62 respectively) (Myles *et al*, 2008).

The subsequent analysis reported by Hussain 2008/2009, with a larger dataset (831 cases and 1298 controls, age ≤60) applied a more refined definition to the exposed group. The exposed group was defined by any subjects who reported having had one exposure of that particular procedure in a specific time period and further filtered according to exposure time (5, 10, 20 years prior to diagnosis). The results suggested that hip/pelvic procedure >5yrs before diagnosis OR 1.89, 95% C.I. 1.16-3.09 and OR, 1.92 95% C.I. 1.09-3.36 if procedure >10yrs before diagnosis and upper leg/thigh X-ray >5yrs the OR 1.90, 95% C.I. 1.05-3.45, >10yrs OR 2.16, 95% C.I. 1.10-4.25 and >20yrs OR 2.46, 95% C.I. 1.10-5.50 is a risk factor for early onset prostate cancer (Hussain, 2008/2009). The author concluded that there are 2 possible reasons to explain why these two results are different including different approaches for data cleaning/filtering of each procedure creating the much smaller number of cases as compared to controls in the second analysis.

This present study has largest sample size as compare to two others and including subjects with ***all ages***. The reason to select all ages is to see the effect of radiation is exclusively in the young-onset prostate cancer, which is less prevalent and mostly familial but also seen in sporadic cases which have an older age of onset and are more prevalent and because of old age they might have exposed to more doses and might have cumulative effect. The different approach (to the first and the second analysis) to define exposed group is applied to reduce any confounding/co-effects of other procedures. Thus subjects will only be eligible to the analysis if they exposed to one procedure only and also further refine the period of having had the procedures 5, 10 and 15 years prior to diagnosis or receiving questionnaire. The analysis did not take into account any subjects who reported having had procedure more than one time because data on dates are incomplete (part of the dataset did not have details on date of subsequent procedure) thus it is impossible to justify if the subsequent procedures were carried out before or after diagnosis. The exposure at greater than 5, 10 and 15 years prior to diagnosis were investigated in order to minimise any chances of being exposed through treatments/diagnosis.

The stratified analysis was also performed in age ≤60 as compare to >60, but the results were not significant and sample size was small in each age group. From the first, second to the present analyses, there are some key differences in each of these sub studies as study progresses over the period of 10 years of data collection such as larger sample size, inclusion of all age groups, a different methodology of defining variables. With the larger samples, the analyses of more refine exposures are viable hence this dataset allows the analysis of an independent effect of a single X-ray procedure (without being confound with other procedures) and the results of hip/pelvic X-ray remains consistent throughout all 3 analyses regardless subject's age and the approach used.

Brenner and colleagues reported that radiation dose ranging from 5-100msv increased risk for solid cancers as compare to dose less than 5msv (Brenner *et al*, 2003). Among these 5 procedures, only barium enema and hip/pelvic X-ray can potentially convey the maximum dose to prostate gland at 25msv and at 5msv respectively. However, barium enema is a rare procedure as shown by only 4% of cases reported ever had barium enema. Hip/pelvic X-ray, on the other hand, is more common in study subjects (11% in case group). Thus it could be the small numbers of subjects that affected the null results of barium enema. Although barium enema was reported as a risk factor by Myles et al, however the subsequent analysis both by Hussain and by author did not show any associations. Hussain also pointed out that it could be due to smaller samples as the result of different approach use to define exposure group. This is also the case for this present analysis. The null associations in other 3 procedures; IVP, barium meal and leg/thigh can be explained by either the rare event (IVP, leg/thigh X-ray-less than 5% of subjects in both procedures) and/or low dose of X-ray that although delivered to the prostate, did not harm the gland.

Berrington de Gonzalez et al, found that cumulative radiation induced risk increases from forty years of age. The most common cancers associated with X-rays are bladder, colon, breast and leukaemia in both sexes and the highest risk procedures are CT scan, barium enema, hip and pelvic X-rays. It is also stated that risk of getting cancer depends upon dose, frequency and radiosenstivity of organ. Although this study explores several other cancers, however, it did not investigate prostate cancer. But as it assess other hormonal cancers such as breast cancer and thyroid cancer, thus we have reason to believe that prostate cancer may be affected in a similar way (Berrington de Gonzalez *et al*, 2004).

Since hip/pelvic X-ray shows a strong risk, the author investigated further on the effect of cumulative dose of hip/pelvic X-ray which may suggest a dose-response association. This can only be examined by the author's data (the second phase thus limit the number of eligible subjects. Moreover, during data audit process, there are problems with incomplete information on dates of having had the exposure in a few subjects particularly if they reported having had exposed to any procedures more than 3 times, very few has completed date of each procedure. This has an effect on identify eligible subjects. When omitted these subjects, the sample size then became too small.

There are very limited evidence from epidemiological studies that radiation exposure in particular X-ray can be the risk factor for prostate cancer. Long term follow-up studies of the United Kingdom cohort of ankylosing spondylitis patients who received X-ray treatment and United Kingdom Atomic Energy Authority (UKAEA) employees who have internal low-LET (linear energy transfer) radiation exposures found no strong evidence for radiation exposure as a risk factor for prostate cancer, this conclusion, was made on the lack of evidence for dose-response in these studies (UNSCER., 2006). In a retrospective study of prostate cancer in the United Kingdom Atomic Energy Authority employees, a strong association was found between exposure of radio-nuclides and prostate cancer risk (Atkinson *et al*, 1994), but according to Atkinson et al.,2004, there is less significant association between prostate cancer risk and radiation dose compared with previous studies (Atkinson *et al*, 2004).

Several studies have been conducted on the survivors of Hiroshima and Nagasaki. The survivors are from the general population and of all age and gender. During first five years the first cancer observed was leukaemia followed

by variety of solid tumours within ten years, with the significant exception of pancreatic, prostate, uterine cancer and chronic lymphoblastic leukaemia. The cancers showing a steady rise are leukaemia, non-melanoma skin and bone cancer (Little, 2009). The possible explanation for high incidence of these cancers following the exposure high dose radiation may be the effect of radiation on rapidly multiplying tissues like blood and bone marrow cells and for skin cancer possibly due to direct penetration of radiation in skin.

The results from several cohort studies remain inconclusive and do not suggest radiation as risk factor for prostate cancer (Carr *et al*, 2002; Iwasaki *et al*, 2003; McGeoghegan & Binks, 2000a; McGeoghegan & Binks, 2000b). In the report from National research Council, National Academy of Science, Advisory Committee on Biological effects of ionising radiation (*BEIR)* of 1990 found only a week association between prostate cancer and radiation exposure (BEIR, 1990).

Another study conducted on 13,136 subjects of the Childhood Cancer Survivor Study revealed 83% of patients had received radiotherapy during their childhood cancer and 59% developed a secondary neoplasm in a previous radiotherapy field with a median elapsed time of 15 years. Although there is higher dose of radiation used for therapeutic purpose as compare to diagnostic radiography but one cannot exclude the chance of getting cancer later in life as evident from the results of this study that there are potential chances of developing prostate cancer with hip X-ray exposure greater than five years.

Since literature on epidemiological evidence of diagnostic procedures and prostate cancer risk are limited, it is therefore worth mentioning the example of other hormone dependent organs such as breast and their vulnerability to acquire cancer after radiography or radiotherapy or screening. The results from case-control study on medical radiation exposure and breast cancer risk from the family cancer registry showed increased risk of breast cancer in those women who had exposed to radiotherapy for other cancers (OR 3.55, 95% C.I. 1.47-4.20) and X-ray chest for lung infection such as tuberculosis and pneumonia (OR 2.49, 95% C.I. 1.82-3.40) and (OR 2.19, 95% C.I. 1.83-3.47). Risk was highest in women with several exposures start from young age and with genetic predisposition (John *et al*, 2007). Another study also revealed that exposure to low level ionising radiation such as X-ray chest at early age and with frequent exposures lead to breast cancer later in life (95 % C.I. 1.11-1.67; P = 0.001) (Miller *et al*, 1989).

In this study the stratified analysis with family history and age <60 years were also performed to see the difference in risk between genetically predisposed and those who are not. The results show no significant difference in both groups thus the results did not present here.

This is the first case-control study evaluating the effects of different X-rays procedures on causation of prostate cancer, there are few limitations of this study:

1- The possible recall bias because the data was based on self-reporting by the participants using self administered questionnaire. Case group are more likely to recall events more accurate than control group, this is evident by the more complete data in case group. Subjects with prostate cancer may feel it is more important and might relate it with some role in their cancer.

2- Data validation should be considered however due to time and resource constraints, records could not be verified in this present study.

In summary hip and pelvic X-ray procedure showed increase risk of prostate cancer.

4.10 Conclusions

Findings of this study suggested increased risk of prostate cancer with exposure to hip/pelvic X-ray and explained the importance of low-dose ionising radiation in the aetiology of prostate cancer.

4.11 Recommendations

- This study provides rationale for large scale case-control studies along with some better tool/assessment of investigating exposure history, as self reported history might lead to recall bias. A validation study particularly for case-control study should be considered.
- Future study with completed information on diagnostic medical procedures would allow the calculation of lifetime cumulative dose.

Chapter 5 Hormone markers

5 Literature review

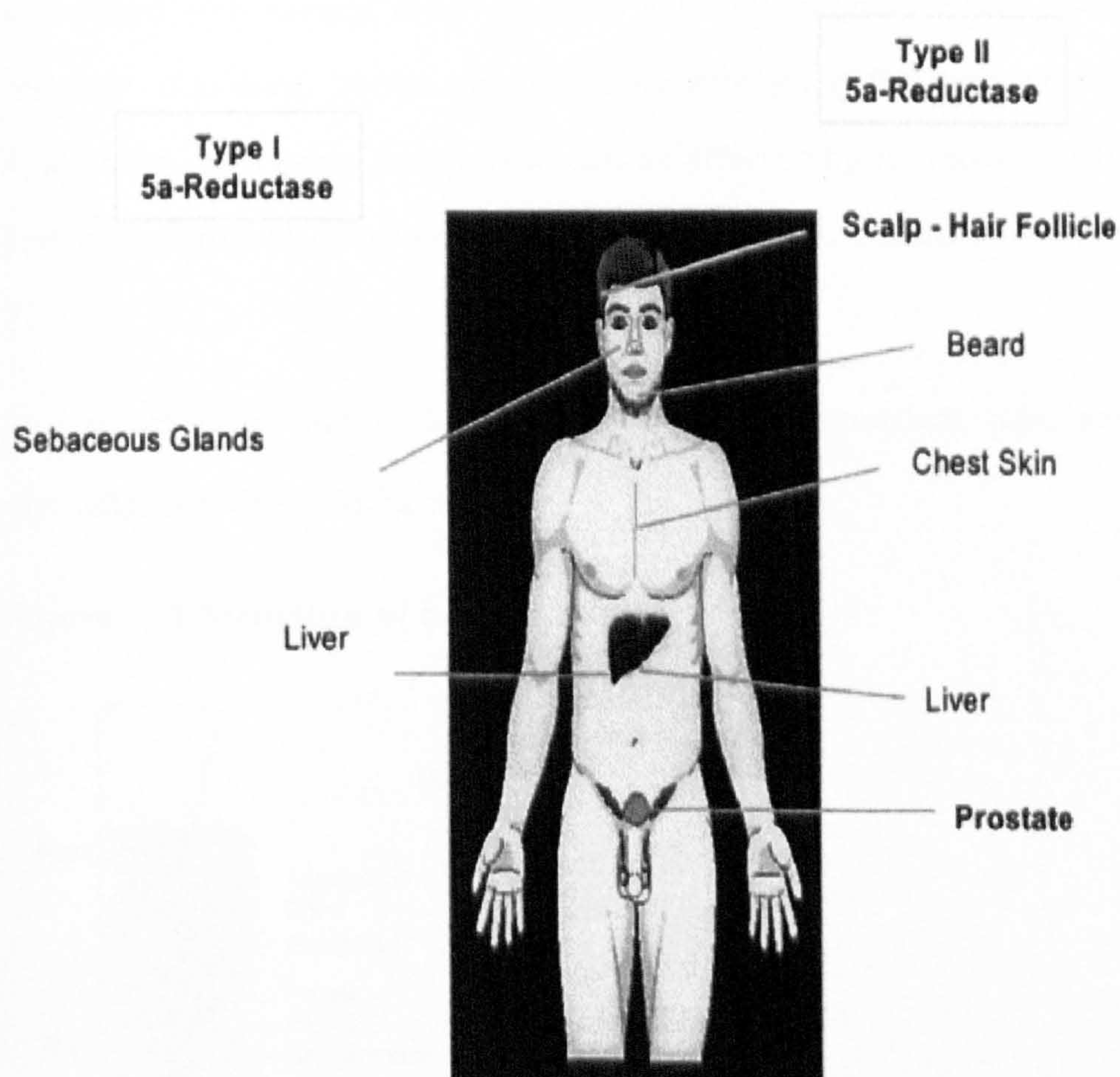
5.1 Androgens

Androgens play an important role in the growth and function of male reproductive organs such as testis and prostate and in the development of secondary sexual characteristics (Hsing *et al*, 2002). Androgens are male sex hormones formed by the testis and adrenal glands, and also from some peripheral tissues like skin and the prostate (Hsing, 2001). It has been postulated that they play a role in growth and progression of prostate cancer, but this association remains obscure in most epidemiological studies, possibly due to methodological issues (Platz *et al*, 2004). There are two main forms of androgens, firstly testosterone, of which about 90% of it is formed from androstenedione and secreted by the Leydig cells of the testis and 5-10% from adrenal glands and which is the major circulating androgen in adult male. The other is its metabolite, 5 alpha-dihydrotestosterone or dihydrotestosterone (DHT) mainly metabolized by 5 alpha-reductase in the skin and prostate (65-75%) and nearly 25% secreted by testis, is mainly found in tissues (Hsing, 2001; Soronen *et al*, 2004). There are two types of 5 alpha-reductase enzymes, type 1 enzyme is encoded by SRD5A1 gene and mainly found in hair and skin and type 2 encoded by SRD5A2 gene is mainly located in genital skin and prostate. This enzyme is responsible for irreversible conversion of testosterone to DHT within the prostate (Hsing, 2001) (see Figure 5-1).

Though the precise mechanism of androgen action in prostatic carcinogenesis is still not clear, the following facts suggest an important role of androgens in prostate cancer aetiology, the response of prostate cancer to hormonal therapy, the rare incidence of prostate cancer in eunuchs, regression of tumour after

androgenic ablation and the observation that prostate cancer never develops in men castrated before puberty and in individuals who are deficient in 5 alpha-reductase (Bosland, 2000; Hsing *et al*, 2002; Kirby & Patel, 2009). To understand the exact role of androgenic hormones in prostatic carcinogenesis Hsing et al, suggested setting up large-scale multidisciplinary investigations by incorporating molecular genetics, histopathology, biochemistry and endocrinology in epidemiological studies (Hsing *et al*, 2002).

Figure 5-1 Spatial distribution of types 1 and 2 5 alpha-reductase (Steers, 2001).

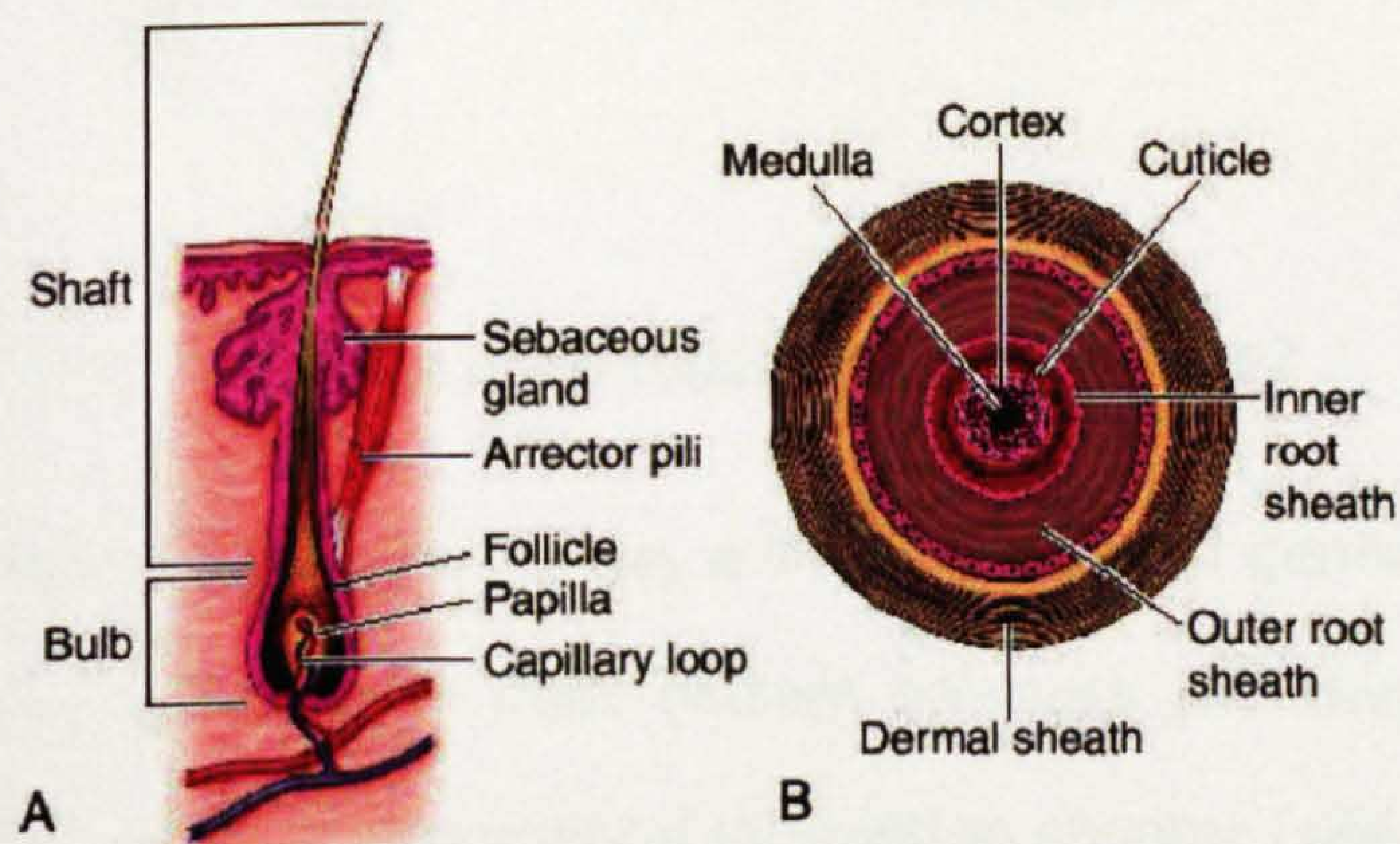


5.2 Baldness (Androgenetic alopecia)

Androgenetic alopecia (AGA) or male pattern baldness (MPB) is a very common condition characterized by a distinct pattern of hair loss from the scalp (Hillmer *et al*, 2005). Those who developed a marked alopecia soon after birth are more prone to develop alopecia on same areas in their adult life. Factors contributing to the pathogenesis of androgenic alopecia are androgens, endocrine stimulation by testis (and ovaries in females), genetic predisposition and aging. Though the most important factor is androgen dependency (Hamilton, 1951), a genetic predisposition which appears to be a polygenic is an important prerequisite for the phenotype (Hillmer *et al*, 2005). It has been suggested that AGA is associated with several other diseases such as coronary heart disease, insulin resistant disorders, benign prosatatic hyperplasia (BPH) and prostate cancer. Apart from androgens, hair growth can be affected by hormones such as thyroid and glucocorticoid (Gonzalez-Gonzalez *et al*, 2009; Lotufo *et al*, 2000; Trueb, 2010).

The structure of hair follicle is like a three-dimensional tube composed of epithelial cells (see Figure 5-2).

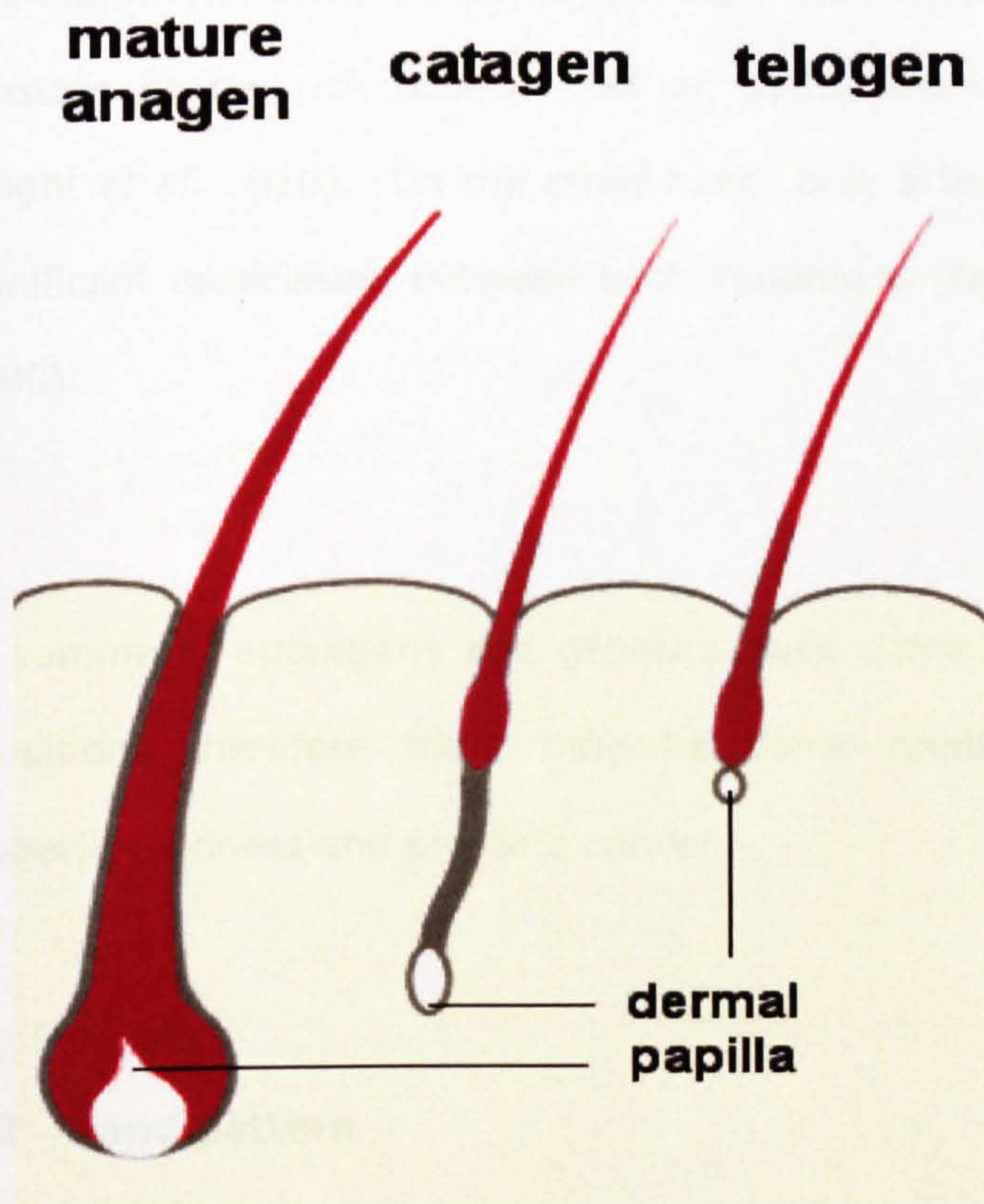
Figure 5-2 Structure of hair follicle



Taken from http://medicaldictionary.thefreedictionary.com/ /viewer.aspx.

There are three phases of hair follicle growth cycle, Anagen-(growth phase), Catagen-(short transitional phase), Tolegen-(resting phase) (see Figure 5-3). At the base of hair follicle are the dermal papillas which are believed to play a key role in controlling this cycle for growth and development of the hair follicle. Androgens act on the dermal papilla and regulate hair growth (Hibberts *et al*, 1998).

Figure 5-3 Hair follicle growth cycle



Taken from http://www.google.co.uk/imgres?

During literature review, a small number of genes have been found which appear to predispose to male pattern baldness and they will be discussed in details in Gene and Environmental interaction chapter (see chapter 6).

5.2.1 Baldness and prostate cancer

Though the exact mechanism and relation between the development of AA/MPB and prostate cancer is still unknown, both share many common factors such as prevalence, aging, androgens and heritability (Hawk *et al*, 2000; Wright *et al*, 2010). There are very few epidemiological studies that have addressed the association between AA/MPB and prostate cancer and results are inconsistent.

The majority of studies showed non significant association between baldness and prostate cancer risk (Cremers *et al*, 2010; Demark-Wahnefried *et al*, 2000; Wright *et al*, 2010). On the other hand, only a few studies showed statistically significant association between both conditions (Giles *et al*, 2002; Hawk *et al*, 2000).

In summary, androgens and genetics have some role in the aetiology of both conditions therefore there may be some relationship between androgenic alopecia/baldness and prostate cancer.

5.3 Hand pattern

Ancient civilizations recognised the study of the hand and its finger pattern in relation to health, longevity and personality traits. Even today, it remains an area of great interest amongst palmists, scientists and researchers. But it was only a decade ago that the second to fourth digit length (2D:4D) was proposed as a marker of prenatal testosterone exposure. Since then several studies have been conducted to establish the association between 2D:4D and human

behaviour, fertility, sexual orientation and different disease risks (McIntyre, 2006).

The 2D:4D ratio is sexually dimorphic, lower in males than females meaning the 4th digit is longer than 2nd digit and this pattern is associated with high levels of foetal testosterone, present at age 2 years and did not change at puberty (Lutchmaya *et al*, 2004; Voracek *et al*, 2005). Initially, the foetal differentiation into male phenotype is dependent on SRY gene on the Y chromosome. Which leads to the formation of foetal testis and the production of testosterone which starts at about 8 weeks of foetal life (Manning & Robinson, 2003). The Second and fourth digit ratio (2D:4D) has been correlated with foetal growth, hand preference, sperm count, family size, high sports ability, autism, Asperger's syndrome, age at myocardial infarction (MI) and breast cancer (Fink *et al*, 2006; Lutchmaya *et al*, 2004; Manning *et al*, 2003; Manning & Taylor, 2001b).

According to Manning et al., 1998, low digit ratio in right hand is associated with high sperm count and increased level of testosterone concentration in men. High digit ratio in both men and women is due to high concentration of luteinizing hormone(LH), oestrogen and prolactin (Manning *et al*, 1998).

The results of a report conducted on 255,116 participants in a BBC internet survey also showed lower mean 2D:4D for right hand as compared to left in men, but lower in left hand than right in women (Manning *et al*, 2007). The data revealed that the sex difference in 2D:4D ratio almost same in all races and is universal (Manning & Robinson, 2003). However it is also evident from other studies that, the mean 2D:4D is higher in Whites, non- Chinese Asians and Mid-

Easterners and lower for Chinese and Blacks. These differences are also significant across sexual orientation but only in men, higher in homosexuals and bisexuals as compare to heterosexuals suggesting low prenatal testosterone exposure in former group (Manning *et al*, 2007).

Though there is a growing body of evidence that many sex-dependent behaviour are associated with the 2D:4D ratio. However, there is no direct evidence for this association and is entirely based on indirect evidence from the characteristics dependent on sex hormones such as congenital adrenal hyperplasia (a genetic disorder associated with high prenatal androgen), maternal smoking during pregnancy supposed to increase foetal testosterone and having sons with a low 2D:4D, waist-hip ratio and 2D:4D and polymorphism in X-linked androgen receptor gene resulting in increased sensitivity to testosterone (Manning *et al*, 2003; Manning *et al*, 2007). A study conducted on 50 men (49 Caucasian, 1 Caucasian/Chinese) showed a positive association with CAG (Cytosine, Adenine and Guanine) number, that men with low 2D:4D in their right hand have AR alleles with low CAG numbers (Manning *et al*, 2003). Twin studies suggest there is also a possible genetic role in addition to any prenatal environmental influence on this hormonally related skeletal ratio in both men and women (Gobrogge *et al*, 2008; Paul *et al*, 2006). To author's knowledge, there is only one cohort study looking at digit ratio and prostate volume, PSA level, and the prostate cancer risk (Jung *et al*, 2010). No other case-control study investigates this marker and prostate cancer risk.

In summary, the morphology of hand pattern (2D:4D) arises in *utero* from the concentration of sex hormones, lower in men than in women meaning 2D length is shorter than 4D length, and negatively associated to prenatal and adult

testosterone and related phenotypes such as sperm counts and positively associated with prenatal oestrogen. The inclusive link between second to fourth digit length and testosterone thus warrants the investigation of this marker with prostate cancer.

5.4 Acne

Acne or acne vulgaris is the most common skin disorder of the pilosebaceous unit (consists of sebaceous gland and hair follicles) affecting nearly 80% of young population aged 11-30 (Leyden, 1995; Toyoda & Morohashi, 2001). There is no standard classification for acne but it has been suggested that it classified in to non- inflammatory (open or closed comedones) and inflammatory (papules, pustules and/or nodules) forms (Bhambri *et al*, 2009). Problems related with acne are disfiguration, permanent scarring, and psychological disturbances ranging from social phobias to clinical depression (Leyden, 1995). The Pathogenesis of acne is multifactorial and factors that can play an important role in the development of acne are genetics, androgens, sebum, immunity and bacterial infection (Mascaro, 2000).

Acne is characterized by androgen stimulated increased sebum production (seborrhoea), abnormal desquamation of follicular epithelium, colonization of *propionibacterium acnes (P.acnes)* and inflammation (Toyoda & Morohashi, 2001). *P.acnes* is a harmless commensal gram-positive anaerobic bacteria found in the sebaceous gland of the skin (Bruggemann *et al*, 2004).

Acne appears at a time of steady increase in production of androgens, that is puberty. But most men and women with acne have normal androgen levels and it is thought to be due to hyper responsiveness of sebaceous glands to androgens (Cunliffe, 1980; Leyden, 1995). Most of the androgens are produced by testis and adrenals, they can also be produced by sebaceous glands from adrenal precursor hormone, dehydroepiandrosterone sulphate (DHEAS), but the main androgens that act on androgen receptors are testosterone and DHT. DHT is 5-10 times more active than testosterone to act on androgen receptors present in the sebaceous gland (Thiboutot & Thiboutot, 2004). Androgens increase sebum production, a critical factor in the development of acne (Leyden, 1995).

Although hormones are important in the development of acne, the mechanism of action of hormones in the pathogenesis of acne is not clear (Thiboutot & Thiboutot, 2004). However, evidence from different studies suggests that acne appears on administering androgens in castrated men or those who have genetic mutations in androgen metabolism. It is found that women with acne can be cured with antiandrogenic therapy and the oral contraceptive pill and acne is often associated with androgenic producing tumours of the adrenals and ovaries (Galobardes *et al*, 2005; Thiboutot & Thiboutot, 2004).

The combined twin and nested cross-sectional study in women reported that acne is a inheritable disease with significant additive genetic effects. Until recently very few candidate genes have been identified and but those that have related to androgen and steroid metabolism. The sample size of these studies however was very small (Bataille *et al*, 2002).

5.4.1 Acne and prostate cancer risk

It has generally been hypothesized that androgens have an important role in the aetiology of both acne and prostate diseases, though the exact mechanism is yet to be established. Acne was used a marker of excess male hormone in a case-control study conducted by Giles et al, observed association between acne and prostate cancer risk (Giles *et al*, 2003).

It is also well established that chronic inflammation can cause several human cancers. The emerging theory that *P.acnes* might be one of the causes of prostate cancer gives a new lead to several combined risk factors in the aetiology of both conditions (Sutcliffe & Platz, 2007).

It is evident from several studies that nearly 20% of adult human cancers are due to chronic infection or inflammation. Many epidemiological, molecular and histopathological studies proved that inflammation plays an important role in prostate cancer pathogenesis but the exact cause of prostate inflammation is still not clear. There may be several reasons for prostatic inflammation and infectious agent could be one of them and there may be several infectious agents leading to chronic inflammation of the prostate (De Marzo *et al*, 2007b).

In summary, three factors are common in the pathogenesis of acne and prostate cancer, androgens, *P.acnes* and heritability may play some role in the aetiology of both conditions. Therefore it is worth investigating the role of acne in development of prostate cancer.

5.5 Hypothesis and aims

Hypothesis

There is an association between male sex hormones surrogate markers and prostate cancer risk including balding, pattern of index to ring finger length and acne.

Aims

To assess the association between male sex hormones surrogate markers and prostate cancer risk including balding, pattern of index to ring finger length and acne.

5.6 Methodology

5.6.1 Grading of Baldness pattern

Hamilton's classification of the pattern of baldness (Baran *et al*, 1991) was used (see section 3 in the questionnaire in appendix). There are seven grades of baldness from normal hair pattern to severe vertex baldness. Grade 1 was assigned for normal hair pattern, which was also used as reference category, grade 2 for frontal baldness and 3-7 for vertex baldness. The reason of merging all grades of vertex baldness is to make sample size stable in that category, because all other have only one grade and vertex baldness has five grades. This approach had been applied by many other studies (Demark-Wahnefried *et al*, 1997; Giles *et al*, 2002). Subjects were asked to record their balding from the

pictures provided during their 20s, 30s and 40s. The analysis was performed with these individual ages and further with the subset with a positive family history of prostate cancer. For the latter, only significant results are presented here due to limitations on space (baldness at age 30) (see Table 5-2).

5.6.2 Right hand pattern

Subjects were asked to identify their finger length pattern on right hand as nearest to series of pictures depicted in the questionnaire. A clear instruction of how best to compare their hand with the pictures provided. There were three illustrations indicating: the index finger longer than the ring finger, the index equally as long as the ring finger and the index shorter than the ring finger. The latter was used as the reference category.

5.6.3 Acne

Subjects were asked to respond yes or no for the presence of acne at puberty, in their 20s and 30s. Acne during these periods was individually analysed to obtain odd ratios.

To investigate an effect of prolonged acne since teens through to 20s and 30s, only subjects who answered all questions related to acne at teens, at 20s and at 30s were eligible for the analysis. A further variable filter was applied to categorise them according to the duration of acne presence i.e. from teens only, teens to 20s etc. Subjects who reported never had acne were used as a reference category.

5.7 Analysis

Unconditional logistic regression was used to generate odds ratios and 95% C.Is. To control for confounding, age and social class were added to the model; age was included as a continuous variable whereas social class was fitted as a categorical variable.

5.8 Results

5.8.1 Pattern of baldness

Results showing pattern of baldness at age 20, 30 and 40

Table 5-1 shows distribution and odds ratios of baldness and prostate cancer risk

Table 5-1 Distribution and odds ratio of pattern of baldness at age 20, 30, and 40

#Baldness at 20	**Case (%)**	**Control (%)**	**OR†**	**95%CI Lower-upper**	**P-value**
No baldness	716(68.3)	1235(69.9)	1.00		
Frontal baldness	234(22.3)	361(20.4)	1.10	0.91-1.33	0.34
Vertex baldness	99(9.4)	172(9.7)	1.02	0.78-1.33	0.88
Total	1049(100.0)	1768(100.0)		*P for trend*	*0.58*
#Baldness at 30					
No baldness	429(40.6)	733(41.3)	1.00		
Frontal baldness	311(29.4)	559(31.5)	0.95	*0.79-1.14*	*0.56*
Vertex baldness	317(30.0)	481(27.1)	1.13	*0.94-1.36*	*0.20*
Total	1057(100.0)	1773(100.0)		*P for trend*	*0.25*
#Baldness at 40					
No baldness	201(18.4)	328(18.0)	1.00		
Frontal baldness	308(28.3)	602(33.0)	0.84	0.67-1.06	0.15
Vertex baldness	581(53.3)	896(49.1)	1.07	0.87-1.31	0.55
Total	1090(100.0)	1826(100.0)		*P for trend*	*0.18*

#No baldness (picture 1), frontal baldness (picture2), vertex baldness (picture 3-7)
†adjusted for age and social class

Results showed that the prevalence of baldness increased with advancing age. At the age 40, nearly 80% of cases and controls had some baldness. Those with frontal and vertex baldness are not at any greater risk compared to no baldness at any age (all confidence intervals include 1).

Cluster analysis of family history of prostate cancer in first degree relatives

Cluster analysis was carried out to investigate an effect modification of family history on balding and prostate cancer risk. Only the results of balding at 30s show the significant association as presented below.

Baldness at age 30s and risk of prostate cancer within familial cases

Table 5-2 shows distribution and risk estimates on baldness at age 30s and prostate cancer among subjects with father and brother affected and not affected by prostate cancer.

Table 5-2 Percentage and odds ratio of pattern of baldness at age 30 within familial cluster

First degree relative affected with prostate cancer	#Baldness at 30	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
No	No baldness	293(41.9)	649(40.2)	1.00		
	Frontal baldness	205(29.3)	519(32.1)	0.86	0.69-1.06	0.16
	Vertex baldness	201(28.8)	447(27.7)	1.01	0.81-1.25	0.96
	Total	699(100.0)	1615(100.0)		*P for trend*	*0.90*
Yes	No baldness	115(38.1)	46(53.5)	1.00		
	Frontal baldness	87(28.8)	18(20.9)	2.06	1.10-3.83	0.02
	Vertex baldness	100(33.1)	22(25.6)	1.85	1.03-3.31	0.04
	Total	302(100.0)	86(100.0)		*P for trend*	*0.03*

#No baldness (picture 1), frontal baldness (picture2), vertex baldness (picture 3-7)
†adjusted for age and social class

The pattern of baldness is almost the same in cases and controls with no family history of prostate cancer. While those with positive family history of prostate cancer, both frontal and vertex balding prevalence is greater in case group. Risk is slightly higher with frontal balding subjects as compared to vertex balding

subjects (OR 2.06, 95% C.I. 1.01-3.83 and OR 1.85, 95% C.I. 1.03-3.31) respectively with positive history of prostate cancer in the family.

5.8.2 Right hand pattern

Distribution values and odds ratios are shown in Table 5-3

Table 5-3 Right hand pattern and prostate cancer risk

Right hand pattern	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Index shorter than ring	862(78.1)	1442(77.4)	1.00		
Index equal to ring	221(20.0)	368(19.8)	1.01	0.83-1.22	0.93
Index longer than ring	21(1.9)	52(2.8)	0.63	0.37-1.07	0.09
Total	1104(100.0)	1862(100.0)			

†adjusted for age and social class

The result indicates risk reduction in subjects with index longer than ring finger with borderline statistically significant (p-value 0.09).

5.8.3 Acne

Acne at different ages in life was explored and the results are presented below.

Acne at puberty

Presence of acne during puberty is shown in Table 5-4

Table 5-4 Acne during puberty and prostate cancer risk

Acne at puberty	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
No	800(72.7)	1393(75.1)	1.00		
Yes	301(27.3)	461(24.9)	1.16	0.97-1.37	0.10
Total	1101(100.0)	1854(100.0)			

†adjusted for age and social class

No association was observed with presence of acne at puberty and prostate cancer risk (confidence interval includes 1).

Acne at 20s

Acne at age 20s is shown in table 5-5

Table 5-5 Acne at 20s and prostate cancer risk

Acne at 20	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
No	974(88.9)	1659(89.9)	1.00		
Yes	121(11.1)	187(10.1)	1.10	0.86-1.41	0.46
Total	1095(100.0)	1846(100.0)			

†adjusted for age and social class

Results showed presence of acne at 20s is not associated with prostate cancer risk.

Table 5-6 shows presence of acne from teen through 20s and prostate cancer risk

Table 5-6 Risk estimates of acne appearance from teen through 20s

Teen through 20	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Never	800(73.3)	1393(75.5)	1.00		
Acne at teen but clear at 20s	172(15.8)	266(14.4)	1.16	0.93-143	0.18
Both at teen and 20s	119(10.9)	187(10.1)	1.11	0.86-1.43	0.42
Total	1091(100.0)	1846(100.0)		*P for trend*	*0.21*

†adjusted for age and social class

The results showed that there is no risk difference amongst those who have had acne from teen through 20s to those who never have had acne. There is no trend of increasing risk across categories (P for trend 0.21).

Acne at 30

Acne at age 30s is shown in table 5-7

Table 5-7 Acne at 30s and prostate cancer risk

Acne at 30	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
No	1026(95.4)	1725(97.1)	1.00		
Yes	49(4.6)	52(2.9)	1.59*	1.06-2.39	0.03
Total	1075(100.0)	1777(100.0)			

†adjusted for age and social class

A greater percentage of case reported having had acne in their 30s as compared to controls (4.6% as compared to 2.9%) and the result showed 60% risk increase as compared to those who reported never had acne.

Presence of acne from teen through to age 30s and risk of prostate cancer is shown in Table 5-8.

Table 5-8 Association between duration of acne appearance from teens through 30s and prostate cancer risk

Teen through 30	Case (%)	Control (%)	OR†	95%CI Lower-upper	P-value
Never have acne	794(72.5)	1389(75.1)	1.00		
Presence of acne at puberty only	186(17.0)	291(15.7)	1.15	0.93-1.41	0.19
Presence of acne at teen and 20s but not 30s	71(6.5)	121(6.5)	1.01	0.74-1.39	0.93
Presence of acne at teen, 20s and 30s	44(4.0)	49(2.6)	1.60*	1.04-2.45	0.03
Total	1095(100.0)	1850(100.0)		*P for trend*	*0.06*

†adjusted for age and social class

Subjects who reported suffered from acne from puberty through to the age of 30s are at greater risk as compared to subjects who never had acne (OR 1.60, 95% C.I. 1.04-2.45) (p-value=0.03). There is a borderline trend of risk increase across categories (p-value =0.06).

5.9 Discussion

Male hormones are hypothesised to associate with prostate cancer therefore this study investigated selected surrogate markers of male hormones and prostate cancer risk. These markers are balding, finger length pattern and acne.

5.9.1 Pattern of baldness

The results showed no associations with either frontal or vertex baldness at any age and prostate cancer risk (all ORs are around 1). Similar findings were reported in a recent case-control study suggesting no statistically significant association between baldness and prostate cancer risk (OR 1.10, 95% C.I. 0.89-1.34) (Cremers *et al*, 2010). Another case-control study using the same method for assessing the pattern of baldness (Illustration of the Hamilton classification) showed some association between early (age 30) and later (age 40) onset vertex baldness and prostate cancer risk, but these risks were, however not statistically significant (OR 2.44, 95% C.I. 0.57-10.46) and (OR 2.11, 95% C.I. 0.66-6.73) respectively (Demark-Wahnefried *et al*, 2000). Several other previous case-control studies also support the above results by showing no significant association between baldness and prostate cancer risk (Demark-Wahnefried *et al*, 1997; Hsieh *et al*, 1999; Oishi *et al*, 1989; Wynder *et al*, 1971).

A recent population-based case-control study showed negative association between baldness and prostate cancer risk. There was 29% risk reduction with hair loss at age 30 for prostate cancer cases (OR 0.71, 95% C.I. 0.56-0.91) but no risk reduction for those who reported hair loss only at referent date (1 year prior to diagnosis in cases and a randomly assigned date that approximated the

distribution of cases diagnosis date for controls). The risk reduction is even greater with hair loss (all types) at age 30 in men aged >60 at referent date (OR 0.55, 95% C.I. 0.33-0.93) (Wright *et al*, 2010). In this study 999 cases and 942 controls were analyzed, using different methodology for selection of controls, for assessing the pattern of baldness such as observing hair loss at age 30 and referent date and using show cards in person interview. The study did not use Hamilton classification and in their primary analysis they used three categories for assessing baldness such as little or no hair loss, loss at forehead only and loss at top of head and forehead (each class contain two pictures) and in their subsequent secondary analysis they used five categories, while using little or no hair loss as referent and other four as an independent categories.

On the other hand, an Australian case-control study of 1446 cases and 1390 controls found a significant increased risk between both frontal and vertex baldness and prostate cancer at age (60-69 years) (OR 1.80, 95% C.I. 1.02-3.16; OR 2.91, 95% C.I. 1.59-5.32) respectively. Combined effect of baldness vs. no baldness is also appeared to be significant (OR 1.95, 95% C.I. 1.10-3.45) (Giles *et al*, 2002). The results of this study were different form present study may be due to differences in methodology.

A prospective study conducted on 4,421 men age 25-75 years old without history of prostate cancer followed for 17-21 years found 421 incident cases of prostate cancer showing the greater age standardized incidence of prostate cancer among men with baldness and there was evidence of increased risk among baldness and prostate cancer risk (RR 1.50, 95% C.I. 1.12-2.00) (Hawk *et al*, 2000). The possible explanation of the difference in results could be the study design and the use of different scale of measurement for baldness.

In this present study, there is very similar prevalence of hair patterns both frontal and vertex balding, in cases and controls. This could explain non significant findings. Male pattern baldness is a very common condition affecting nearly 25-30% of men by the age of 30 and 80% of all males by age of 80 years (Cancer Research UK, 2010a; Ellis *et al*, 2001). More common in Caucasians as compare to other races (Hamilton, 1951) with the highest prevalence of 30% occurring by the age 30 years (Kabai & Kabai, 2008). According to the figures from the NHS, UK, nearly 6.5 million men are bald in the UK and by age 60, the majority of men have some degree of hair loss (National Health Service, 2010).

The analysis of the subset with a family history of prostate cancer in first degree relatives suggested statistically significant results for frontal and vertex baldness in those with positive family history (OR 2.06, 95% C.I. 1.01-3.83 and OR 1.85, 95% C.I. 1.03-3.31 respectively). These results indicate a genetic association in both balding phenotypes. To the author's knowledge there are no case-control studies reporting baldness as a risk factor in the aetiology of prostate cancer relative to the presence of prostate cancer in the family. But it is evident from genetic studies that family history and genetic factors play an important role in both conditions (Hawk *et al*, 2000).

This study used self reported baldness using pictures present in questionnaire. This method was proved reasonably precise by a study conducted by Littman and White, 2005 (Littman & White, 2005). Taylor et al, 2004, also suggested self reporting as the method of choice in the absence of trained staff for assessing baldness using Hamilton-Norwood classification (Taylor *et al*, 2004). This may be due to its cosmetic and social importance.

In conclusion, the results from this study supported no association between pattern of hair loss and prostate cancer risk at any age. There was, however, a positive association between baldness and prostate cancer risk in those with a positive family history of prostate cancer, suggesting a potential shared genetic role in the aetiology of both conditions which might influence hormonal mechanism.

5.9.2 Right hand pattern

The common phenotype of male finger pattern is index shorter than ring finger (approximately 77.4% in controls). This phenotype is used as reference. The result indicated a risk reduction, although not statistically significant, between men with index longer than ring finger and men with index shorter than ring finger (OR 0.63, 95% C.I. 0.37-1.07). No association was found in men with ring equal to index finger as compared to men with index shorter than ring finger (OR 1.01, 95% C.I. 0.83-1.22).

The only study investigated the relationship between digit length pattern and prostate cancer is the Korean Cohort study. Jung et al, showed a significant negative association between digit ratio and PSA (r=-0140, p=0.007). Those with lower digit ratio had higher mean PSA level and had higher risk of prostate biopsy (OR 1.75, 95% C.I. 1.07-2.84) and prostate cancer (OR 3.22, 95% C.I. 1.33.7.78) (Jung *et al*, 2010). These results are consistent with the present study.

In our previous analysis whereby 3 data sets from a series of prostate cancer studies conducted by our study group including "The Gene-environment

interaction study from the first (1999-2004) and second phase (2007-2009) " Gene-environmental interactions in prostate cancer" and "Prostate cancer: A case-control study of lifestyle and dietary factors using BPH and community-based controls "(1999-2002), a PhD thesis by Dr Artitaya Lophatananon, were merged to provide a large number with 1524 cases and 3044 population-based controls had shown the negative association between the hand pattern with index finger longer than ring finger (high 2D:4D) and prostate cancer risk, indicative of a protective effect with a 33% risk reduction (OR 0.67, 95% C.I. 0.57-0.80) (Rahman *et al*, 2010) (article in press). In this present analysis, the author included only data from the Gene-environment interactions in prostate cancer study (both phase I and II) as the author was directly involved with the data collection/validation process and to make all analyses of all variables consistent throughout. This results in a smaller number of subjects, 1104 cases and 1862 controls.

Pictures of the right hand were provided to aid the response as there is a greater sex difference in 2D:4D on the right hand side than on the left hand (Williams *et al*, 2000). The procedure was particularly successful in terms of response rate (99% of eligible subjects responded to the question).

It has been suggested that intra-uterine exposure of hormones has an impact on the development of other adult-onset diseases (Manning & Bundred, 2000) including a large study on finger pattern and osteoarthritis risk, where lower digit ratio was associated with osteoarthritis (Zhang *et al*, 2008). In this study, digit lengths were physically measured on the hand radiographs using vernier callipers to allow for actual measures with a high degree of accuracy and repeatability. Although such an approach allows for an exact ratio to then be

calculated, it was considered impractical and unethical for this present study as hand radiographs were not available. Instead, we used a more pragmatic way to identify the pattern of 2nd and 4th finger by self reported comparison of the hand with pictures. The self-reported finger length, however, raises a possible concern over measurement error as discussed by Caswell and Manning. In their study, they used two different approaches to measure 2D:4D including finger length measured from photocopies of the ventral surface of hands (photo 2D:4D) and self-reported finger length measured directly from the finger (S-R 2D:4D). The results suggested that S-R 2D:4D showed more extreme values when compared to photo 2D:4D. The authors, however, concluded that a large sample size would reduce the effect size of this and thus this possible error is unlikely to have a large effect in our study (Caswell & Manning, 2009). Another validation assessment was made by Zhang et.al. In their study, a questionnaire was used with hand pictures and this was compared with exact finger length measurements from radiographs. Both methods showed similar results with a lower digit ratio associated with increased risk of osteoarthritis of knee and hip (Zhang *et al*, 2008).

The finger length relationship seen in our study is also in keeping with equivalent studies on breast cancer risk based on current understanding of the role of hormonal patterns *in utero*. Women with a high ratio of 2D:4D (indicative of higher prenatal oestrogen exposure) are at greater risk of breast cancer. Women with the more "feminine" pattern of digit length (2D:4D high - ring finger closer in length or shorter than the index finger), were also more likely to present at a younger age (Manning & Bundred, 2000).

Although finger length in humans has been studied for decades, its relationship with hormones has only been established only recently. In humans, the growth and pattern of digits and the differentiation of gonads is controlled by the homeobox genes *HOXA* and *HOXD*. Therefore, gonadal foetal products such as testosterone may influence finger morphology (Manning *et al*, 2003). Many studies have shown that a high concentration of testosterone, indicating high prenatal testicular activity was inversely related with 2D:4D ratio (higher the prenatal testicular activity and lower the 2D:4D ratio). The negative correlation between digit ratio and hormone profile has been used as a marker to predict offspring sex ratio and sporting ability (Manning *et al*, 2002; Manning & Taylor, 2001a; Robinson & Manning, 2000; Williams *et al*, 2000). 2D:4D ratio is greater in the right hand compared with the left and has a higher sensitivity with foetal androgens than the left hand (Williams *et al*, 2000). Manning and colleagues demonstrated that high 2D:4D ratio in male right hands was associated with germ cell failure (GCF) due to azoospermia or oligospermia with no motility. They also demonstrated that testosterone assays from 58 male subjects were inversely associated with 2D:4D ratio in the right hand (p= 0.03). The association was absent in left hand (Manning *et al*, 2000).

In summary, the indicative negative association suggested some potential role of lower prenatal activity of testosterone which showed some protection against prostate cancer later on in life.

5.9.3 Acne

The results indicate that acne at puberty and at age 20s is not a risk factor for the prostate cancer (OR 1.16, 95% C.I. 0.97-1.37 and OR 1.10, 95% C.I. 0.86-1.41, respectively). There was no trend of increasing risk with the presence of

acne from teens through to their 20s (p for trend 0.21). The results from a cohort study conducted on 11, 232 male students in Glasgow University who participated in voluntary health checks reported history of acne participants during 1948-1968, contradicting the results of this study, showed that students with acne at a young age had higher risk of prostate cancer mortality than at a later age (hazard ratio=1.67, 95% C.I. 0.79-3.55) (Galobardes *et al*, 2005).

In the present study, men who reported suffering from acne in their 30s are at greater risk for developing prostate cancer (OR 1.59, 95% C.I. 1.06-2.39) compared to men who never had acne. A test for trend suggests increasing risk of prostate cancer with the longer duration of acne "from puberty till age 30" (p for trend 0.06). Since acne is not exclusively related to high level of androgen and inflammation, this result supported that long term suffering from acne potentially indicatives prolonged and persistent high level of androgens is a risk factor for prostate cancer and/or may be suggestive the role of chronic inflammation in the causation of prostate cancer.

To the author's knowledge, only a case-control study by an Australian group investigated the association between markers of body growth, size, changes including acne and prostate cancer risk on 1476 cases and 1409 controls. As they classified acne in different manner, it is not possible to compare results with present study, however, a negative association was found in their results of never had acne vs. ever had acne (OR 0.71, 95% C.I. 0.52-0.97) and acne onset before puberty vs. never had acne (OR 0.67, 95% C.I. 0.45-0.99). Results of acne after puberty vs. never had acne proved insignificant (Giles *et al*, 2003).

As mentioned in the literature review, another emerging theory is the role of inflammation in the causation of prostate cancer (De Marzo *et al*, 2007a; De Marzo *et al*, 2007b; Sutcliffe & Platz, 2007). It is evident from previous studies that chronic inflammation plays some role in the aetiology of cancers of many other organs such as oesophagus, stomach, colon, liver and urinary bladder. It is also evident from epidemiological studies that prostatitis and sexually transmitted diseases may increase the risk of prostate cancer (Sugar, 2006). There could be several reasons for prostatitis ranging from invasion of organ by microorganism to cell injury due to physical and chemical trauma, hormonal variations and dietary factors (De Marzo *et al*, 2007b; Vasto *et al*, 2008; Wagenlehner *et al*, 2007). A cohort study conducted at the Johns Hopkins Bloomberg School of Public Health revealed a possible role of *P.acnes* as a causative agent for acne in the development of prostate cancer (RR. 1.70, 95% C.I. 1.03-2.80) (Sutcliffe *et al*, 2007). Cohen et al in their study also found *P.acnes* in 35% samples of prostate cancer patient underwent radical prostectomy for localized tumour (*P*=0.007) (Cohen *et al*, 2005).

5.10 Recommendations

- For the hand pattern measurement of actual ratio between 2 digits are recommended.

- Bearing in mind the important role of hormones in baldness, defining hand pattern, acne with prostate cancer, there is a timely need for more large-scale multidisciplinary investigations incorporating molecular genetics, histopathology, biochemistry and endocrinology in epidemiological studies to further investigate how these conditions can be used as biomarkers for prostate cancer.

- In addition the role of *P.acnes* in the development of prostate cancer should be evaluated in a more detailed manner using inflammatory markers and by culturing biopsies taken for histopathological grading.

Chapter 6 Gene and environmental interaction in prostate cancer

6 Literature review

It is suggested that cancer is not exclusively the outcome of endogenous or exogenous carcinogens but their interaction with genes is suggested to play a role in carcinogenesis. This may be particularly so in "sporadic" cancer which may be the result of exposure to environmental factor along with polymorphism in genes indicative of increased susceptibility (Kotnis *et al*, 2005). About 99.9% of Deoxyribonucleic acid (DNA) is identical in every human genome and only 0.1% variation is responsible for inter-individual differences and exclusive phenotype of each individual. These small genetic variations in the genome are known as **single nucleotide polymorphisms (SNPs)** (Kotnis *et al*, 2005). The key concept of genetic research is to discover the association of sequence variation with heritable phenotypes and SNPs which are considered the most common variations that may have functional significance (Smigielski *et al*, 2000).

The majority of chronic diseases are likely to be the outcome of gene and environmental interaction and the most suitable approach to investigate the association between multiple genes and environmental factors is a standard case-control study (Kellen *et al*, 2005). For gene-environmental interaction studies, there still is a shortage of good quality data sets. The results to date are varied and this is due to the requirement of a large sample size to allow such investigation.

Table 6-1 Rationale for the study of gene-environment interactions

• Obtain a better estimate of the population-attributable risk for genetic and environmental risk factors by accounting for their joint interactions. • Strengthen the associations between environmental factors and diseases by examining these factors in genetically susceptible individuals. • Help to dissect disease mechanisms in humans by using information on susceptibility (and resistance) genes to focus on the biological pathways that are most relevant to that disease, and the environmental factors that are most relevant to the pathways. • Determine which specific compounds in the complex mixtures of compounds that humans are exposed to (such as diet or air pollution) cause disease. • Use the information on biological pathways to design new preventive and therapeutic strategies. • Offer tailored preventive advice that is based on the knowledge that an individual carries susceptibility or resistance alleles.

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Taking forward the results of exposure analysis in previous chapters, two particular groups of genes were investigated further relating to X-ray damage, and hormone markers including balding and acne. The following literature describes the roles of these genes and their possible relationship to prostate cancer.

6.1 DNA repair genes

6.1.1 DNA damage repair machinery

DNA is commonly subjected to damage caused by endogenous as well as exogenous mutagens such as cigarette smoking, ionising radiation, ultra-violet rays and other chemicals. There are several genome stability pathways for the repair of damage to DNA due to different agents (Bulman *et al*, 2006). There are now more than 150 DNA repair genes directly involved in these repair pathways in humans (Agalliu *et al*, 2010).

Different genes function within different pathways during DNA replication or DNA repair. These pathways include recognising and deleting the DNA lesions, giving tolerance to DNA damage, and providing protection from errors (Ronen & Glickman, 2001). If the damage remains unrepaired it may leads to apoptosis, unregulated cell growth and cancer (Goode *et al*, 2002).

Different types of damage to DNA such as exposure to ionising radiation can cause oxidation and fragmentation of DNA bases. Subsequently, it affects the formation of DSBs (Bulman *et al*, 2006). It has been evident from animal studies that DSBs initially repaired by non-homologous end joining process which is prone to error. Cells with multiple DSBs may cause chromosomal rearrangements and several other major changes leading to radiation carcinogenesis (Little, 2000).

To perform a critical repair function, there are five different types of DNA repair pathways such as homologous recombination repair (HRR), non-homologous end joining (NHEJ), nucleotide excision repair (NER), base excision repair (BER) and mismatch repair (MMR) (Bernstein *et al*, 2002). DNA damage caused by ionising radiation is however, repaired by HRR and NHEJ pathways (Goode *et al*, 2002). These repair systems protect genome stability by repairing modified bases, DNA adducts, crosslinks and DSBs (Bulman *et al*, 2006).

Although these two pathways are mainly involved in repairing insults caused by ionising radiations, we are also looking at genes involved in other pathways.

6.1.2 DNA repair genes and prostate cancer risk

DNA repair pathways play a vital role in retaining the genome stability by preventing and protecting DNA from injury. Damage to these pathways may lead to many cancers. With aging, there could be several different factors accumulated and may result in DNA damage such as oxidative stress, inflammatory process, exposure to different environment carcinogens and/or dwindling DNA repair capacity may increase the prostate cancer risk (Agalliu *et al*, 2010).

In this chapter, DNA pathways are reviewed and after extensive literature searches, the following genes were selected to investigate further on the basis of their potential interactions between X-ray exposures and the selected single nucleotide polymorphisms. The summary of studies is depicted in table 6-2 (see

Table 6-2) and the summary of selected SNPs of these genes is illustrated in table 6-3 (see Table 6-3).

A study conducted by Hirata et al, showed evidence that the chemokine CXCL12G801A polymorphism may be associated with prostate cancer risk, they studied this particular mutation on the basis of evidence from previous studies that chemokines have an important role in the metastasis of tumour cells (Hirata *et al*, 2007a). The XPD (Xeroderma Pigmentosum Group D) gene is mainly involved in nucleotide excision repair (NER) pathway, and it has been suggested that this gene has a vital role in environmentally induced cancers. Mutations in this gene may halt the important steps leading to removal of DNA adducts from the affected organ and especially polymorphism in *XPD codon 312* which is associated with increased level of DNA adducts in breast cancer tissue (Rybicki *et al*, 2004). The *XPC Lys939Gln* (Xeroderma Pigmentosum Group C) is also works principally through the NER pathway, a pathway mainly responsible to fix bulky DNA adducts. It is evident from studies that *XPC codon 939* polymorphism may be associated with increased risk of bladder and lung cancer and might be risk factor for prostate cancer (Hirata *et al*, 2007b). Ritchey et al, studied *XRCC3 Thr241Met* (X-ray repair cross completing group 3), which act through HRR pathway, a pathway responsible for repairing DSBs and found some association with prostate cancer risk, although they analysed the combined effect of environmental factor such as diet and genotype (Ritchey *et al*, 2005). Ritchey et al, also found some association of *MGMT (O-6-methylguanine-DNA methyltransferase) MGMT -Leu84phe&MGMT-Ile143Val* which act through direct damage reversal mechanism with prostate cancer risk (Ritchey *et al*, 2005). It is evident from several studies that *XRCC1Arg399 Gln* (X-ray repair cross completing group 1) genotype involved in BER pathway are associated with

increased prostate cancer risk (Hirata *et al*, 2007b; Ritchey *et al*, 2005; Rybicki *et al*, 2004), while Hirata et al (b) performed first study to see the association between polymorphism in *XRCC1 Arg194Trp* and found it to be a possible risk factor for prostate cancer (Hirata *et al*, 2007b). The *XRCC7G6721T* (X-ray repair cross completing group7) is one of the genes which acts through NHEJ pathway and it was found that polymorphism in this gene may be associated with glioma. In this study they investigated polymorphism XRCC7 and two other genes *XRCC1Arg399 Gln, XRCC1 Arg194Trp and XPC codon 939* (mentioned above) and found no association of XRCC7 with prostate cancer risk (Hirata *et al*, 2007b).

Table 6-2 Summary studies on X-ray DNA repair genes

No	Gene name	Description	Study description and results [OR(95%CI)]	Frequency of polymorphism in controls	Reference
1	XPD	Xeroderma Pigmentosum Group D	637cases vs. 480 controls Caucasian [***XPD codon 312*** OR 1.59 (1.01-2.51) p=0.05 **XPD codon 751** OR 1.20 (0.74-1.95) p= 0.46]	***@ XPD codon 312*** allele Freq % *ASP/ASP* 180 41.2 *ASP/ASN* *218* 49.9 *ASN/ASN* *39* 8.9 ***@ XPD codon 751*** *Lys/Lys* 178 40.9 *Lys/Gln* 205 47.1 *Gln/Gln* 52 12.0	(Rybicki *et al*, 2004)
2	XRCC1 (BER)	X-ray repair cross completing group 1	165 cases vs. 165 controls [OR 1.28(0.93-1.78)] [OR 1.06(0.76-1.50)]	***Arg194Trp*** *Arg* 232 70 *Trp* 98 30 ***Arg399 Gln*** *Arg* 241 73 *Gln* 89 27	(Hirata *et al*, 2007b)
3	XPC *Lys939Gln*	Xeroderma Pigmentosum Group C	165 cases vs. 165 controls [OR 1.28(0.93-1.78)]	*Lys* 214 65 *Gln* 116 35	(Hirata *et al*, 2007b)
4	CXCL12*G801A*		167casesvs167Controls [OR1.34(0.63-2.86)] [OR1.58(1.03-2.43)] [OR1.39(1.00-1.94)]	*GG* 91 54 *GA* 63 38 *AA* 13 8 *GA+AA* 76 46 *G* 245 73 *A* 89 27	(Hirata *et al*, 2007b)
5	XRRC7G6721T	X-ray repair cross completing group7	165 cases vs 165 controls [OR 0.84(0.60-1.17)]	*G* 91 28 *T* 239 72	(Hirata *et al*, 2007b)

No	Gene name	Description	Study description and results [OR(95%CI)]	Frequency of polymorphism in controls	Reference
6	MGMT-*Leu84phe* MGMT-*Ile143Val* (directdamage reversal)	O-6-methylguanine-DNA methyltransferase	162cases vs. 251 controls 1.00 [OR1.95(1.15-3.30)] [OR3.39(0.30-38.1)] [OR1.99(1.19-3.34)] 1.00 [OR1.55(0.44-5.47)] - [OR1.85(0.55-6.20)]	***MGMT-84*** allele Freq % *CC* 213 86.6 *CT* 32 13.0 *TT* 1 0.4 *CT+TT* 33 ***MGMT-143*** *AA* 243 98.0 *AG* 5 2.0 *GG* 0 *AG+GG* 5	(Ritchey *et al*, 2005)
7	XRCC3 Thr241Met	X-ray repair cross completing group 3	162cases vs. 251 controls 1.00 [OR0.84(0.45-1.58)] [OR2.23(0.36-13.7)] [OR0.92(0.51-1.68)]	 *CC* 214 86.6 *CT* 31 12.6 *TT* 2 0.8 *CT+TT* 33	(Ritchey *et al*, 2005)

Table 6-3 DNA repair genes and the SNPs analysed in this study

X-ray Gene	Description of the gene	SNPs	Reference
CXCL12G801A	chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)	rs 1801157	(Hirata *et al*, 2007a)
XPD XPD codon ASP312 Asn XPD codon lys751 Gln	Xeroderma Pigmentosum Group D	 rs1799793 rs13181	 (Ritchey *et al*, 2005; Rybicki *et al*, 2004)
XPC Lys939Gln	Xeroderma Pigmentosum Group C	rs2228001 rs3731055	(Hirata *et al*, 2007b)
XRCC3 Thr241Met	Involved in the homologous recombination repair (HRR) pathway of double-stranded DNA, thought to repair chromosomal fragmentation, translocations and deletions	rs861539	(Ritchey *et al*, 2005)
MGMT MGMT-Leu84phe MGMT-Ile143Val	O-6-methylguanine-DNA methyltransferase	 rs12917 rs2308321	(Ritchey *et al*, 2005)
XRCC1 XRCC1 Arg194Trp XRCC1-Arg399 Gln	X-ray repair cross completing group 1	 rs1799782 rs25487	(Hirata *et al*, 2007b; Ritchey *et al*, 2005; Rybicki *et al*, 2004)
XRCC7G6721T	X-ray repair cross completing group7	rs7003908	(Hirata *et al*, 2007b)

6.2 Genes related to sex steroid hormones

6.2.1 Genes related to balding and prostate cancer

It is evident from the previous studies that androgens play an important role in the development of baldness but it is also evident that genetic predisposition may also have some role in the aetiology of baldness (Hamilton, 1951; Hillmer *et al*, 2005). Although the genetic basis for baldness remains unclear, the following facts may be of importance: the genes encoding 5 alpha-reductase isonzymes (SRD5A1 and SRD5A2) or genes encoding enzymes that act in the earlier stages of the androgen pathway such as 3β-hydroxysteroid dehydrogenase appear to have a role. A study by Ellis et al in 2001, revealed a significant association between AGA/MPB and androgen receptor (AR) gene polymorphisms (*StuI*) in 54 young and 392 older cases of baldness and 107 older controls without baldness with AGA. The AR gene *StuI* was found in 98.1% of young bald men age 18-30 ($p=0.0005$) and in 92.3% of older men ≥50 years with baldness ($p=0.000004$) and found only in 76.6% of 107 controls ≥50 years without baldness. The AR exon 1 triplet repeat polymorphisms CAG and GGC (which have also an association with prostate cancer) were also prevalent in bald men ($p=0.03$) (Ellis *et al*, 2001). In their recent large population-based Caucasian cohort study they also found strong association between androgen receptor gene SNP (rs6152) with baldness ($p=0.0001$), but no association was found with polyglutamine CAG or polyglycine GGN triplet repeat ($p=0.13$) (Ellis *et al*, 2007). The results from the study by Hillmer et al suggested that for early onset AGA, genetic variation in the androgen receptor gene is the key requirement and for functional effects there is possible role of a polyglycine-encoding GGN repeat in exon 1. They also suggested a maternal line of inheritance for AGA due to X-chromosomal location of AR (Hillmer *et al*, 2005).

Hillmer et al, in their genome-wide association study(GWAs) in 296 subjects with baldness and 346 population based control, investigated thirty SNPs and found a strong association for five SNPs on chromosome 20p11 (Hillmer *et al*, 2008).

In another genome-wide association study on 1125 men with baldness, the authors found new susceptibility locus at 20p11 (rs1160312) with increased risk in the Twin UK cohort and also with Icelandic and Dutch cohort. In their combined analysis, the results were also highly significant (Richards *et al*, 2008).

It is evident from previous studies that sex hormones particularly androgens play an important role in the growth of the prostate and may be associated with prostate cancer carcinogenesis (Platz *et al*, 2005; Travis *et al*, 2009). Twin studies suggest an inherited component for serum concentration of sex hormones; however, there is limited epidemiological evidence about the steroid hormone gene variants in prostate cancer aetiology. Travis et al, studied genetic variations at the CYP19A1 locus in relation to prostate cancer risk and with circulating steroid hormone concentrations in men in the Breast and Prostate Cancer Consortium (BPC3), a large collaborative prospective study. BPC3 aimed to investigate role of the common variants in CYP19A1 by targeted resequencing and dense genotyping; selected haploytype-tagging single nucleotide polymorphism (htSNP) in U.S. and European whites, Latinos, Japanese Americans, and native Hawaiians. The results of this study found no association with prostate cancer risk (Travis *et al*, 2009).

Table 6-4 Genes and selected SNPs in association with baldness and prostate cancer risk.

Hormone Gene	SNPs	Reference
Chromosome 20p11	rs2180439	(Hillmer *et al*, 2008)
Chromosome 20p11	rs1998076	(Hillmer *et al*, 2008)
Chromosome 20p11	rs1160312	(Richards *et al*, 2008)
Chromosome 20p11	rs11603132	(Richards *et al*, 2008)
CYP19A1	rs2414096	(Travis *et al*, 2009)

6.3 Hypothesis and aims

Hypothesis

There are possible interactions between environmental exposures including low dose diagnostic radiation, balding, acne and selected single nucleotide polymorphisms.

Aims

1. To assess prevalence of sixteen selected SNPs in study population.
2. To explore risk estimates of subjects who carry these alleles.
3. To explore potential gene-environment interactions between:

- Medical radiation exposure and DNA repair genes.
- Genes involved in androgen pathways (particularly for baldness).

The selection of SNPs in this study was based on following criteria:

An extensive review was carried out for 150 DNA repair genes and balding genes and those selected genes and their SNPs were based on their associations with prostate cancer risk in previous studies.

6.4 Methodology

DNA extraction from 5 ml blood samples was commercially prepared by Gen Probe Company and subsequently DNA were deposited with the study partner at the Royal Marsden Hospital (RMH). Since DNA extraction process was carried out in batches throughout the period of data collection which is still ongoing thus not all of study subjects in this study had their DNA extracted by the time of genetic analysis request. To prepare DNA for SNPs analysis, the list of study subjects were sent to the researcher at the RMH, DNA samples of 633 cases (57%) and 1438 (77%) controls were successfully matched and ready for genotype.

6.4.1 SNPs analysis

SNPs analysis was out sourced with the KBiosciences who specialise in low volume DNA analysis (5 nanograms DNA in 75 micro litre of sample normalisation). Eleven SNPs from seven DNA repair genes and four SNPs for baldness were identified. Two other suggested genes were steroid hormone genes, however only one SNP result was received. The other SNP result (of acne gene) showed only one genotype (G: G), and was therefore excluded from analysis.

In total, 11 SNPs from 7 DNA repair genes and 5 SNPs from 2 genes related to balding were analysed (see Table 6-3 and Table 6-4).

6.4.2 SNPs statistical analysis

Frequency statistics were run to check genotype prevalence in each SNP, then each genotype as a trichotomous variable was transformed into numeric e.g. if one SNP has genotype G: G, G: A and A: A, they were transformed into 1, 2 and 3 (the most common allele was recoded as 1 and was used as reference category). All missing values and samples which were failed at genotyping process were re-coded as 9 and 99 respectively and were considered as missing. ORs of each SNPs was obtained by running unconditional logistic regression.

6.4.3 Interaction analyses

Botto and Khoury suggested different ways of analysis such as combining gene and environmental interaction and analysing separate (case or control only) and joint analysis (multiplicative or additive interaction)(Botto & Khoury, 2001). They also suggested that the best way to present interaction is by creating a two- by- four table which summarises both gene and environmental factors in dichotomous variable (Botto & Khoury, 2001; Kellen *et al*, 2005).

The following explains the operational details for creating variables for gene and environmental interaction.

To assess the effect of genotype and environmental interaction on prostate cancer risk, dichotomous variables were created from both genotype and environmental exposure. For genotype, a new dichotomous variable was created by keeping the most common genotype as 1; while two other alleles were merged into one group and labelled as 2.

For environmental exposures, there are two main exposures in the GE analysis including diagnostic radiation exposure and markers of male hormones, dichotomous variables were created for both exposures. However, the scheme of coding was slightly differed from the previous chapter due to the requirement of large sample size for Gene and environment interaction analysis. The following describes the process involved in creating the exposure variables for GE analysis.

6.4.4 Diagnostic X-ray procedure

As previously reported, hip/pelvic X-ray procedure appeared to be a strong risk factor for prostate cancer not only in this present study but also in our two previous interim data analysis by Myles and Hussain (Hussain, 2008/2009; Myles *et al*, 2008) . The first approval was made by using hip/pelvic X-ray for the exposure analysis as presented in chapter 4 (for recalling coding scheme see chapter 4 page 71). However, while filtering variables to create categories for subjects with presence on both exposure and gene, the sample size was too small in most of the newly created dichotomous variables. A further approach was therefore applied by creating a new dichotomous variable so called "universal hip and pelvic X-ray" taking into account only two categories of subjects including ever had hip/pelvic X-ray in life time and never had hip/pelvic X-ray at all.

Since one of the *a-priori* hypotheses was that a diagnostic radiation insult to the prostate gland could potentially lead to prostate cancer, an interaction between the exposure of any X-ray procedures during subjects' lifetime on prostate cancer risk and X-ray DNA repair gene polymorphisms was investigated. A

dichotomous variable "Universal X-ray" was created. Subjects were categorised into two groups; ever had any of 5 procedures and never had any procedures at all.

6.4.5 Balding

This variable was investigated in 2 ways, firstly at each decade (20s, 30s and 40s) and secondly by scoring overall balding. A dichotomous variable for balding at age 20s, 30s and 40s was created; subjects with balding (by merging frontal and vertex baldness) and subjects with no balding at that particular age. For the overall balding variable, a new dichotomous variable "universal baldness" was created by recoding subjects into subjects with no baldness versus subjects with baldness occurring at any age.

Once both exposure and gene variables had been coded into dichotomous, all included subjects were classified according to their presence/absence of both variables. The basic layout was as follows:

Table 6-5 Shows basic layout for a case-control study assessing the effect of a genotype and environmental factors (Botto & Khoury, 2001)

Genotype	Environmental factors	Cases	Controls	OR		Contrast	Main information
+	+	A	B	ah/bg	A	A vs. D	Joint genotype and environmental factor vs. none
+	-	C	D	ch/dg	B	B vs. D	Genotype alone vs. None
-	+	E	F	eh/fg	C	C vs. D	Environmental factor alone vs. None
-	-	G	H	1	D		Common reference

Botto and Khoury described several advantages of this two-by-four table including the fact that it displays primary data clearly, helps efficiently to calculate risk estimates alone and for joint exposure, highlights sample size issues, attributable fractions can be calculated alone for each individual exposure and for the joint exposure, case only and control only. Risk estimates can be calculated easily and multiplicative and additive models of interactions can also be obtained (Botto & Khoury, 2001).

From the table, to assess the separate effect of exposures (in the absence of gene), odds ratios were computed by EH/FG and to calculate risk for genetic polymorphisms in the absence of exposure, odds ratios were computed by CH/DG. For joint effect (presence of both gene and exposure), the risks were computed by AH/BG. All of these formulae are shown in column 5th of the table (column label as "Odd ratio").

Multiplicative interaction is the ratio of the joint effect to the product of the independent effects. To assess multiplicative interaction, the following formula was used [OR = A/ (B*C)]. All analyses was performed by logistic regression and adjusted for age, social class and family history.

In summary, the gene and environment interaction analysis consisted of 633 cases and 1438 controls. Subjects were classified according to presence or absence of their genes and their exposure. A 2 by 4 cross tabulation table was created. Subjects with most common variant and not exposed to exposure were used as reference category.

6.5 Results

6.5.1 DNA repair genes

Prevalence of selected SNPs (X-ray DNA repair genes) is presented in table 6-6.

Table 6-6 Distribution of seven DNA repair genes (eleven SNPs) polymorphism and prostate cancer risk

Gene name	Selected SNPs	Genotype	Cases (%)	Controls (%)	OR†	95%C.I.	P
CXCL12G801A	rs 1801157	G:G	412(67.2)	935(66.1)	1.00		
		G:A	186(30.3)	426(30.1)	1.04	0.83-1.30	0.76
		A:A	15(2.4)	53(3.7)	0.59	0.32-1.10	0.10
XPD		A:G	274(45.0)	631(45.1)	1.00		
XPD codon ASP312 Asn	rs1799793	G:G	281(46.1)	604(43.1)	1.10	0.88-1.37	0.39
		A:A	54(8.9)	165(11.8)	0.77	0.53-1.11	0.17
XPD codon lys751 Gln	rs13181	G:T	290(46.8)	654(47.0)	1.00		
		T:T	257(41.5)	556(39.9)	1.00	0.81-1.25	0.98
		G:G	73(11.8)	182(13.1)	0.77	0.55-1.08	0.13
XPC Lys939Gln	rs2228001	C:A	281(46.3)	674(48.9)	1.00		
		A:A	220(36.2)	489(35.5)	1.06	0.84-1.33	0.65
		C:C	106(17.5)	214(15.5)	1.23	0.92-1.64	0.17
	rs3731055	G:G	616(99.7)	1420(99.2)	1.00		
		G:A	02(0.3)	12(0.8)	0.63	0.14-2.86	0.55
XRCC3 Thr241Met	rs861539	T:C	292(47.7)	639(45.4)	1.00		
		C:C	243(39.7)	571(40.5)	0.97	0.78-1.21	0.80
		T:T	77(12.6)	199(14.1)	0.80	0.58-1.11	0.19
MGMT	rs12917	C:C	491(79.4)	1077(76.9)	1.00		
MGMT-Leu84phe		T:C	119(19.3)	305(21.8)	0.82	0.63-1.06	0.13
		T:T	08(1.3)	19(1.4)	0.86	0.35-2.15	0.75
MGMT-Ile143Val	rs2308321	A:A	452(73.4)	1036(73.7)	1.00		
		G:A	152(24.7)	346(24.6)	1.09	0.86-1.39	0.47
		G:G	12(1.9)	24(1.7)	1.14	0.53-2.46	0.73
XRCC1		C:C	528(86.4)	1246(87.4)	1.00		
XRCC1 Arg194Trp	rs1799782	C:T	78(12.8)	171(12.0)	0.90	0.66-1.24	0.53
		T:T	05(0.8)	09(0.6)	1.17	0.35-3.89	0.80
XRCC1-Arg399 Gln	rs25487	G:A	282(45.3)	630(44.7)	1.00		
		G:G	265(42.6)	570(40.5)	1.03	0.83-1.28	0.80
		A:A	75(12.1)	208(14.8)	0.79	0.57-1.09	0.15
XRCC7G6721T	rs7003908	A:A	270(44.6)	627(45.6)	1.00		
		C:A	269(44.5)	614(44.6)	1.10	0.89-1.38	0.38
		C:C	66(10.9)	135(9.8)	1.15	0.80-1.64	0.46

†adjusted for age, social class and family health

Prevalence of genotype in all polymorphisms is similar in both case and control groups, none of the allele mutations is associated with prostate cancer risk (all confidence interval include 1).

Universal hip/pelvic X-ray and selected SNPs

The separate assessment of the effects of individual and joint risk factors (universal hip/pelvic X-ray and X-ray DNA repair genes) is shown in table 6-7.

Table 6-7 Distribution and risk estimates of genotype and universal hip/pelvic X-ray and prostate cancer risk

Gene	SNPs	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
CXCL12G801A	**rs1801157**	NO	NO	236	666	1.00			1.00		
		NO	**YES**	**121**	**182**	**1.88**	**1.43-2.47**	**<0.001**	**2.00**	**1.49-2.70**	**<0.001**
		YES	NO	123	333	1.04	0.81-1.35	0.75	1.11	0.84-1.47	0.45
		YES	**YES**	**61**	**102**	**1.69**	**1.19-2.40**	**<0.001**	**1.73**	**1.18-2.54**	**0.01**
		Total		541	1283						
XPD codon ASP312 Asn	**rs1799793**	NO	NO	169	452	1.00			1.00		
		NO	**YES**	**78**	**115**	**1.81**	**1.29-2.54**	**<0.001**	**1.88**	**1.29-2.73**	**<0.001**
		YES	NO	189	542	0.93	0.73-1.19	0.57	0.99	0.76-1.28	0.92
		YES	**YES**	**99**	**166**	**1.60**	**1.18-2.16**	**<0.001**	**1.73**	**1.24-2.41**	**<0.001**
		Total		535	1275						
XPD codon lys751 Gln	**rs13181**	NO	NO	188	478	1.00			1.00		
		NO	**YES**	**76**	**110**	**1.76**	**1.25-2.46**	**<0.001**	**1.96**	**1.36-2.82**	**<0.001**
		YES	NO	178	506	0.89	0.70-1.14	0.36	0.86	0.66-1.11	0.25
		YES	**YES**	**105**	**169**	**1.58**	**1.18-1.12**	**<0.001**	**1.53**	**1.11-2.10**	**0.01**
		Total		547	1263						
XPC Lys939Gln	**rs2228001**	NO	NO	173	461	1.00			1.00		
		NO	YES	74	149	1.32	0.95-1.84	0.10	1.42	0.99-2.03	0.05
		YES	NO	185	515	0.96	0.75-1.22	0.72	0.97	0.75-1.27	0.84
		YES	**YES**	**105**	**125**	**2.24**	**1.64-3.06**	**<0.001**	**2.30**	**1.64-3.22**	**<0.001**
		Total		537	1250						

Gene	SNPs	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
XPC Lys939Gln	**rs3731055**	NO	NO	359	1010	1.00			1.00		
		NO	**YES**	**184**	**279**	**1.86**	**1.49-2.32**	**<0.001**	**1.95**	**1.53-2.48**	**<0.001**
		YES	NO	1	6	0.47	0.06-3.91	0.48	0.79	0.09-6.67	0.83
		YES	YES	0	5	0.00	0.00	1.00	0.00	0.00	1.00
		Total		544	1300						
XRCC3 Thr241Met	**rs861539**	NO	NO	179	448	1.00			1.00		
		NO	**YES**	**80**	**125**	**1.60**	**1.15-2.23**	**0.01**	**1.49**	**1.04-2.14**	**0.03**
		YES	**NO**	**179**	**553**	**0.81**	**0.64-1.03**	**0.09**	**0.77**	**0.59-1.00**	**0.05**
		YES	**YES**	**100**	**155**	**1.62**	**1.19-2.19**	**<0.001**	**1.76**	**1.27-2.45**	**<0.001**
		Total		538	1281						
MGMT-Leu84phe	**rs12917**	NO	NO	282	767	1.00			1.00		
		NO	**YES**	**145**	**208**	**1.90**	**1.47-2.44**	**<0.001**	**1.99**	**1.52-2.62**	**<0.001**
		YES	NO	81	228	0.97	0.73-1.29	0.82	0.93	0.68-1.28	0.66
		YES	YES	35	70	1.36	0.89-2.09	0.16	1.32	0.82-2.11	0.25
		Total		543	1273						
MGMT-Ile143Val	**rs2308321**	NO	NO	267	742	1.00			1.00		
		NO	**YES**	**128**	**202**	**1.76**	**1.36-2.29**	**<0.001**	**1.84**	**1.38-2.45**	**<0.001**
		YES	NO	95	257	1.03	0.78-1.35	0.85	1.09	0.81-1.46	0.59
		YES	**YES**	**52**	**78**	**1.85**	**1.27-2.70**	**<0.001**	**2.05**	**1.36-3.08**	**<0.001**
		Total		542	1279						
XRCC1 Arg194Trp	**rs1799782**	NO	NO	309	875	1.00			1.00		
		NO	**YES**	**156**	**255**	**1.73**	**1.37-2.20**	**<0.001**	**1.76**	**1.36-2.28**	**<0.001**
		YES	NO	44	134	0.93	0.65-1.34	0.70	0.76	0.51-1.13	0.17
		YES	**YES**	**28**	**31**	**2.56**	**1.51-4.33**	**<0.001**	**2.50**	**1.40-4.45**	**<0.001**

Gene	SNPs	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
		Total		537	1295						
XRCC1Arg399 Gln	**rs25487**	NO	NO	169	438	1.00			1.00		
		NO	**YES**	**93**	**129**	**1.87**	**1.36-2.57**	**<0.001**	**1.98**	**1.40-2.80**	**<0.001**
		YES	NO	194	573	0.88	0.69-1.12	0.29	0.83	0.64-1.08	0.15
		YES	**YES**	**86**	**155**	**1.44**	**1.05-1.98**	**0.03**	**1.37**	**0.97-1.95**	**0.07**
		Total		542	1295						
XRCC7G6721T	**rs7003908**	NO	NO	152	442	1.00			1.00		
		NO	**YES**	**86**	**127**	**1.97**	**1.42-2.74**	**<0.001**	**2.20**	**1.53-3.15**	**<0.001**
		YES	NO	203	532	1.11	0.87-1.42	0.41	1.24	0.94-1.62	0.12
		YES	**YES**	**91**	**150**	**1.76**	**1.28-2.43**	**<0.001**	**1.94**	**1.37-2.75**	**<0.001**
		Total		532	1251						

†adjusted for age, social class and family health

The exposure to universal hip/pelvic X-ray alone was a strong risk factor for prostate cancer for all 11 selected SNPs, odds ratios ranging from 1.49-2.20 and p-value between 0.03-<0.001.

Subjects who carried allele mutation of XRCC3 Thr241Met gene (rs861539) also showed a significant risk reduction (OR 0.77, 95% C.I. 0.59-1.00) (p-value=0.05).

Statistically significant joint effects (presence of both polymorphism and hip/pelvic x-ray exposure) were observed in 9 out of 11 selected SNPs including rs1801157 (CXCL12G801A), rs1799793 (XPD codon ASP312 Asn), rs13181 (XPD codon lys751 Gln), rs2228001 (XPC Lys939Gln), rs861539 (XRCC3 Thr241Met), rs2308321 (MGMT-Ile143Val), rs1799782 (XRCC1 Arg194Trp), rs25487 (XRCC1Arg399 Gln) and rs7003908 (XRCC7G6721T) (for ORs and C.Is see Table 6-7). Odds ratio for joint effect between SNP rs3731055 (XPC Lys939Gln) and universal hip/pelvic X-ray could not be calculated as sample size is zero in case group.

There was no evidence of joint effect between SNP rs12917 (MGMT-Leu84phe) and universal hip/pelvic X-ray and prostate cancer risk (OR 1.32, 95% C.I. 0.82-2.11).

Multiplicative interaction between DNA repair genes, hip-pelvic x-ray and prostate cancer risk

Table 6-8 shows risk estimates for multiplicative interaction between DNA repair genes and universal hip/pelvic exposure in prostate cancer

Table 6-8 Multiplicative interaction between DNA repair genes (SNPs) and prostate cancer with universal hip/pelvic exposure

DNA SNPs	OR	95% CI	p-value	OR†	95% CI	p-value
rs1801157	0.86	0.54-1.38	0.54	0.78	0.47-1.29	0.33
rs1799793	0.94	0.60-1.48	0.80	0.93	0.57-1.53	0.79
rs13181	1.01	0.64-1.58	0.98	0.91	0.56-1.48	0.71
rs2228001	**1.77**	**1.13-2.78**	**0.01**	**1.66**	**1.02-2.71**	**0.04**
rs3731055	0.00	0.00	1.00			
rs861539	1.24	0.80-1.95	0.34	1.54	0.95-2.51	0.08
rs12917	0.74	0.43-1.28	0.28	0.71	0.39-1.28	0.25
rs2308321	1.02	0.62-1.68	0.93	1.03	0.60-1.76	0.92
rs1799782	1.59	0.82-3.07	0.17	1.88	0.91-3.86	0.09
rs25487	0.89	0.57-1.39	0.60	0.86	0.53-1.39	0.53
rs7003908	0.81	0.52-1.27	0.35	0.71	0.44-1.17	0.18

†adjusted for age, social class and family health

The multiplicative test of interaction between universal hip/pelvic X-ray and DNA repair genes SNPs showed significant interaction with modest increase risk only for SNP rs2228001 (XPC Lys939Gln gene) (OR 1.66, 95% C.I. 1.02-2.71).

Universal X-ray exposure

Table 6-9 shows individual and joint effects of SNPs (DNA repair genes) and being exposed to universal X-ray

Table 6-9 Shows distribution and risk estimates of SNPs (DNA repair genes) and universal X-ray exposure

Gene	SNPs	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
CXCL12G801A	**rs1801157**	NO	NO	159	440	1.00			1.00		
		NO	**YES**	**229**	**460**	**1.38**	**1.08-1.75**	**0.01**	**1.34**	**1.03-1.74**	**0.03**
		YES	NO	87	229	1.05	0.77-1.43	0.75	1.07	0.76-1.48	0.71
		YES	YES	107	233	1.27	0.95-1.70	0.11	1.30	0.95-1.79	0.10
		Total		582	1362						
XPD codon ASP312 Asn	**rs1799793**	NO	NO	110	308	1.00			1.00		
		NO	**YES**	**154**	**300**	**1.44**	**1.07-1.92**	**0.02**	**1.42**	**1.03-1.96**	**0.03**
		YES	NO	136	357	1.07	0.80-1.43	0.67	1.12	0.81-1.55	0.48
		YES	YES	176	386	1.28	0.96-1.69	0.09	1.34	0.98-1.82	0.07
		Total		576	1351						
XPD codon lys751 Gln	**rs13181**	NO	NO	133	312	1.00			1.00		
		NO	YES	147	314	1.10	0.83-1.46	0.52	1.16	0.86-1.58	0.33
		YES	NO	119	345	0.81	0.61-1.08	0.15	0.83	0.60-1.13	0.23
		YES	YES	189	370	1.20	0.92-1.57	0.19	1.22	0.91-1.62	0.18
		Total		588	1341						
XPC Lys939Gln	**rs2228001**	NO	NO	132	324	1.00			1.00		
		NO	YES	138	321	1.06	0.79-1.40	0.71	1.06	0.78-1.45	0.70
		YES	NO	119	331	0.88	0.66-1.18	0.40	0.91	0.67-1.25	0.57
		YES	YES	189	349	1.33	1.02-1.74	0.04	1.31	0.98-1.75	0.07
		Total		578	1325						

Gene	SNPs	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
XPC Lys939Gln	**rs3731055**	No	No	250	674	1.00			1.00		
		No	YES	333	692	1.03	1.07-1.58	0.01	1.28	1.04-1.59	0.02
		YES	NO	02	05	1.08	0.21-5.59	0.93	1.74	0.33-9.14	0.52
		YES	YES	0	07	0	0	1	0	0	1
		Total		585	1378						
XRCC3 Thr241Met	**rs861539**	NO	NO	129	309	1.00			1.00		
		NO	YES	153	305	1.20	0.91-1.59	0.20	1.10	0.81-1.50	0.53
		YES	NO	121	360	0.81	0.60-1.08	0.14	0.75	0.55-1.04	0.08
		YES	YES	176	383	1.10	0.84-1.45	0.49	1.11	0.83-1.49	0.49
		Total		579	1357						
MGMT-Leu84phe	**rs12917**	NO	NO	194	513	1.00			1.00		
		NO	**YES**	**268**	**519**	**1.37**	**1.09-1.70**	**0.01**	**1.34**	**1.05-1.70**	**0.02**
		YES	NO	58	149	1.03	0.73-1.45	0.87	0.95	0.65-1.39	0.80
		YES	YES	65	167	1.03	0.74-1.43	0.86	1.00	0.70-1.43	0.99
		Total		585	1348						
MGMT-Ile143Val	**rs2308321**	NO	NO	186	507	1.00			1.00		
		NO	**YES**	**242**	**498**	**1.33**	**1.06-1.66**	**0.02**	**1.27**	**0.99-1.63**	**0.06**
		YES	NO	64	165	1.06	0.76-1.48	0.74	1.07	0.74-1.63	0.73
		YES	**YES**	**92**	**185**	**1.36**	**1.00-1.83**	**0.05**	**1.46**	**1.05-2.02**	**0.03**
		Total		584	1355						

Gene	SNPs	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
XRCC1 Arg194Trp	**rs1799782**	NO	NO	205	589	1.00			1.00		
		NO	**YES**	**295**	**612**	**1.39**	**1.12-1.71**	**<0.001**	**1.38**	**1.09-1.73**	**0.01**
		YES	NO	36	88	1.18	0.77-1.79	0.45	1.01	0.64-1.59	0.97
		YES	YES	43	85	1.45	0.98-2.17	0.07	1.23	0.79-1.92	0.35
		Total		579	1374						
XRCC1Arg399 Gln	**rs25487**	NO	NO	120	297	1.00			1.00		
		NO	YES	155	303	1.27	0.95-1.69	0.11	1.27	0.93-1.74	0.14
		YES	NO	96	290	0.82	0.60-1.12	0.21	0.78	0.55-1.09	0.15
		YES	YES	123	288	1.06	0.78-1.43	0.72	1.00	0.72-1.38	0.99
		Total		494	1178						
XRCC7G6721T	**rs7003908**	NO	NO	103	314	1.00			1.00		
		NO	**YES**	**153**	**288**	**1.62**	**1.20-2.18**	**<0.001**	**1.71**	**1.23-2.36**	**<0.001**
		YES	**NO**	142	338	1.28	0.95-1.72	0.10	**1.45**	**1.05-2.01**	**0.03**
		YES	**YES**	**175**	**385**	**1.39**	**1.04-1.84**	**0.03**	**1.48**	**1.08-2.03**	**0.01**
		Total		573	1325						

†adjusted for age, social class and family health

The results showed that the independent effect of environmental exposure was a risk factor for prostate cancer for six SNPs and ORs were ranging from 1.34-1.71 and p-value=0.06-<0.001.

Individuals carrying the variant allele of SNP rs7003908 (XRCC7G6721T) without environmental exposure are at modest risk for getting prostate cancer (OR 1.45, p-value =0.03).

The joint effects of both gene and environmental exposure were only significant with rs2308321 (MGMT-Ile143Val) (OR 1.46, 95% C.I. 1.05-2.02) and rs7003908 (XRCC7G6721T) (OR 1.48, 95% C.I. 1.08-2.03).

Multiplicative interaction between DNA repair genes, universal X-ray and prostate cancer risk

Distribution and risk estimates of multiplicative interaction between SNPs (DNA repair genes) and prostate cancer with universal X-ray exposure are shown in table 6-10

Table 6-10 Multiplicative interaction between SNPs (DNA repair genes) and prostate cancer with universal X-ray exposure

SNPs	OR	95% CI	p-value	OR†	95% CI	p-value
rs1801157	0.88	0.58-1.33	0.54	0.92	0.58-1.43	0.70
rs1799793	0.83	0.56-1.24	0.36	0.84	0.55-1.29	0.42
rs13181	1.35	0.90-2.00	0.14	1.21	0.79-1.85	0.39
rs2228001	1.43	0.96-2.12	0.08	1.35	0.88-2.07	0.17
rs3731055						
rs861539	1.14	0.77-1.69	0.52	1.33	0.87-2.04	0.19
rs12917	0.73	0.46-1.17	0.20	0.78	0.47-1.31	0.35
rs2308321	0.97	0.62-1.51	0.89	1.08	0.66-1.74	0.77
rs1799782	0.89	0.50-1.59	0.70	0.89	0.47-1.67	0.72
rs25487	1.12	0.84-1.48	0.45	1.08	0.79-1.47	0.63
rs7003908	**0.67**	**0.45-1.00**	**0.05**	**0.60**	**0.39-0.93**	**0.02**

†adjusted for age, social class and family health

A negative multiplicative interaction was seen in SNP rs7003908 (XRCC7G6721T) (OR 0.60, 95% C.I. 0.39-0.93).

6.5.2 Genes related to baldness

Prevalence of selected SNPs in the study subjects is presented in table 6-11

Table 6-11 Distribution of genotype from two Hormone genes (five SNPs) and prostate cancer risk

Gene name	Selected SNPs	Genotype	Cases (%)	Controls (%)	OR†	95%C.I.	p-value
Chromosome 20p11	rs2180439	T:C	306(50.1)	687(49.1)	1.00		
		T:T	202(33.1)	483(34.5)	0.94	0.74-1.19	0.61
		C:C	103(16.9)	228(16.3)	0.98	0.72-1.33	0.88
Chromosome 20p11	rs1998076	G:A	306(50.6)	683(48.9)	1.00		
		G:G	200(33.1)	491(35.2)	0.89	0.70-1.13	0.33
		A:A	99(16.4)	222(15.9)	0.92	0.67-1.25	0.59
Chromosome 20p11	rs1160312	G:A	297(49.7)	710(50.6)	1.00		
		A:A	158(26.5)	395(28.2)	0.89	0.69-1.15	0.38
		G:G	142(23.8)	298(21.2)	1.01	0.77-1.34	0.93
Chromosome 20p11	rs11603132	G:A	309(50.4)	649(46.8)	1.00		
		A:A	205(33.4)	482(34.8)	0.83	0.65-1.06	0.13
		G:G	99(16.2)	255(18.4)	0.82	0.60-1.10	0.18
CYP19A1	rs2414096	G:A	337(54.0)	719(51.5)	1.00		
		A:A	140(22.4)	352(25.2)	0.87	0.67-1.13	0.28
		G:G	147(23.6)	324(23.2)	0.90	0.69-1.17	0.43

†adjusted for age, social class and family health

The distributions of genotypes are very similar between cases and controls. None of the allele mutations is associated with prostate cancer risk.

Balding exposure at different ages in life

Table 6-12 shows the separate and joint effect between baldness SNPs and baldness at age 20s, 30s and 40s in prostate cancer risk

Table 6-12 Individual and joint effects of baldness SNPs and baldness in prostate cancer risk

Gene	SNPs	Age	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
Chromosome 20p11	**rs 2180439**	**20s**	NO	NO	202	474	1.00			1.00		
			NO	YES	91	178	1.20	0.89-1.62	0.24	1.07	0.75-1.51	0.72
			YES	NO	198	447	1.04	0.82-1.31	0.75	1.00	0.77-1.30	0.99
			YES	YES	84	226	0.87	0.65-1.18	0.37	0.83	0.59-1.16	0.27
			Total		575	1325						
		30s	NO	NO	124	289	1.00			1.00		
			NO	YES	170	363	1.09	0.83-1.44	0.54	1.00	0.73-1.37	0.99
			YES	NO	112	271	0.96	0.71-1.31	0.81	0.88	0.63-1.25	0.48
			YES	YES	172	405	0.99	0.75-1.30	0.94	0.94	0.69-1.29	0.71
			Total		578	1328						
		40s	NO	NO	55	136	1.00			1.00		
			NO	YES	248	536	1.14	0.81-1.62	0.45	1.06	0.71-1.57	0.79
			YES	NO	54	122	1.09	0.70-1.71	0.69	1.08	0.66-1.79	0.75
			YES	YES	241	570	1.05	0.74-1.48	0.80	0.95	0.64-1.40	0.78
			Total		598	1364						

†adjusted for age, social class and family health

Gene	SNPs	Age	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
Chromosome 20p11	**rs1998076**	**20s**	NO	NO	201	474	1.00			1.00		
			NO	YES	92	174	1.25	0.92-1.69	0.15	1.10	0.78-1.56	0.59
			YES	NO	194	447	1.02	0.81-1.30	0.85	0.95	0.73-1.24	0.70
			YES	YES	82	227	0.85	0.63-1.15	0.30	0.79	0.56-1.11	0.17
			Total		569	1322						
		30s	NO	NO	121	290	1.00			1.00		
			NO	YES	173	358	1.16	0.88-1.53	0.30	1.04	0.76-1.43	0.79
			YES	NO	110	271	0.97	0.72-1.32	0.86	0.86	0.61-1.22	0.39
			YES	YES	168	406	0.99	0.75-1.31	0.95	0.91	0.67-1.25	0.56
			Total		572	1325						
		40s	NO	NO	54	135	1.00			1.00		
			NO	YES	249	533	1.17	0.82-1.66	0.38	1.03	0.69-1.53	0.88
			YES	NO	53	122	1.09	0.69-1.71	0.72	0.98	0.59-1.62	0.93
			YES	YES	236	571	1.03	0.73-1.47	0.86	0.88	0.59-1.31	0.53
			Total		592	1361						

†adjusted for age, social class and family health

Gene	SNPs	Age	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
Chromosome 20p11	**rs1160312**	**20s**	NO	NO	190	480	1.00			1.00		
			NO	YES	91	193	1.19	0.88-1.61	0.25	1.01	0.72-1.42	0.96
			YES	NO	200	444	1.14	0.90-1.44	0.29	0.98	0.75-1.28	0.88
			YES	YES	79	211	0.95	0.70-1.29	0.72	0.83	0.58-1.17	0.28
			Total		560	1328						
		30s	NO	NO	119	288	1.00			1.00		
			NO	YES	163	383	1.03	0.78-1.37	0.84	0.91	0.66-1.25	0.56
			YES	NO	113	273	1.00	0.74-1.36	0.99	0.83	0.59-1.17	0.29
			YES	YES	168	387	1.05	0.79-1.39	0.73	0.90	0.65-1.23	0.50
			Total		563	1331						
		40s	NO	NO	55	134	1.00			1.00		
			NO	YES	237	560	1.03	0.73-1.46	0.86	0.92	0.62-1.36	0.67
			YES	NO	55	124	1.08	0.69-1.69	0.73	0.94	0.57-1.54	0.79
			YES	YES	237	550	1.05	0.74-1.49	0.79	0.86	0.58-1.28	0.46
			Total		584	1368						

†adjusted for age, social class and family health

Gene	SNPs	Age	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
Chromosome 20p11	**rs11603132**	**20s**	NO	NO	202	437	1.00			1.00		
			NO	YES	91	171	1.15	0.85-1.56	0.36	1.11	0.79-1.56	0.56
			YES	NO	199	475	0.91	0.72-1.15	0.41	0.88	0.68-1.15	0.34
			YES	YES	84	232	0.78	0.58-1.06	0.11	0.71	0.50-1.00	0.05
			Total		576	1315						
		30s	NO	NO	118	288	1.00			1.00		
			NO	**YES**	**177**	**322**	**1.34**	**1.01-1.78**	**0.04**	1.22	0.89-1.68	0.21
			YES	NO	119	268	1.08	0.80-1.47	0.60	0.95	0.67-1.34	0.76
			YES	YES	166	440	0.92	0.7.-1.22	0.56	0.85	0.62-1.17	0.32
			Total		580	1318						
		40s	NO	NO	49	131	1.00			1.00		
			NO	YES	253	501	1.35	0.94-1.94	0.10	1.16	0.78-1.73	0.47
			YES	NO	60	126	1.27	0.81-2.00	0.29	1.06	0.64-1.76	0.81
			YES	YES	238	595	1.07	0.75-1.53	0.72	0.90	0.60-1.34	0.59
			Total		600	1353						

†adjusted for age, social class and family health

Gene	SNPs	Age	Gene	Environment	case	control	OR	95% CI	p-value	OR†	95% CI	p-value
CYP19A1	**rs2414096**	**20s**	NO	NO	220	483	1.00			1.00		
			NO	YES	93	204	1.00	0.75-1.34	1.00	0.98	0.70-1.36	0.90
			YES	NO	189	433	0.96	0.76-1.21	0.72	0.97	0.75-1.27	0.85
			YES	YES	85	201	0.93	0.69-1.25	0.63	0.82	0.59-1.15	0.26
			Total		587	1321						
		30s	NO	NO	131	287	1.00			1.00		
			NO	YES	186	402	1.01	0.77-1.33	0.92	1.04	0.76-1.41	0.82
			YES	NO	111	267	0.91	0.67-1.23	0.55	0.96	0.68-1.35	0.81
			YES	YES	164	368	0.98	0.74-1.29	0.87	0.93	0.68-1.27	0.63
			Total		592	1324						
		40s	NO	NO	59	127	1.00			1.00		
			NO	YES	271	577	1.01	0.72-1.42	0.95	0.93	0.64-1.37	0.72
			YES	NO	52	128	0.87	0.56-1.37	0.56	0.85	0.52-1.40	0.53
			YES	YES	230	528	0.94	0.66-1.33	0.72	0.84	0.57-1.23	0.37
			Total		612	1360						

†adjusted for age, social class and family health

Baldness at age 30s showed an increase risk in the absence of allele mutation (SNP rs11603132, OR 1.34, 95% C.I. 1.01-1.78) however the result was no longer statistically significant in the full adjusted model (when age, social class and family history of prostate cancer were adjusted for).

Multiplicative interaction between genes related to balding, balding and prostate cancer risk

Risk estimates in multiplicative interaction for baldness and sex steroid hormone genes in prostate cancer

Table 6-13 Multiplicative interactions between baldness and sex steroid hormone genes in prostate cancer

Baldness SNPs	Age	OR	95% C.I	p value	OR†	95% CI	p value
rs 2180439							
	20s	0.90	0.77-1.07	0.23	0.93	0.78-1.10	0.38
	30s	0.97	0.83-1.13	0.68	0.96	0.81-1.13	0.59
	40s	0.89	0.73-1.08	0.24	0.90	0.71-1.12	0.34
rs1998076							
	20s	0.88	0.75-1.02	0.10	0.89	0.75-1.06	0.19
	30s	0.89	0.78-1.02	0.10	0.92	0.79-1.08	0.31
	40s	0.90	0.77-1.07	0.24	0.95	0.78-1.16	0.62
rs1160312							
	20s	0.93	0.81-1.07	0.30	0.95	0.83-1.10	0.51
	30s	1.00	0.88-1.14	0.99	1.07	0.93-1.24	0.35
	40s	1.04	0.86-1.27	0.68	1.06	0.86-1.31	0.60
rs11603132							
	20s	0.95	0.81-1.12	0.56	0.89	0.73-1.07	0.22
	30s	0.94	0.81-1.09	0.40	0.92	0.78-1.08	0.31
	40s	0.86	0.71-1.03	0.09	0.86	0.71-1.05	0.14
Steroid hormone SNP	**Age**	**OR**	**95% C.I**	**p value**	**OR†**	**95% CI**	**p value**
rs2414096							
	20s	1.03	0.84-1.28	0.75	1.06	0.84-1.35	0.61
	30s	1.13	0.92-1.40	0.25	1.12	0.89-1.41	0.34
	40s	0.95	0.76-1.19	0.65	0.97	0.76-1.23	0.77

†adjusted for age, social class and family health

There was no multiplicative interactions for all five SNPS (all confidence intervals include 1).

6.6 Discussion

The purpose of this work was to explore the prevalence of polymorphisms in DNA repair and hormonal genes in the study population and to investigate how genetic and environmental factors might jointly influence the risk of developing prostate cancer. The analysis consisted of 633 cases and 1438 controls. The genetic factors examined included seven DNA repair genes polymorpisms, CXCL12G801A (rs 1801157), XPD codon ASP312 Asn (rs1799793), XPD codon lys751 Gln (rs13181), XPC Lys939Gln (rs2228001 rs3731055), XRCC3 Thr241Met (rs861539), MGMT-Leu84phe (rs12917), MGMT-Ile143Val (rs2308321), XRCC1 Arg194Trp (rs1799782), XRCC1Arg399 Gln (rs25487), XRCC7G6721T (rs7003908) and baldness genes Chromosome 20p11 (rs2180439, rs1998076, rs1160312, rs11603132) and steroid hormone gene CYP19A1 (rs2414096).

To the author's knowledge, this is the first study looking at both polymorphisms in DNA repair genes and genes related to balding and their interactions with exposures on a large scale case-control study.

6.6.1 DNA repair genes

The prevalence of polymorphisms was similar for both cases and controls and risks for subjects with these possible risk alleles were not different from subjects with common allele. Previous studies reported similar findings. A case-control study conducted on 167 cases and same number of control by Hirata et al, 2007a, found G:G (54.0%) was the most common variant followed by G:A

(38.0%) and A:A (8.0%) in CXCL12 G801A polymorphism in their controls, which is similar to our study G:G (66.1%) , G:A (30.1%) and A:A (3.7%), however the distribution in cases and controls was different to the present study. They too suggested none of the gene alleles were associated with prostate cancer risk. However, when two alleles were combined (GA+AA), the prevalence was higher in prostate cancer cases as compared with controls and a modest risk was observed (OR 1.58, C.I. 1.03-2.43) (Hirata *et al*, 2007a). The difference in results might be due to difference in ethnicity (Japanese origin), smaller size of study participants and different methodological approach.

Rybicki et al, conducted a study on 637 cases and 480 controls out of 506 Caucasians sibships identified through a brother with prostate cancer, to see the effect of XPD codons 312 and 751 polymorphisms on prostate cancer risk. They found moderate risk with XPD codon 312 *Asn* allele when two copies of the allele were present (OR 1.59, 95% C.I. 1.01- 2.51) (Rybicki *et al*, 2004). These results were not in keeping with the results of the present study. However Ritchey found no effect of XPD codons 751 genotype on prostate cancer risk (Ritchey *et al*, 2005). Another case-control study comprising 118 cases and 132 age matched controls from South Australia, found no association between XPD codon 312 *Asn* and prostate cancer risk (Dhillon *et al*, 2009).

Hirata et al, 2007b, in their small scale case-control study based on 165 cases and 165 controls conducted in Japan, found no association between XPC Lys939Gln polymorphism and prostate cancer risk. These results are similar with the results of present study (Hirata *et al*, 2007b).

The results from a population-based case-control study conducted on 162 prostate cancer cases and 251 age (5 year interval) and frequency matched controls in Shanghai, China, found the same prevalence in both cases and controls for XRCC3 –Thr241Met polymorphism. No genotype was associated with prostate cancer risk; these results are in accordance with the present study. The results from the same study for MGMT-84 polymorphism found a different pattern of distribution among the cases and controls as compare to present study. C: T genotype with 22.4% prevalence in cases and 13.0% in controls appeared to be a risk factor for prostate cancer (OR 1.95, C.I. 1.15-3.30). Our results suggested that C:T allele is more prevalent in our controls (22%).

For MGMT-143 polymorphism, the most common genotype was A: A with very similar prevalence in cases and controls. No significant association was found between prostate cancer risk and any of genotype. These results are consistent with the results reported by Ritchey and colleagues(Ritchey *et al*, 2005).

For XRCC1 genes, the findings suggested no association with prostate cancer risk. The results of pooled data from two case-control studies (1,457 cases and 1,351 controls) conducted by Agalliu, et al. found no association between prostate cancer risk and 28 SNPs in nine DNA repair genes including XRCC1 (Agalliu *et al*, 2010). However, other previous studies suggested an association of these variant alleles with prostate cancer. Ritchey et al, found almost similar distribution of variant genotypes in both case and controls in XRCC1-399 and A:A genotype was associated with prostate cancer risk (OR 2.18, 95% C.I. 0.99-4.81) (Ritchey *et al*, 2005). Hirata et al, suggested XRCC1-Arg399Gln and T-A haplotype of XRCC1 Arg194Trp might have some role in prostate carcinogenesis (Hirata *et al*, 2007b). Furthermore, they reported a potential interaction

between XRCC1 codon 399 *Gln* allele and XPD codon 312 *Asn* allele when both alleles were present in homozygous states (OR 4.81, 95% C.I. 1.66-13.97) (Rybicki *et al*, 2004).

For XRCC7 gene, the findings are similar to those reported by Hirata and colleagues. Hirata et al, 2007b, found almost identical distribution of genotype in cases and controls with XRCC7 G6721 and found no association between gene polymorphism and prostate cancer (Hirata *et al*, 2007b).

6.6.1.1 GE interaction (DNA repair genes, universal hip and pelvic X-ray and prostate cancer)

The multiplicative test for interaction between universal hip/pelvic X-ray and DNA repair genes SNPs showed a significant increased risk only for SNP rs2228001 (XPC Lys939Gln) in prostate cancer risk (OR 1.66, 95% C.I. 1.02-2.71). To date, there is no study reporting an interaction between this particular SNP and hip/pelvic X-ray exposure thus this is the first study to report an interaction between these two components. It is evident from previous studies that this particular DNA repair gene plays important role in removing bulky DNA adducts and it's polymorphism is associated with cancers such as bladder and lung and might therefore be risk for prostate cancer (Hirata *et al*, 2007b). Kotnis et al, stated that there could be range of susceptibilities with additive or multiplicative interaction of each allele, those have always a minute genotypic risk (Kotnis *et al*, 2005).

6.6.1.2 GE interaction using universal X-ray

Analyses were conducted using universal X-ray exposure as an environmental factor and DNA repair genes. Results of individual and joint effects of each SNP (DNA repair genes) and environmental risk factor and the possible risk of prostate cancer were provided in Table 6-9. Table 6-9 shows that independent effect of environmental exposure appeared to be a risk factor for prostate cancer in most SNPs rs1801157 (CXCL12G801A), rs1799793 (XPD ASP312 Asn), rs12917 (MGMT-Leu84phe), rs2308321 (MGMT-Ile143Val), rs1799782 (XRCC1 Arg194Trp) and rs7003908 (XRCC7G6721T) and ORs were ranging from 1.27-1.71 and p-value=0.06-<0.001.

Multiplicative interaction of SNP rs7003908 (XRCC7G6721T) and "universal X-ray" assessment was appeared to show a negative association (OR 0.60, 95% C.I. 0.39-0.93). To date, there have been two studies looking at this SNPs mutation in association with cancer but none has investigated further the interactions with environment exposures. The first study conducted by Wang et al, showed an association of XRCC7G6721T with glioma (Wang *et al*, 2004) in subsequent study Hirata et al, 2007b, looked at XRCC7G6721T in connection with prostate cancer but found no association (Hirata *et al*, 2007b). The only explanation that could be offered for this negative association is that when multiplicative effects was calculated, the joint effect was used as numerator and separate effect from both gene and exposure was used as denominator. The fact that these risk estimates are very similar particularly the risk of joint effect could contribute to the negative multiplicative effect.

In sum, little is known of a direct effect of DNA repair capacity on prostate cancer risk but there is a growing body of evidence that environmental exposure to toxins leads to formation of DNA adducts in prostate and with faulty DNA repair machinery may lead to prostate carcinogenesis (Rybicki *et al*, 2004). Therefore hip/pelvic X-ray exposure might play a role in a same way by either affecting DNA repair capacity which can lead to translocations, gene rearrangements, amplifications and deletion (Ritchey *et al*, 2005) or by help formation of DNA adducts in prostate.

Most of the previous studies were just looking at polymorphism in DNA repair genes and its effect on prostate cancer not the gene-environment interaction and risk of prostate cancer. In this study, the author demonstrated the potential roles of DNA repair genes and their interaction with low dose radiation in prostate cancer including X-ray repair cross completing group7 and Xeroderma Pigmentosum Group C. Further studies using large sample sizes are warranted.

6.6.2 Genes related to Baldness

There was no effect of all five variant genotypes and prostate cancer risk.

For SNP rs11603132, baldness appeared to be a risk factor for prostate cancer at age 30's (OR 1.34, 95% C.I. 1.01-1.78). There was no evidence for a joint effect between baldness at any other age and SNPs (rs 2180439, rs1998076, rs1160312, rs11603132). The results from the fully adjusted model showed a non-significant multiplicative interaction between both baldness at any age and

the SNPs (rs2180439, rs1160312, rs1998076, rs11603132, rs2414096) with prostate cancer risk.

There is no case-control study on interactions of these genes and balding factors available to compare results of the present study with. Most of the previous studies have investigated SNPs role in direct relation to baldness. The case-control study by Hillmer et al, found significant association between rs2180439 (OR 1.82, 95% C.I. 1.45-2.30) and baldness in their genome wide association study (GWAS) and also reported an OR of 2.17 with 95% C.I. 1.70-2.78 in their replication analysis however after combined studies (the German samples from GWAS+ replication analyses), they found highly significant association between rs2180439 and baldness ($p=2.67*10^{-15}$). The results also suggested that rs1998076 showed a significant risk increase both with GWAS (OR 1.90, 95% C.I. 1.50-2.41) and with replication analysis (OR 2.13, 95% C.I. 1.66-2.73). They found rs1998076 as the best SNP ($p=1.3*10^{-7}$) located outside the androgen receptor locus (AR) (Hillmer *et al*, 2008).

For steroid hormone gene CYP19A1 (rs2414096), there is only one study looking at mutation of CYP19A1 and prostate cancer risk. The study included 8,166 prostate cancer cases and 9079 age and ethnicity matched controls and results suggested that germ line variation in CYP19A1 htSNPs were responsible for significant difference in sex steroid hormone concentrations in men but they did not have measureable effects on prostate cancer risk (Travis *et al*, 2009). Previous studies showed that CYP19 gene plays an important role in the biosynthesis of the most active biological oestrogen, the oestradiol. It has been postulated that polymorphisms of CYP19 could increase oestradiol level and have some role in breast cancer risk (Mucci *et al*, 2001).

In summary, no association was found with baldness genes, baldness and prostate cancer risk.

6.7 Conclusion

This study has identified gene and environment interactions between two mutations of DNA repair genes including XPC and XRCC7, low dose ionising radiation and prostate cancer risk. This observation, if further substantiated, has the potential to make substantial contribution to our understanding the roles of genetic susceptibility in prostate cancer.

6.8 Recommendations

As linkage studies lack power to detect alleles with moderate effects on risk, a large scale case-control studies are more efficient and the hypotheses supported by such evidence can then be further investigated in cohort studies and clinical trials.

Studies on molecular level for identifying individuals with higher risk carrying genetic polymorphisms with reduced DNA repair capacity should be identified and this could help in cancer prevention by targeting those individuals.

Chapter 7 Summary of work

This thesis forms part of the study of Gene-Environment Interactions in Prostate Cancer. The study was a population based case-control study. Cases were recruited from a variety of prostate cancer centres and controls were sourced from general male population and were age and geographically matched. The study sets out to investigate exposures associated with risk and also to explore genetic components involved in disease aetiology and their interactions. The Trent Multi Ethics approval was obtained. Exposures data were collected using self completed questionnaire. 18 ml Blood samples and toe nail clippings were also collected. Data of 1112 prostate cancer cases with symptomatic prostate cancer of all ages and 1872 population-based controls were analysed.

The study obtained a good response rates, 85.0% in case group and 74.4% in control group. The study population were predominantly Caucasian (>95%). The median age for cases and controls were 60 years and 59 years respectively suggesting good age matching between cases and controls. Furthermore, social class and education level distributions were similar between the two groups. These general demographic factors indicate that the study design was good.

This thesis presents results with two main parts, environmental factors and genetic factors.

For environmental factors, the results suggested that probands with positive family history of prostate cancer in first degree relatives were at greater risk compared to those with no family history of any cancer. A slightly lower risk, as compared to prostate cancer proband, was observed when the proband had a

family history of breast cancer in first degree relatives. The findings suggest strong genetic components in the development of prostate cancer.

The findings on X-ray exposure suggested that hip/pelvic X-ray increased the risk of developing prostate cancer. These results indicate that low dose radiations delivered to the anatomical site of the prostate gland are a potential cause prostate cancer later on in life.

Balding of any ages was not associated with prostate cancer risk overall however, an investigation of baldness in the subset of subjects with a positive family history of prostate cancer indicated that baldness was a risk factor for prostate cancer in this subgroup, suggesting a combined genetic role in the aetiology of both the conditions.

The results of hand pattern (second digit length (2D) compare to fourth digit length (4D) showed that men with index finger longer than ring finger (high 2D:4D) indicative of higher oestrogen at conception were less likely to develop prostate cancer as compared to men with index finger shorter than ring finger (low 2D:4D) indicative of higher testosterone at conception though the results were borderline statistically significant. This finding suggests the role of prenatal androgenic influences on prostate cancer risk later on in life.

Acne appeared at age 30 and presence of acne from teens through to age 30s showed an increased risk. This suggested a possible role of prolonged exposure to high levels of androgens and/or chronic inflammatory process in prostate carcinogenesis.

For the genetic investigations, low penetrance genes may be in part responsible for about 90% of cases (10% are inherited with high penetrance genes) were targeted and subsequently identified based on previous associations reported. Eleven selected single nucleotide polymorphisms (SNPs) from seven genes involved in DNA repair pathways and five selected SNPs from two genes involved in balding were analysed. The risk estimates of each SNPs was obtained and none of these SNPs showed any significant association with prostate cancer. The multiplicative interaction analysis on hip/pelvic X-ray suggested one SNP (rs2228001) of Xeroderma Pigmentosum Group C (XPC DNA repair gene) showed a modest risk increase. In contrast, the multiplicative analysis of universal X-ray exposures suggested negative associations between DNA repair genes (X-ray repair cross completing group7 (XRCC7G6721T rs7003908), X-ray exposures and prostate cancer risk. No interaction was observed between balding, balding gene mutations and prostate cancer risk.

This research was based on analysing the large UK case-control data set of many novel potential risk factors for prostate carcinogenesis including environmental and genetic factors. This large dataset allows not only the better power to detect any significant associations of exposures but also the investigation of gene and environment interactions. The newly identified environmental risk factors include low dose medical diagnostic radiological procedure, acne and balding in subjects with positive prostate cancer family history. The finding on gene and environment interaction suggested that subjects who carry the mutation allele on XPC DNA repair gene are at greater risk of developing prostate cancer, however, subjects who carry X-ray repair cross completing group7 allele mutation and who were exposed to low dose radiation are less likely to develop prostate cancer. This is the first study to look at DNA X-ray

repair and potential candidate hormone marker gene polymorphisms and their interactions with exposures.

The findings of this study provide several new leads in the field of cancer epidemiology of prostate cancer which could be applied further to investigate the aetiology of prostate cancer. The findings from gene and environmental analyses provide new means of evaluating prostate cancer and many other cancers and better understanding of potential combined effects of gene and environmental effects on human cancer. The completion of whole genome project includes various populations around the world will allow the identification of a much wider range of SNPs and genes. This will enable future work to expand SNPs selection according to study population and extend the investigations stated here on the effects both alone and in combination of genetic and environmental factors in prostate cancer aetiology.

This work has indicated some new leads in prostate cancer aetiology that should be further investigated in other large epidemiological studies. The Gene-environment interaction work has also demonstrated the potential of such an approach but required very large sample sizes that would be provided by prostate cancer case-control study consortia. Such consortium has recently been established (Collaborative Oncological Gene-environment Study-COGs) to which these data have been contributed and the lead identify in this dissertation and others will be investigated further in due course.

Chapter 8 References

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Chapter 9 Appendix

National Research Ethics Service

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06 June 2007

Professor Kenneth Muir
Professor in Epidemiology
University of Nottingham
Division of Epidemiology and
Public Health School of
Community Health Sciences
Queen's Medical Centre.
NOTTINGHAM
NG7 2UH

Dear Professor Muir

Full title of study:Investigation of Environmental, Lifestyle and Genetic Risk Factors for Prostate Cancer in Younger Men
REC reference number: 07/MRE04/29

Thank you for your letter of 23 May 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by

the Chair. **Confirmation of ethical opinion**

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has designated this study as exempt from site-specific assessment (SSA. There is no requirement for any Local Research Ethics Committees to be informed or for site-specific assessment to be carried out at each site.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

This research Ethics Committee is an advisory committee to East Midlands Strategic Health Authority

The National Research Ethics Service (NRES) represents the NRES Directorate within the National Patient Safety Agency and Research Ethics Committees in England

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	Version 5.3	05 April 2007
Investigator CV	Version 2	01 January 2007
Covering Letter		05 April 2007
Questionnaire: F1001 Reminder	Version 1	01 March 2007
Questionnaire: Gene-Environment interactions in Prostrate Cancer	Revised 5th Draft	06 February 2007
Letter of invitation to participant	P3001- Introduction study procedures- Version 1	01 March 2007
Letter of invitation to participant	2 - F1003 – Control	23 May 2007
Participant Information Sheet: P2002 - Controls	2	23 May 2007
Participant Information Sheet: P2001 - Cases	2	23 May 2007
Participant Consent Form: F1002 Reminder	Version 1	01 March 2007
Participant Consent Form: P2004 - UKCPCS - Cases	2	23 May 2007
Participant Consent Form: P2003 - Consultants/GP-Cases/Controls	2	23 May 2007
Response to Request for Further Information		23 May 2007
Letter to nurses - P3002, Instructions for blood sample taking	Version 1	01 March 2007
Headed Paper 2		
Headed Paper 1		
Evidence of Insurance letter, Marsh Ltd	Version 1	23 October 2006
Letter from Medical Research Council		19 October 2004
Letter from The Prostate Cancer Research Foundation		01 February 2006
Procedures Manual	Version 1	04 April 2007
Invitation Letter- P1002, Obtaining permission to contact consultant cases	Version 1	01 March 2007
Invitation Letter-P1001, UKGPCS Cases	Version 1	01 March 2007
Invitation Letter - P1002a, Consultant/GP Cases	Version 1	01 March 2007
Letter to consultant/GP - C1002	Version 1	01 March 2007
Letter to GP - C1001, Instructions for controls selection	Version 1	01 March 2007
Letter to Consultant - 00001, Initial Approach	Version 1	01 March 2007

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://vvww.rdforum.nhs.uk/rdform.htm.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

Feedback on the application process

Now that you have completed the application process you are invited to give your view of the service you received from the National Research Ethics Service. If you wish to make your views known please use the feedback form available on the NRES website at:

https://www.nresform.org.uk/AppForm/Modules/Feedback/EthicaiReview.aspx

We value your views and comments and will use them to inform the operational process and further improve our service.

071MRE04129 Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Your sincerely

Dr Robert Bing

Email: jill.marshali@derwentsharedservices.nhs.uk

Enclosures: *Standard approval conditions [SLAC2]*

Copy to: Professor Kenneth Muir, Division of Epidemiology and Public Health, University of Nottingham

ID number

Official use only

The University of
Nottingham

Gene-Environment Interactions in

Prostate Cancer

This study is being conducted by the Division of Epidemiology and Public Health, University of Nottingham, Institute of Cancer Research and the Royal Marsden Hospital NHS Trust. We are investigating factors that may be involved in the occurrence of prostate disease.

We would be very grateful if you could complete this questionnaire. This should only take about 30-45 minutes and we hope you will find it interesting. Your information will be ***treated in the strictest confidence***.

Please ***DO NOT*** write your name anywhere on the questionnaire. You will be identified only by the unique ID number at the top of this page.

Please ***return*** the completed questionnaire at your earliest convenience in the enclosed prepaid envelope - ***no stamp is required***.

Thank you for your help.

Dr Aneela Rahman (Researcher) Tel: 0115-8230495

Study Team from The University of Nottingham
Prof. Ken Muir (Principal Investigator)
Dr Artitaya Lophatananon (Research Officer)
Dr Aneela Rahman (Researcher)
Ms Jo-Fen Liu (Research Officer)

Study Team from The Institute of Cancer Research/The Royal Marsden Hospital NHS Trust
Dr Rosalind Eeles (Principal Investigator)
Prof. Douglas Easton (Co- Investigator)
Prof. David Dearnaley(Consultant Oncologist)

ID Number [ ]

use only

Section 1: About you

We would like to ask about your personal details.

1) Date of birth

☐☐/☐☐/☐☐

Date Month Year

2) Your marital status *(please tick the appropriate box)*

☐ Married ☐ Widowed ☐ Single

☐ Divorced ☐ Separated ☐ Other, please specify.............

3) Please indicate which group you belong to *(please tick the appropriate box)*

☐ White ☐ Black- Caribbean

☐ Black- African ☐ Black- other

☐ Indian ☐ Pakistani

☐ Jewish ☐ Sephardic

☐ Ashkenazi ☐ Chinese

☐ Other, please specify.........................

4) In which country were you born? *(Please tick the appropriate box)*

☐ UK ☐ Other, please specify...

5) Have you always lived in the UK? *(Please tick the appropriate box)*

☐ Yes *(go on to question 7)* ☐ No *(go on to question 6)*

6) How long have you been living in the UK? *(Please specify number of years)*

........................years

7) What is the highest educational qualification you have obtained?

(Please tick the appropriate box)

☐ None

☐ GCSEs, "O" levels or equivalent

☐ "A" Levels, higher or equivalent

☐ Higher or professional qualifications e.g. degree, HND

☐ Other, please specify...

Section 2: Employment

This section is about the jobs you have had since you left school.

8) Can you briefly describe all the jobs you have had for ***more than 1 year***.

(Please start with your current job or your latest job).

Job title and description of duties	Full time (FT) or Part time (PT)	Started (year)	Finished (year)	Self-Employed (SE) or Employed (E)	Did you supervise any others? (Y or N)
1					
2					

Job title and description of duties	Full time (FT) or Part time (PT)	Started (year)	Finished (year)	Self-Employed (SE) or Employed (E)	Did you supervise any others? (Y or N)
3					
4					
5					
6					
7					
9					
10					
11					
12					
13					
14					

9) Have you ever been exposed to chemical substances in any of your jobs?

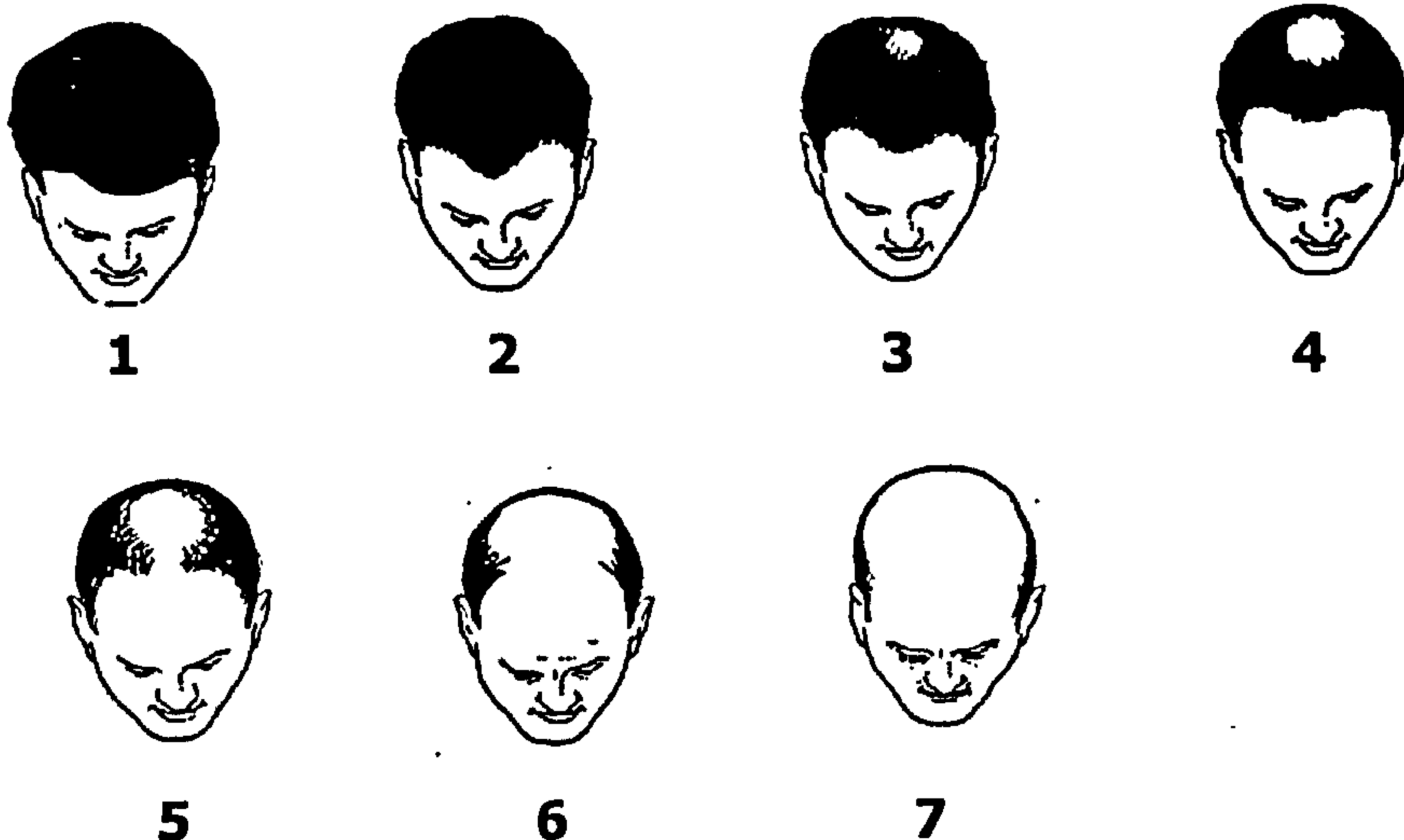
☐ Yes (p*lease complete the table below)* ☐ No (*go on to Section3)*

Chemical substances	(Y/N)	Degree of exposure i.e. *high, intermediate or background*	Regularity i.e. *daily, weekly*	Total number of years exposed	From which job? *– please give the job number from the list above*
Paints/varnishes /lacquers					
Solvents/ degreasing agents					
Petrol/diesel/ hydrocarbons					
Weed killers/ herbicides					
Radiation					

Section 3: Your hormones

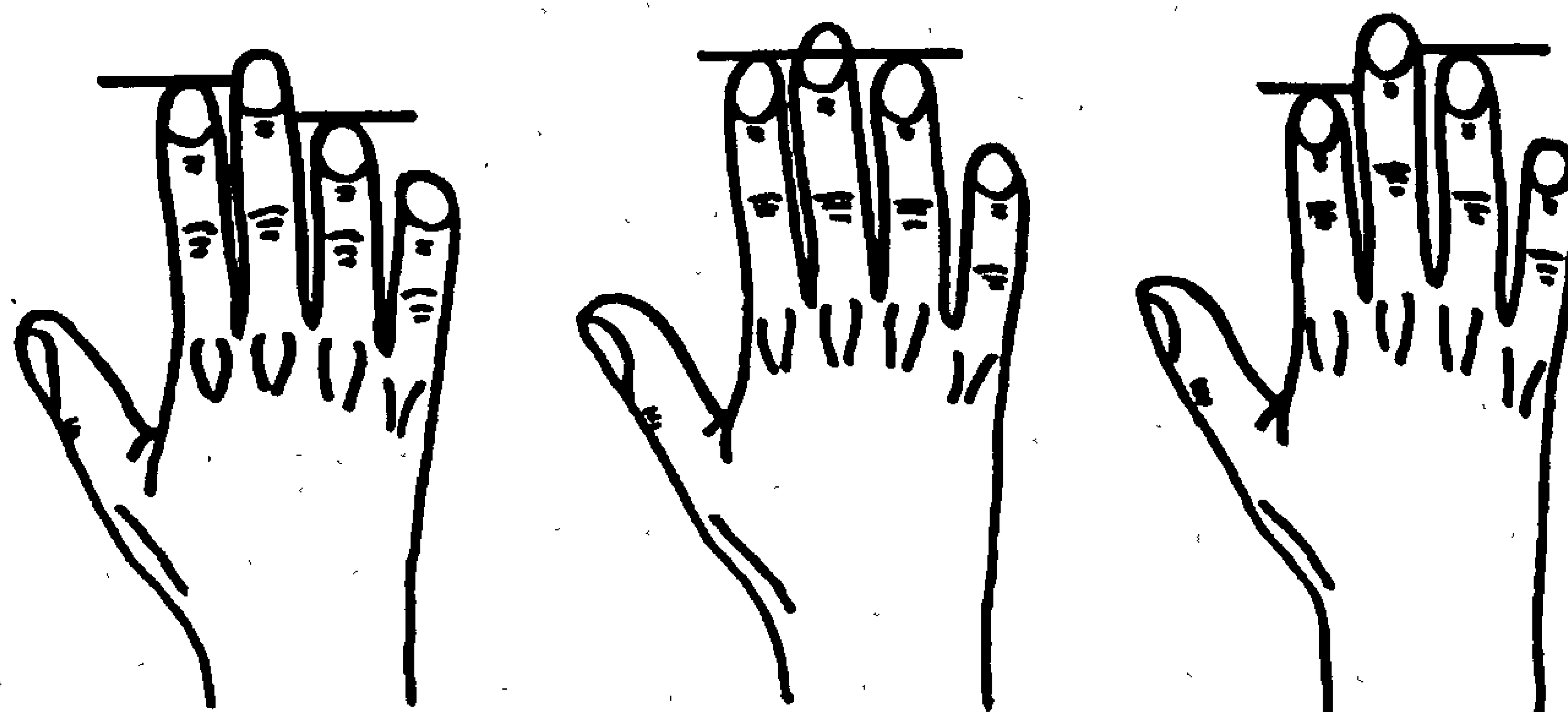
Evidence has suggested a possible relationship between male hormones and prostate disease. The effect of hormones can be seen physically, for example, pattern of hair loss, frequency of shaving, acne or hand pattern. In this section we would like to ask you about these factors at various ages.

Please choose the **NUMBER** corresponding to the hair pattern nearest to your own at the ages below. Please select one answer to each question. If you can't remember precisely, please make your best estimate.



10) In your 20s ☐ 11) In your 30s ☐ 12) In your 40s ☐

13) From the picture below, could you please look at the ***index*** and the ***ring*** fingers on your ***right hand*** by putting your hand on the table and compare these to the patterns below. Please tick the appropriate box for the pattern that is nearest to your own.



Right hand 1 ☐ **Right hand 2** ☐ **Right hand 3** ☐

14) In your 20s, how often, on average, did you need to shave in order to keep clean shaven?

☐ Once a day ☐ Twice a day ☐ Every other day

☐ Less than every other day ☐ Do not shave

15) Did you have acne when you were young?

☐ Yes *(if yes go on to question 16)* ☐ No *(go on to Section 4)*

16) Did you still have acne when you were:

	Yes	No
In your 20s	☐	☐
In your 30s	☐	☐

Section 4: Smoking

We would like to know a bit more about your smoking habit in this section. Please recall your smoking habits **prior to your diagnosis**

17) How would you describe yourself? *(Please tick one box only)*

☐ Current smoker, smoke daily *(go on to question 18)*

☐ Current smoker, smoke occasionally *(go on to question 18)*

☐ Ex-smoker, don't smoke at all now *(go on to question 21)*

☐ Never smoked *(go on to Section 5)*

Smokers only

18) In a day, I usually smoke (*please tick the box – you can tick more than one box and write down the number of cigarettes/ cigars or amount of pipe tobacco you smoke per day or per week)*

☐ Cigarettes number........per day or number........per week *(go on to questions 19,20)*

☐ Cigar number........per day or number........per week *(go on to questions 19)*

☐ Pipe amount ....per day or amount........per week *(go on to questions 19)*

19) The cigarettes I normally smoke are: *(Please tick appropriate box)*

☐ High tar level ☐ Middle tar level ☐ Low tar level

20) I have been a smoker for........years (*please write down a number and go to next section)*

Ex-smokers only

21) I have been an ex-smoker for: (*please tick appropriate box)*

☐ Less than a year ☐ 1-3 years

☐ 4-10 years ☐ over 10 years

22) When I was smoking, I used to smoke (*please tick the box – you can tick more than one box and write down the number of cigarettes/ cigars or amount of pipe tobacco you smoke per day or per week)*

☐ Cigarettes number........per day or number.........per week

☐ Cigar number.........per day or number.........per week

☐ Pipe amount ....per day or amount.........per week

Section 5: About sex

The prostate gland is responsible for producing fluid that helps sperm to survive when they enter the female reproductive tract following ejaculation. Changes in the prostate gland may occur depending on how often you have sexual intercourse or masturbate. Some sexual activities may also be related to hormone levels or may lead to an increased risk of infection. To help us find out if there is an association between prostate changes and sexual activities we need to know about past and present sexual practices.

We realise that this is a very sensitive subject but we would be very grateful if you could complete this section. ***Please answer these questions only if you feel able to do so***.

All your answers will be treated as STRICTLY CONFIDENTIAL AND NO INFORMATION WILL BE PASSED ON TO ANYONE OUTSIDE THE STUDY INCLUDING YOUR FAMILY DOCTOR.

23) At what age did you first have sexual intercourse? (*Please tick appropriate box)*

☐ Never ☐ Under 15 years old

☐ 15-19 years old ☐ 20-24 years old

☐ 25-29 years old ☐ 30 years or older

24) How often on average did you have sexual intercourse? *(Please tick one box and indicate yes or no, as appropriate)*

In your	Neve r	Less than once a month	Once to three times a month	Once a week	Two to three times a week	Four to six times a week	Daily	Condom normally used	
								Yes	No
20s								☐	☐
30s								☐	☐
40s								☐	☐
50s								☐	☐

25) In your lifetime, how many women ***in total*** have you had sexual intercourse with? (*Please tick appropriate box)*

☐ None ☐ One ☐ Two

☐ Three to five ☐ Six to ten ☐ Eleven to twenty

☐ More than twenty

26) From your answer to question 25, how many of them would you have classified as your "partner" *(i.e. someone you have/had sexual intercourse with once a week or more for a period of 3 months or longer).*

☐ None ☐ One ☐ Two

☐ Three to five ☐ Six to ten ☐ Eleven to twenty

☐ More than twenty

27) In your lifetime, have you ever paid money to women for sexual intercourse? (*Please tick appropriate box)*

☐ Yes *(go on to question 28)* ☐ No *(go on to question 29)*

28) Did you normally use condoms on those occasions?

☐ Yes ☐ No

29) At what age did you first masturbate? (*Please tick appropriate box)*

☐ Never ☐ Under 15 years old

☐ 15-19 years old ☐ 20-24 years old

☐ 25-29 years old ☐ 30 years or older

30) How often on average did you masturbate?

In your	Never	Less than once a month	1-3 times a mont h	Once a week	2-3 times a week	4-6 times a week	Daily
20s							
30s							
40s							
50s							

31) Overall, did you regard yourself has having a problem with sexual activity at different ages? *(please tick appropriate box)*

In your 20s ☐ Yes ☐ No

30s ☐ Yes ☐ No

40s ☐ Yes ☐ No

50s ☐ Yes ☐ No

32) In your 20s, 30s, 40s and 50s, did you encounter any of the following statement(s) that might have restricted you from sexual activity? ***(you can tick √ more than 1 statement)***

<u>**Statements**</u>	**In your**			
	20s	**30s**	**40s**	**50s**
1. Were not in any relationships	☐	☐	☐	☐
2. Your partner had physical/ emotional difficulties	☐	☐	☐	☐
3. You suffered from the following conditions which restricted your sexual activity. (*You can tick more than 1 box.*)	☐	☐	☐	☐
- depression	☐	☐	☐	☐
- diabetes (high blood sugar)	☐	☐	☐	☐
- high blood pressure	☐	☐	☐	☐
- arthritis or rheumatism	☐	☐	☐	☐
- prostate cancer	☐	☐	☐	☐
- enlarged or swollen prostate	☐	☐	☐	☐
- back problem	☐	☐	☐	☐
- impotence / erectile dysfunction	☐	☐	☐	☐
- lack of desire/ too tired	☐	☐	☐	☐
- other, please specify	☐	☐	☐	☐

33) In your lifetime, have you ever attended a sexually transmitted disease (STD) or special (VD) clinic? (*Please tick appropriate box)*

☐ Yes ☐ No

34) Have you ever been told by a doctor that you had any of the following conditions, even if it was a long time ago? (*Please tick appropriate box)*

Gonorrhoea	☐ Yes	☐ No
Syphilis	☐ Yes	☐ No
Genital herpes	☐ Yes	☐ No
Genital warts *(ie.warts on your penis/anal area)*	☐ Yes	☐ No
Non-specific urethritis (NSU)	☐ Yes	☐ No
Any other type of venereal disease	☐ Yes	☐ No

35) Have you ever had sores or ulcers on your penis?

☐ Yes (*go on to question 36)* ☐ No (*go on to Section 6)*

36) Have you ever had sexual intercourse while you had sores or ulcers on your penis? (*Please tick appropriate box)*

☐ Yes ☐ No

Section 6:About your skin and sun exposure

There is growing evidence on the relationship between UV radiation exposure from sunlight and prostate diseases. Thus we would like to ask you questions about your skin colour and also lifetime sun exposure.

Please tick appropriate box for each question:

37) What type of complexion do you have?

☐ Oily ☐ Dry ☐ Combination ☐ Normal

38) What is your skin colour when you are not sun tanned?

☐ Very fair ☐ Fair ☐ Medium

☐ Olive ☐ Very dark

39) What happens when you stay in the sun too long?

☐ Painful, bad blistering and peeling

☐ Blistering followed by peeling ☐ Burns sometimes

☐ Rarely burns ☐ Never had burns

40) On average looking back at the various stages of your life, in the daytime, how long were you out of doors during your working and non working hours? (*If during the last 5, 10 or 20 years you did not work please answer only non working time.)*

*Please recall your sunlight exposure **prior to your diagnosis**

In your		**Less than 1 hour**	**1-2 hours**	**3-4 hours**	**More than 5 hours**
20s	Working				
	Non- working				
30s	Working				
	Non- working				
40s	Working				
	Non- working				
During the last 5 years*	Working				
	Non- working				

41) On average looking back at the various stages of your life in the day time when outdoors, did you generally try one of the following? *Please put √ under the activity. You can answer more than one activity. If you did not spend time outdoors at all, please put √ under the far right column*

* Please recall your activity **prior to your diagnosis**

In your	**When outdoors, you........**				**Did not spend time outdoors at all**
	Always seek a sun tan	**Wear very little**	**Wear normal summer clothing**	**Try to cover yourself up from the sun**	
20s					
30s					
40s					
During the last 5 years*					

42) Did you use suntan oil, lotion or cream to protect your skin when you were out in the sun? *Please tick √*

In your	**Always**	**Sometimes**	**Rarely**	**Never**
20s				
30s				
40s				
During the last 5 years*				

Section 7: About the health of your family

Some prostate diseases may be hereditary. We would like to ask if any of your family have ever been diagnosed with prostate problems or any type of cancer.

43) Have any male members of your family been told by a doctor that he has/had any of the following? (*If there is no one, please go on to question 44)*

Identify relationship to you

☐ Yes *(Please answer the following)* ....

☐ A swollen or enlarged prostate ....

(benign prostatic hyperplasia) ....

....

☐ Prostatitis (infection of the prostate) ....

....

☐ No (*please go on to question 44)*

Certain cancers are known to have a genetic or familial component. Please record below any cancers that you are aware of and that have occurred in your **first degree relatives (parents, siblings or your children).**

44) Have any of your first degree relatives have cancer of any type?

☐ Yes *(go on to question 45)* ☐ No *(go on to Section 8)*

45) If yes, please specify their relationship to you and type of cancer that they have (including ***prostate cancer***).

Relationship to you	Type of cancer	Age at diagnosis (if known)	Date of birth
1			
2			

Relationship to you	Type of cancer	Age at diagnosis (if known)	Date of birth
3			
4			
5			
6			
7			
8			
9			
10			

Section 8: Physical activity

In this section we would like you to think about the physical activity you have undertaken in a typical day at various stages of your life.

On average have you undertaken *at least 30 minutes of* ***moderate*** *physical activity per day –* ***either*** *at home or at work.* (These activities can be made up of many components, for example, moving a table, pushing a vacuum cleaner, bowling or playing golf).

* Please recall your activity **prior to your diagnosis**

46) In your 20s ☐ Yes ☐ No ☐ Not applicable

47) In your 30s ☐ Yes ☐ No ☐ Not applicable

48) In your 40s ☐ Yes ☐ No ☐ Not applicable

49) During the last 5 years* ☐ Yes ☐ No ☐ Not applicable

On average have you undertaken *20 minutes or more of **energetic** activity at least 3 times per week whilst NOT at work.* (These include, for example, keep fit, dancing or exercises, swimming or other brisk sport, long walks, jogging or running, hard work in a job at home or in the garden, cycling).

* Please recall your activity **prior to your diagnosis**

50) In your 20s ☐ Yes ☐ No ☐ Not applicable

51) In your 30s ☐ Yes ☐ No ☐ Not applicable

52) In your 40s ☐ Yes ☐ No ☐ Not applicable

53) During the last 5 years* ☐ Yes ☐ No ☐ Not applicable

Section 9: Your general health and medication

In this section we would like to know more about your general health, medication (use of steroids, hormone treatments, or pain killers etc), as well as any X-ray procedures you have ever had at various stage of your life time.

Please recall your general health and medication **prior to your diagnosis**

54) Have you had a vasectomy?

☐ Yes (*go on to question 55*) ☐ No (*go on to question 56)*

55) How old were you? ☐☐

56) Have you ever taken any of the following? *(If no please go on to question 57)*

	Yes/No	At age	Treatment for	Duration of use (mm/yy)
Androgens or testosterone				
Anabolic steroids				
Oestrogen				
Cortisone not as a skin cream				
Cortisone or corticosteroids as a skin cream				
Thyroid drugs				

One of the questions researchers want to know is whether the exposure of medical diagnostic procedures such as X-ray, is associated with prostate disease. In order to answer this question, we will need to collect detailed information about any X-ray or radiological procedures you have ever had.

Please recall any procedures you have had **prior to your diagnosis**

57) Have you ever had any of the following x-ray procedures? (if yes, please give details with your best estimates)

Procedure	Yes/ No	Number of times	Details of procedure		
				At age / date	Purpose of x-ray and site (if applicable)
Barium meal i.e. x-rays of your **stomach** taken after swallowing a glass of chalky liquid			1		
			2		
			3		
			4		
			5		

Procedure	Yes/ No	Number of times	Details of procedure		
				At age / date	Purpose of x-ray and site (if applicable)
Barium enema i.e., a special X-ray test used to examine the **large bowel**(colon and rectum)			1		
			2		
			3		
			4		
			5		
Cholecystogram *i.e.* x-ray of your **gall bladder** taken after swallowing a glass of thick liquid			1		
			2		
			3		
			4		
			5		
IVP Kidney X-ray following an injection			1		
			2		
			3		
			4		
			5		
X-ray of hand, shoulder or arms			1		
			2		
			3		
			4		
			5		
X-ray of upper leg or thigh			1		
			2		
			3		
			4		
			5		

Procedure	Yes/ No	Number of times	Details of procedure		
			At age / date		Purpose of x-ray and site (if applicable)
X-ray of hips/pelvic region			1		
			2		
			3		
			4		
			5		
Lymphangiogram *i.e.* x-ray taken of different parts of the body after dye has been injected			1		
			2		
			3		
			4		
			5		
CAT scan *i.e.* x-ray of your body taken inside a machine where the equipment rotates around you			1		
			2		
			3		
			4		
			5		
NMR or MRI (magnetic resonance imaging) Scan *i.e.* where you are put inside a large magnet			1		
			2		
			3		
			4		
			5		
Radioactive or isotope injections with pictures or x-ray taken afterwards			1		
			2		
			3		
			4		

Procedure	Yes/ No	Number of times	Details of procedure		
			At age / date		Purpose of x-ray and site (if applicable)
			5		
Venogram *i.e.* x-rays of vein after dye has been injected			1		
			2		
			3		
			4		
			5		
Angiogram or arteriogram *i.e.* an x-ray to view your heart or body blood vessels taken after a tube has been passed into your arm or groin			1		
			2		
			3		
			4		
			5		

58) Have you ever been told by doctor that you have/had any of the following conditions?

Conditions	Yes/No	Age at diagnosis
Diabetes		
Heart disease		
Hypercholesterolaemia (high blood cholesterol)		
High blood pressure		
Other please specify		

Some medications may be associated with prostate diseases. In order to study this question in detail, we would like to ask you some questions about your use of prescription or non-prescription painkillers in the past.

Please try to recall the time period **prior to your diagnosis**

59) Have you ever regularly taken statin (e.g, Atorvastatin, Cerivastatin, Simvastatin) in the past 10 years?

☐ Yes *(go on to question 60)* ☐ No *(go on to question 61)*

60) If Yes, could you please let us know

(a) Which type of statin (or brand name) you have taken?

....

a. The dosage of pills or capsule? .... mg or .... µg
b. Roughly how often do you take the medicine? ....
c. For how many years have you been taking the medicine? .... years
d. Reason for taking statin? ....

61) Have you ever regularly taken any non-prescription painkillers bought over the counter from a chemist or a supermarket in the last 10 YEARS? **prior to your diagnosis**

(*By regularly, we mean at least one tablet per week for more than three months.*)

☐ Yes *(go on to question 62)* ☐ No *(go on to question 63)*

62) We would like to know more details about the painkiller(s) you have regularly taken. Could you please let us know:

a) Which type of painkiller(s) you have taken?

b) Do you recall the dose?

c) Roughly how often do you take the tablets or medicine?

d) For how many years have you been taking the tablets or medicine?

e) For what reason do you take them?

Please provide the information in the table

a) Name of Painkiller	b) Dose	c) Average frequency Tick one box per line			d) Duration	
	Dosage of pills/capsules or teaspoons each time	Never or less than ce a month	At least once a month but not every day	At least once a day	Number of years of taking painkillers	*e) Reason for taking painkillers*
1 Aspirin or preparation containing aspirin eg Alka-Seltzer, Disprin						
2 Ibuprofen – e.g. Nurofen, Ibufen, Advil, Migrafen						
3 Paracetamol or preparation containing Paracetamol – eg Panadol, Co-proxamol, Co-codamol						
4 Other pain medication (please specify)						

63) Do you have any side-effects if you take aspirin?

☐ Yes *(go on to question 64)* ☐ Don't know/ I don't use aspirin *(go on to question 65)*

☐ No (*go on to question 65)*

64) Do the side effects make you stop taking aspirin?

☐ Yes (*go on to question 65)*

☐ No. I still take aspirin because ....

....

In this section, we would like you to think about the three most commonly used painkillers in various stages in your adult life. This includes painkillers available in pharmacy or supermarket (i.e., over the counter; OTC), as well as those prescribed by doctor. Please try to recall the period **prior to your diagnosis**

65) Have you been taken any painkilling medication on a regular basis (at least once a week for more than three months) during your adult life, either prescribed by your GP or bought over the counter (OTC).

☐ Yes (*go on to question 66)* ☐ No *(go on to section 10)*

66) If Yes, please can you give us more details

In your	**A** Painkiller 1			**B** Painkiller 2			**C** Painkiller 3		
	Name	From	No of years used	Name	From	No of years used	Name	From	No of years used
20s		GP ☐ OTC ☐			GP ☐ OTC ☐			GP ☐ OTC ☐	
30s		GP ☐ OTC ☐			GP ☐ OTC ☐			GP ☐ OTC ☐	
40s		GP ☐ OTC ☐			GP ☐ OTC ☐			GP ☐ OTC ☐	
50s		GP ☐ OTC ☐			GP ☐ OTC ☐			GP ☐ OTC ☐	

Section 10: Further details about you

In this section we would like to know more about your body size and body shape. This includes the changes of your weight or trouser size in the past years. Please give as approximate estimates if you can and

67) Please can you tell me your current weight and height?

My weight is.......... Stones............Pounds or .... Kilograms

My height is........... Feet.................Inches or.............. Centimetres

68) What was your weight **prior to your diagnosis?**

My weight was ....Stones...........Pounds or ....Kilograms

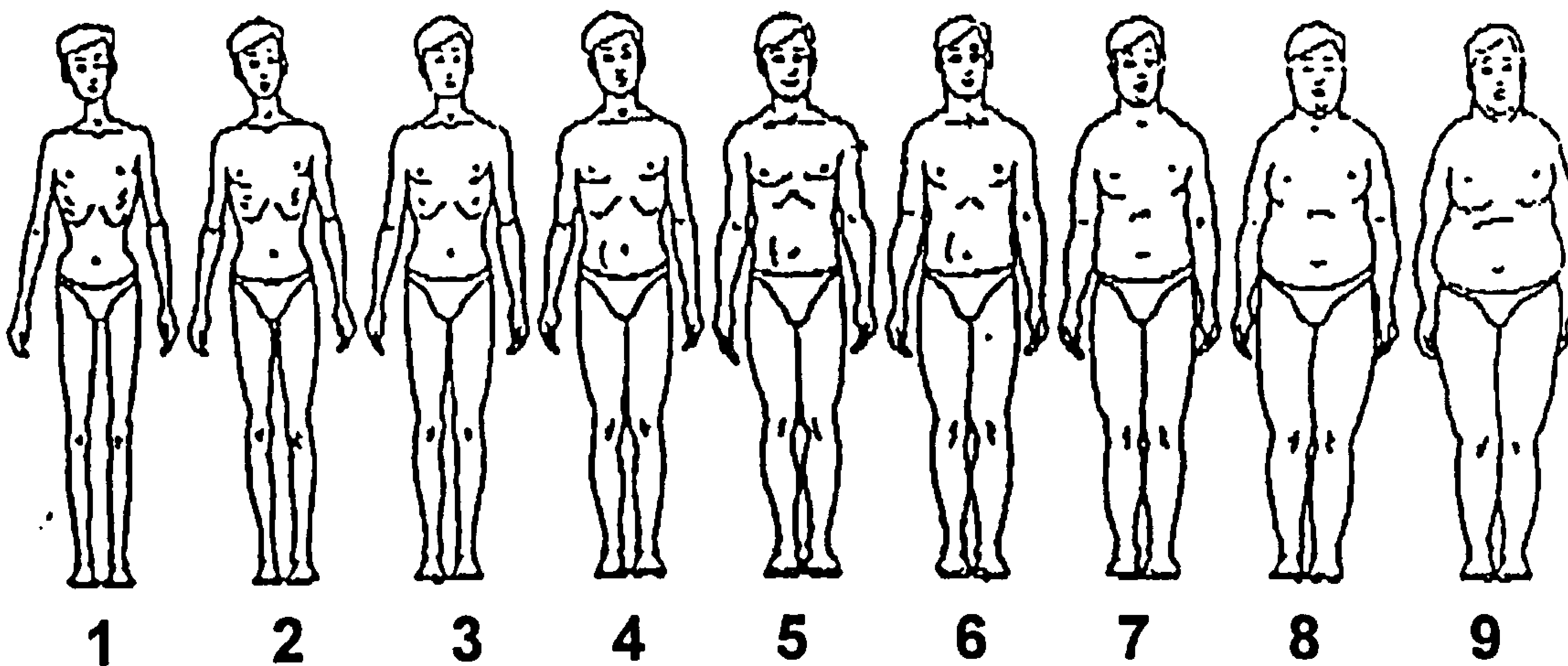
69) What is your collar-size?

	inches

70) Please can you tell me your waist and your approximate hip circumference, either in inches or in centimetres? If you cannot remember your waist circumference, can you recall your trouser size (for example size 30)?

	Waist/ Trouser Size		**Hip**	
	inch	**cm**	**inch**	**cm**
In your 20s				
In your 30s				
In your 40s				
During the last 5 years before your diagnosis				

Please select the shape you think you were at different ages. *(Please write down the number you think you were).*



71) In your 20s ☐

72) In your 30s ☐

73) In your 40s ☐

74) During the last 5 years ☐

(**Before your diagnosis**)

Overall please select one of the descriptions below that suit you the most **before your diagnosis** *(please write down number in the box)*

1.Apple shape- where your body fat is distributed mainly around your tummy area.

2. Pear shape- where your body fat is distributed mainly on your hip and thigh.

3. Oval shape- where your body is distributed around your neck, your chest, your tummy area and also your thigh.

4. Symmetric shape- where you are lean with no fat distribution around your body.

75) My body shape was ☐

May we have your permission

- To contact you if we need further information to resolve any queries?

☐ Yes   Contact telephone number:...

☐ No   Email:..

- To look at your medical record

☐ Yes   ☐ No

If you are planning to move house in the near future, please may we have your new address?

My new address will be..

.... Post code.....................

New telephone number (if known)...

E-Mail: ....

Thank you very much once again for taking the time and trouble to fill in this questionnaire, your help is really appreciated and will be invaluable to this research project.

Please return your answers in the pre-paid envelope as soon as possible to:

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