DETERMINANTS OF PROSTATE CANCER:

The Birmingham Prostatic Neoplasms Association Study

By

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Abstract

This Birmingham Prostatic Neoplasms Association Study (BiPAS) was initiated to investigate determinants of prostate cancer. The study recruited 314 prostate cancer patients, 381 active surveillance patients, 201 hospital controls and 175 population controls. By comparing groups of varying risk, the aetiology of the disease was investigated.

Within the BiPAS dataset, sun exposure, physical activity and obesity were analysed. The association with occupation was assessed by performing a meta analysis of 7, 762 cases and 20, 634 controls. Finally, a replication study on genetic polymorphisms on 8q24 using 277 cases and 282 controls from the Netherlands Cohort Study (NLCS) is presented.

A protective effect was observed for high sun exposure in early adulthood and high intensity exercise. An increased risk was observed for low intensity exercise and men classed as obese at age 20. The meta analysis suggested moderately increased and decreased risks associated with a number of job titles, however none were statistically significant. The results for allele A on the single nucleotide polymorphism rs1447295 were replicated; however a decreased risk was detected for allele -8 on the microsatellite DG8S737. No significant difference was detected for analysis comparing prostate cancer or high PSA cases.

PUBLICATIONS AND PRESENTATIONS ARISING FROM THIS THESIS

Publications in press

1. **Khan HS**, Zeegers MP, Schouten LJ, van Dijk BAC, Goldbohm RA, Schalken J, Shajahan S, Pearlman A, Oddoux C, van den Brandt PA, Ostrer H. Genetic marker polymorphisms on chromosome 8q24 and prostate cancer in the Dutch population: DG8S737 may not be the causative variant. **Eur J Hum Genet. 2011 Jan;19(1):118-20**

2. Eeles RA, Kote-Jarai Z, Al Olama AA, Giles GG, Guy M, Severi G, Muir K, Hopper JL, Henderson BE, Haiman CA, Schleutker J, Hamdy FC, Neal DE, Donovan JL, Stanford JL, Ostrander EA, Ingles SA, John EM, Thibodeau SN, Schaid D, Park JY, Spurdle A, Clements J, Dickinson JL, Maier C, Vogel W, Dörk T, Rebbeck TR, Cooney KA, Cannon-Albright L, Chappuis PO, Hutter P, Zeegers M, Kaneva R, Zhang HW, Lu YJ, Foulkes WD, English DR, Leongamornlert DA, Tymrakiewicz M, Morrison J, Ardern-Jones AT, Hall AL, O'Brien LT, Wilkinson RA, Saunders EJ, Page EC, Sawyer EJ, Edwards SM, Dearnaley DP, Horwich A, Huddart RA, Khoo VS, Parker CC, Van As N, Woodhouse CJ, Thompson A, Christmas T, Ogden C, Cooper CS, Southey MC, Lophatananon A, Liu JF, Kolonel LN, Le Marchand L, Wahlfors T, Tammela TL, Auvinen A, Lewis SJ, Cox A, FitzGerald LM, Koopmeiners JS, Karyadi DM, Kwon EM, Stern MC, Corral R, Joshi AD, Shahabi A, McDonnell SK, Sellers TA, Pow-Sang J, Chambers S, Aitken J, Gardiner RA, Batra J, Kedda MA, Lose F, Polanowski A, Patterson B, Serth J, Meyer A, Luedeke M, Stefflova K, Ray AM, Lange EM, Farnham J, **Khan HS**, Slavov C, Mitkova A, Cao G; UK Genetic Prostate Cancer Study Collaborators/British Association of Urological Surgeons' Section of Oncology; UK ProtecT Study Collaborators; PRACTICAL Consortium, Easton DF. Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. **Nature Genetics. 2009 Oct; 41(10):1116-21.**

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6. **Khan HS**, Brinkman M, Wallace M, Luscombe C, Campbell MJ, Murray P, Cheng KK, McManus RJ, Zeegers MP. Is body mass index associated with Prostate Cancer? Results from the Birmingham Prostatic Neoplasms Association Study

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List of Abbreviations

ADAM	Androgen deficiency in ageing males
AJCC/UICC	American joint committee on cancer/international union against cancer
BiPAS	Birmingham prostatic neoplasms association study
BPH	Benign prostate hyperplasia
COREC	Centre of research ethical campaign
CRT	Conformal radiotherapy
CZ	Central zone
DNA	Deoxyribonucleic acid
DRE	Digital rectal examination
EDTA	Ethylenediaminetetraacetic acid
FDA	The Food and Drug Administration
HPC1	Hereditary Prostate Cancer 1
HPX	Hereditary Prostate Cancer X
ICH-GCP	International conference of humanization guidelines on good clinical practice
IMRT	Intensity modulated radiotherapy
LUTS	Lower urinary tract symptoms
M	Metastasis
MidReC	Midlands research practices consortium
N	Lymph nodes
NCCN	National Comprehensive Cancer Network
OR	Odds ratio
PCA3	Prostate cancer antigen 3

PIN	Prostatic intraepithelial neoplasia
PRACTICAL	Prostate cancer association group to investigate cancer associated alterations in the genome
PSA	Prostate specific antigen
PZ	Posterior zone
QED	Quick early diagnosis
RR	Risk ratio
SEER	Surveillance, epidemiology and end results
SNP	Single nucleotide polymorphism
SOC2000	Standard occupational classification 2000
SOP	Standard operating procedures
SOR	Summary odds ratio
T	Tumour
TRUS	Transrectal ultrasound
TZ	Transition zone
UVR	Ultraviolet radiation
WMCIU	West Midlands cancer intelligence unit
95%CI	95% confidence intervals

INTRODUCTION

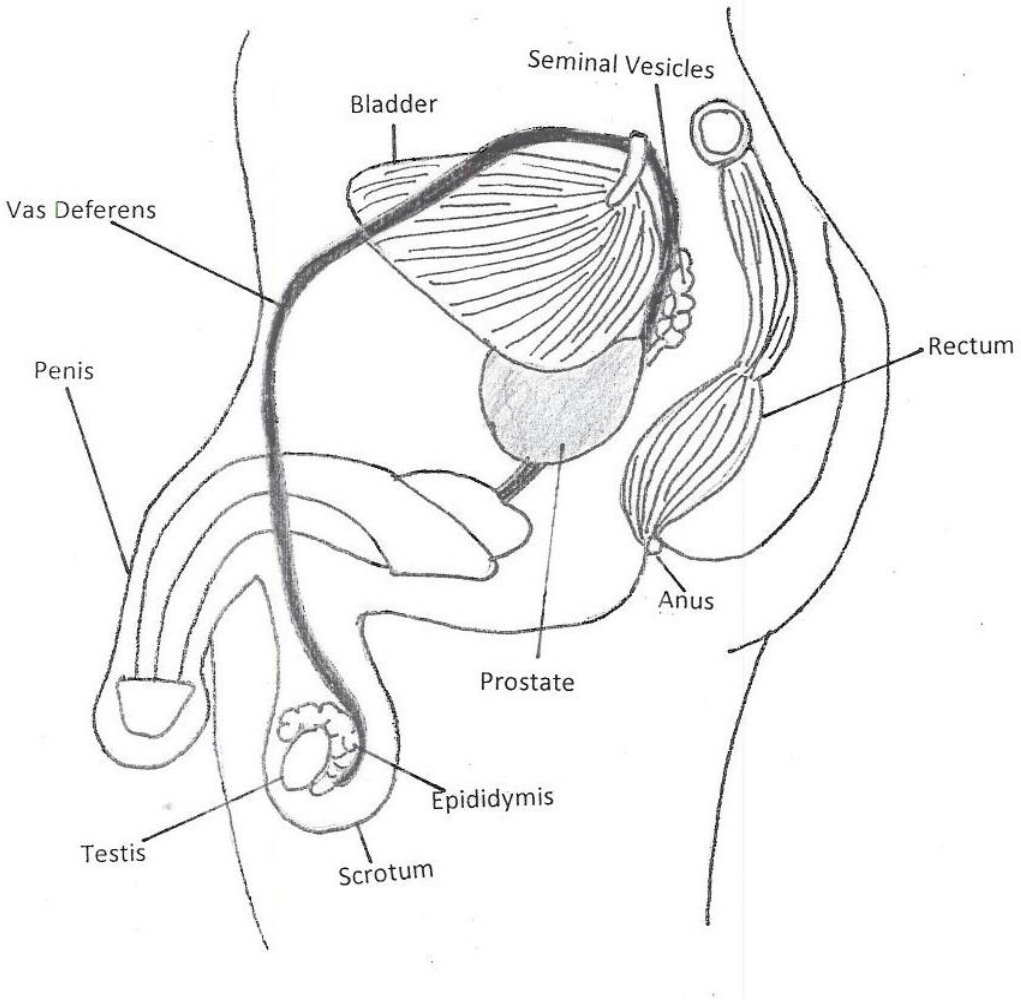
CHAPTER 1

1.1 The Prostate

1.1.1 Anatomy

The prostate is the male sexual accessory gland. It is located on the floor of the pelvis and surrounds the neck of the bladder and urethra (see Figure 1.1). In men, the urethra serves two purposes; urination and ejaculation. It runs from the bladder through the prostate and to the tip of the penis. The section of the urethra running through the prostate is known as the *prostatic urethra*. After being produced in the testicles, sperm moves into a coiled mass, known as the *epididymis* for maturation. It then goes into two muscular tubes known as the *vas deferens,* which coil around the bladder and seminal vesicles. The seminal vesicle can house the sperm for several days until ejaculation. During ejaculation, the prostate muscles contract and expel the sperm into the prostatic urethra towards the tip of the penis.

Figure 1.1 The Prostate Gland



The average weight of a healthy prostate is approximately 11grams, ranging between 7 and 16grams (1). It is encapsulated by a fibroelastic tissue layer, leading to septa extending inwards and dividing the prostate into different lobes. The lobes accommodate nearly 50 irregularly branched saccular glands, excretory ducts, stroma (connective tissue cells), blood vessels and nerves. The glands are lined with two epithelial cell layers, the outer layer is composed of cuboidal epithelia (simple cube shaped) and the inner layer is composed of tall columnar epithelia. This transitional epithelium or urothelium has the ability to contract and expand according to the volume of fluid within.

The main male hormone is testosterone and is produced in the testicles. The prostate is regulated by dihydrotestosterone. Dihydrotestosterone is synthesized from testosterone in the peripheral tissue.

1.1.2 Prostate Function

The primary function of the prostate gland is to store part of seminal fluid and assist ejaculation during sexual activity. The smooth muscles in the prostate help to expel semen during ejaculation. The slightly alkaline fluid produced by the prostate makes up 25% of seminal fluid and allows sperm motility and viability. The vaginal tract is acidic therefore the alkalinity of the semen neutralizes the environment to allow the sperm to stay viable. A major constituent of prostatic secretion is prostate specific antigen (PSA), along with citrate (18.7 mg/ml), zinc (488 µg/ml), spermine (243 mg/ml) and cholesterol (78 mg/ml) (2).

1.1.3 Prostate Structure

The prostate can be classified by two different systems; zones or lobes. The zonal classification is used more in pathology; classifying the prostate into four different regions. The peripheral zone (PZ) forms about 70% of the prostate and surrounds the urethra. Nearly 80% of prostatic cancers develop in the PZ. The central zone (CZ) surrounds the ejaculatory ducts and forms 25% of the prostate. Only 2.5% of prostatic cancers arise in this region, however the cancers that do develop here are more aggressive (3). The transition zone (TZ) accounts for around 20% of prostatic cancers and surrounds the proximal urethra. The TZ grows larger over time; benign prostatic enlargement originates in this region. The final region, the anterior fibro-muscular zone consists of muscle and fibrous tissue only.

The lobe classification system also divides the prostate into four different regions, the anterior lobe (roughly the same as the TZ), posterior lobe (comparable to the PZ), lateral lobes (spans all zones) and the median or middle lobe (CZ). This classification is usually used when describing the anatomy of the prostate.

1.2 Prostate Carcinogenesis

Cancers are described as unregulated growth and consequent spread of cells to other parts of the body (4). All types of cells can undergo such malignant changes and become cancers, however only epithelial cells can become carcinomas. The normal cell cycle is disrupted and the new “tumour” cells overgrow in a localised region at first, then spread to surrounding tissue and finally to other parts of the body via the lymphatic system and vascular system (5-6).

In the process of carcinogenesis, normal cells are transformed into cancer cells due to an uncontrolled cell division. Normal cell division maintains a balance between proliferation and cell death with tightly regulated processes. Mutations in DNA can disturb these processes, leading to the cell to rapidly divide and therefore proliferate at a much higher rate. The resulting mass can either be benign, which does not spread to other parts of the body, or be malignant which can invade other organs and spread to distant locations.

A possible precursor of prostatic carcinoma is prostatic intraepithelial neoplasia (PIN). PIN involves the abnormal development of the epithelial cells which line the prostate glands. Low grade PIN is characterized by crowded and irregularly spaced epithelial cells where the nuclei are hyperchromatic (with elevated chromatin) and pleomorphic (where there is variation in size and shape). In high grade PIN, a higher level of hyperchromatisms and pleomorphism exists. PIN is distinguished from adenocarcinoma by the involvement of a cluster of rounded cells, resembling a raspberry shape, known as acini (7). Presence of PIN suggests an increased risk for adenocarcinoma however it can be up to 10 years before prostate carcinoma presents (8).

Adenocarcinoma is a type of cancer arising from epithelial cells of the secretary glands lining the prostatic ducts. The cytological features include enlarged hyperchromatic nuclei, as in PIN (9).

1.2.1 Molecular changes

Most of the human genome is non-coding DNA; therefore the majority of genetic changes are harmless. The coding DNA makes up approximately 3% of the genome and genes are also split into introns (non-coding) and exons (coding). Only mutations in the exon regions of the genome are subject to harmful changes which may affect protein composition (4).

Although the genetics of prostate cancer are poorly understood, we know cancers almost always arise from a single somatic cell, that undergoes a number of genetic changes which cause a change in gene activity and therefore phenotype (10). Cancer causing mutations usually arise in genes involved in the regulation of cellular growth or death (11). Since there are over 100 types of cancers and each tumour has a number of different subtypes, the complexity makes it difficult to pinpoint origin of disease. The past two decades have seen extensive research in the molecular, biochemical and cellular processes involved in the transformation of normal cells to malignant cancer cells. The vast majority of cancer cells have six different capabilities; self sufficiency in growth signals, insensitivity to anti growth signals, evasion of apoptosis, infinite replication ability, sustained angiogenesis and ability to invade tissue and metastasise (12). Normal cells monitor their external environment and stimulate cell division when necessary. Cancer cells, however, produce their own signals which liberate them from the growth limitations of normal cells. The second capability is insensitivity of anti-growth signals, which works in the same way as the previous stage, as cancer cells do not receive signals to inhibit growth. The third feature is the acquired capability of sustained growth. A normal cell usually stops replicating after 60 or 70 times, which is controlled by the telomeres. These segments of DNA are shortened by each round of

DNA replication, and eventually, when they are too short for another round of the cycle, the cell undergoes apoptosis (cell death). Cancer cells are able to maintain the length of their telomeres, allowing them replicate infinitely. The next feature is evasion of apoptosis, which is usually exerted by p53. In cancer cells, the p53 gene is often mutated and therefore apoptosis does not occur as normal. Angiogenesis is the formation of new blood vessels. These are essential for supplying the tumour with oxygen and nutrients. And the final capability is tissue invasion and metastasis, where cancer cells attach themselves to other cells and move around the body (13).

Cancer genes can be classified into three main categories; oncogenes, tumour suppressor genes and cells involved in DNA repair. Oncogenes were the first cancer causing genes identified and lead to unregulated cell growth (10). Most arise from genes known as proto oncogenes responsible for normal cell growth. They are generally dominant and common mutations include an increase in protein activity or loss of regulation, increase in protein concentration or chromosomal translocation causing gene expression of the different cell type. Examples of oncogenes include *ras* (mutated in about 15% of cancers), *myc* and *abl* (14-15). Tumour suppressor genes are also known as anti-oncogenes and are usually inactivated by loss of function mutations. In 1971, Knudson studied sporadic and familial retinoblastoma and formulated the two hit model of carcinogenesis which demonstrates the loss of function changes. In familial retinoblastoma, there is a 50% chance of a child inheriting the condition from an affected parent and in sporadic there is no additional risk (16). The inherited form is not known to cause a predisposition of tumour development due to germline mutations in one copy of the tumour suppressor gene (17). A somatic mutation of the second copy will cause

tumour progression. In sporadic retinoblastoma, two different "hits" are required within the same cell to develop a tumour. The p53 gene *TP53* is one of the most important tumour suppressor genes involved in key cancer control pathways, such as cell cycle control, apoptosis, angiogenesis and genetic stability. And the final category is genes responsible for DNA repair mechanisms, which allow normal DNA replication. Mutations in this process often result in genetic instability leading to abnormal chromosome numbers or breaks (18).

A mutation specific for prostate cancer is yet to be identified. Also, common mutations in oncogenes and tumour suppression genes for various other cancers are surprisingly rare in primary prostate cancer (13).

1.2.2 Prostate Specific Antigen

Prostate specific antigen (PSA) is a glycoprotein produced by the prostate acinar cells and is unique to the prostate gland (19). The function of PSA is to dissolve the seminal clot after ejaculation in order to facilitate the transport of spermatozoa along the female reproductary tract. PSA may be complexed to serum proteins, when it is known as "complexed PSA" or it can be free, known as "free PSA". Both complexed PSA and free PSA are combined to give a measure of total PSA. Although PSA is present in high concentrations in seminal fluid (0.5 to 2.0 mg/mL) it has a much lower concentration in the blood, almost 1000 times lower. Although the variations in concentration are independent of other proteins, it is sensitive to changes in serum testosterone levels (20). Age specific normal ranges are used to identify elevated levels; however these vary according to the assay used.

PSA was first described as a prostate cancer marker in 1982 and the first reports of its use as a screening test were in 1991 (21-22). The incidence of prostate cancer has had a gradual increase in most Western countries over the last 30 years, however the use of PSA testing caused a spike in new diagnoses in the USA in the early 1990s (23). A good screening test should be sensitive, safe, cheap and should be used for diseases in which early detection improves prognosis. There has been some debate over the last criterion for PSA testing, although it has been developed extensively in order to increase specificity and sensitivity (24-26). As benign prostatic enlargement can also be responsible for increasing PSA, an adjusted value called PSA density, is often used. For this measure, the serum PSA value is divided by prostate volume. The main advantage of this test is that it guides decision making for prostate biopsies. However, as a transrectal ultrasound (TRUS) is required for the measurement of prostate volume, it increases the discomfort for the patient and it is costly (27). Free PSA can be measured by a blood test and is usually lower in prostate cancer patients, however the reasons for this are unclear (23). Men with a free PSA of less than 15% have a higher risk of prostate cancer, yet in men with 25% or higher free PSA, the risk is significantly reduced (28).

As PSA increases with age, cut off values are age dependent (see Table 1.1). There are also different reference ranges for different ethnicities (29). Although these reference ranges are easy to use, they can fail to detect high grade prostate cancer in older men (30).

Table 1.1 PSA Reference ranges for Caucasian men, using the Roche E170 assay

Age (years)	Reference Ranges (ng/mL)
50-59	0-3.5
60-69	0-4.5
70-79	0-6.5

Taken from Oesterling JE *et al*, *Serum prostate-specific antigen in a community-based population of healthy men. Establishment of age-specific reference ranges* JAMA. 1993 270(7): p. 860-4.

1.2.3 Symptoms and Diagnosis

Prostate tumours are usually slow growing and symptoms may not occur for many years. In the early stages of prostate cancer, there are often no symptoms. However, due to its location surrounding the urethra, symptoms for the disease most commonly affect urination. Prostate cancer symptoms include frequent urination, increased urination during the night, (nocturia), difficulty in maintaining a steady stream of urine, blood in the urine (hematuria) and painful urination (dysuria). It can also affect sexual function, for example difficulty in achieving erection or painful ejaculation. If the cancer is advanced, it can spread to other organs, causing bone pain in the pelvis or ribs. Many of the urinary symptoms also occur in other prostate diseases, such as benign prostate hyperplasia, along with an enlargement of the prostate. Prostate tumours are only felt in a small percentage of cases during a digital rectal examination (DRE). Diagnosis of prostate cancer must be confirmed by a needle biopsy. The International Classification of Diseases version 10 (ICD10) classifies malignant neoplasm of the prostate as code C61 (31).

1.2.4 Tumour staging

Once a patient has been diagnosed with a prostate tumour, the cancer must be staged to determine if it has spread beyond the prostate. Staging also provides a better insight into the risk of the disease spreading further so the correct treatment option is selected. The TNM stage was developed by the American Joint Committee on Cancer/International Union Against Cancer (AJCC/UICC) (32). It is used to evaluate the extent of the primary tumour (T), the affected regional lymph nodes (N) and if it has spread or metastasized (M). There are four stages; in stage I only a small part of the prostate is cancerous, most of the cells are normal

and the gland feels normal. In stage II, a lump can be felt in the prostate to the examining finger and a larger part of the prostate is affected. In stage III, the tumour has spread beyond the prostate and in stage IV; it has spread to lymph nodes or nearby organs. A more detailed view can be found in Table 1.2.

Table 1.2 Staging of Prostate Cancer

Primary Tumour (T)	**TX**	Primary tumour cannot be assessed
	T0	No evidence of primary tumour
	Ta	Non invasive papillary carcinoma
	Tis	Carcinoma in situ: "flat tumour"
	T1	Tumour invades sub epithelial cells
	T2	Tumour invades muscle
		T2a Tumour invades superficial muscle (inner half)
		T2b Tumour invades deep muscle (outer half)
	T3	Tumour invades perivesical tissue
		T3a Microscopically
		T3b Macroscopically
	T4	Tumour invades prostate
		T4a Tumour invades prostate
		T4b Tumour invades pelvic wall or abdominal wall
Regional Lymph Nodes (N)	**NX**	Regional lymph nodes cannot be assessed
	N0	No regional lymph node metastasis
	N1	Metastasis in a single lymph node 2cm or less in greatest dimension
	N2	Metastasis in a single lymph node 2cm but no more than 5cm in greatest dimension
		Multiple lymph nodes, none more than 5cm in greatest dimension
	N3	Metastasis in a lymph node no more than 5cm in greatest dimension
Distant Metastasis (M)	**MX**	Distant metastatic cannot be assessed
	M0	No distant metastatic
	M1	Distant metastatic

1.2.5 Tumour Grading

Tumours are graded to allow better predictions for prognosis. The Gleason Grading System is the most commonly used system, where cancers are scored according to their appearance under a microscope. During biopsy, a sample of the prostate tissue is obtained and prepared on microscope slides. Two grade scores are assigned for the two most common tumour patterns, and these scores added together for a final Gleason sum. Gleason scores range from 1 to 5, where 5 has the poorest prognosis and Gleason sums range from 2 to 10. The Gleason patterns are detailed Table 1.3. For the primary grade, pathologists identify which pattern corresponds with at least 50% of the tumour and the secondary grade represents the minority of the tumour.

The prognosis for prostate cancer can be variable. More aggressive tumours, with Gleason sum 8, 9 or 10, can lead to death in a short space of time, however lower grades, with Gleason sum of 6 or lower, may not see any clinical consequences (23). Albertsen *et al* conducted a series of studies on a cohort of 767 untreated cancer patients. Men with tumours of Gleason sum 5 had between 6% to 11% cancer mortality at 20 years. Patients with tumours with a higher Gleason sum had up to 70% (Gleason score 7 or 8) and 87% (Gleason score 10) rates of death from prostate cancer, with very few of the entire cohort surviving more than 15 years after diagnosis (33-34).

Table 1.3 Gleason Patterns

Pattern 1	The cancerous prostate cells closely resemble normal prostate cells. The glands are small, well-formed, and closely packed.
Pattern 2	The glands are larger and have more tissue between them
Pattern 3	The tissue still has recognizable glands, but the cells are darker. Some cells have left the glands and have started to invade the surrounding tissue.
Pattern 4	The tissue has few recognizable glands. Many cells are invading the surrounding tissue
Pattern 5	The tissue does not have recognizable glands. There are often just sheets of cells throughout the surrounding tissue.

1.2.6 Treatment

Three standard treatments for localised prostate cancer exist; surgery, radiotherapy and active surveillance.

Radical prostatectomy surgery is the removal of the prostate gland and any surrounding cancerous tissue (35). This is usually a good treatment option for patients whose cancer has not yet spread outside the prostate (stages I and II) (36). It can be achieved by either open surgery or laparoscopic surgery. In open surgery, the surgeon will make a small incision either in the groin (perineal approach) or in the lower abdomen (retropubic approach) (37). The retropubic approach is the most common method for treating prostate cancer; however the recovery time is longer compared with the perineal approach (38).

In laparoscopic surgery, several small incisions are made in the abdomen and a laparoscope is inserted to allow the tumour to be viewed. The surgeon will then remove the prostate by one of the other incisions. Men undergoing laparoscopic surgery will lose less blood compared to open surgery and also have a shorter recovery time (38).

Radical prostatectomy is very effective in the treatment of early stage cancer (39). With the prostate removed and if the cancer has not spread, PSA levels can drop to zero. A recent randomized controlled trial by Bill-Axelson *et al* reported radical prostatectomy was associated with a reduction in the rate of death from prostate cancer, as well as a reduced risk of metastases compared to the watchful waiting or active surveillance group (40). However in some cases the tumour cannot be completely removed and disease can recur. Adverse effects

of radical prostatectomy usually occur within 30 days of surgery and include erectile dysfunction and urinary incontinence. These effects can either be short (resolved within 90 days) or long term (continuing for up to 12 months after surgery). As it is major surgery, additional general surgery risks exist such as blood clots, reactions to anaesthesia, blood loss and infection of the wound (41).

The second treatment option is radiotherapy. In conformal radiotherapy (CRT), the high energy x-rays are carefully shaped to match the shape of the prostate gland, focussing only on the affected area and protecting surrounding tissue (38). Intensity modulated radiotherapy (IMRT) allows radiation to be adjusted around the target to protect adjacent organs (42).

Short term adverse effects of radiotherapy include bowel disturbances and urinary symptoms such as irritative voiding, incontinence and urinary retention (43). Long term erectile dysfunction can often occur for up to two years following surgery (42).

For men affected by small low grade tumours, active surveillance is often a preferable initial treatment option. It involves the close monitoring of patients with the intention of avoiding unnecessary treatment until disease progression occurs or until the patient requests treatment (35). Not all patients are able to live comfortably with an untreated tumour. A major disadvantage of the active surveillance strategy is the presence of an undetected larger or higher grade tumour that might have been missed at the time of biopsy. In terms of adverse effects, patients undergoing active surveillance often develop erectile dysfunction and urinary

obstruction at the same rate as age matched men without prostate cancer in the general population.

Androgen deprivation therapy has been used to treat metastatic prostate cancer for many years (44). Combined hormone treatment and external beam radiotherapy is reported to cause improvements in advanced prostate cancer. In this process, the locally advanced tumour is reduced and metastatic disease in surrounding areas is eradicated (45).

1.2.7 PSA as a diagnostic test

There are certain conditions for a diagnostic test to be successful, there must be a significant burden of the disease, the natural history of the disease must be known, and the test must be accurate and have a positive effect on treatment. As previously mentioned, the incidence for prostate cancer is increasing and therefore it poses a significant burden on public health. In 1994, Whitmore estimated that a 50 year old man with a 25 year life expectancy has a 42% risk of having prostate cancer, a 9.5% risk of having a clinically evident cancer and a 2.9% risk of dying with prostate cancer (46). We can therefore conclude that many more men die with the disease rather than of the disease. This poses the question of whether a testing programme would be useful as more men would be diagnosed, however, not all would benefit from treatment. Asymptomatic tumours are common in men over the age of 60 years, these are slow growing and often do not require treatment. However, fast growing tumours can spread to surrounding tissue very quickly. A testing programme would be most effective for cancers confined to the prostate to begin with, which later metastasise (47). A simple serum PSA test costs around £5, more accurate tests, such as the ratio of free to complexed PSA, can

cost up to £10. A study by Valeri *et al* in 2002 measured serum PSA in relatives of over 400 men with prostate cancer over a period of two years. A PSA of over 0.004 mg/L was detected in 12.4% of men over the age of 50 years. Ten prostate cancer cases were detected and nine of these were localised tumours, (Gleason scores 5 – 7) (48). A further prostate cancer study in Finland identified 103 prostate cancer families. In this sample of 209 first degree unaffected and asymptomatic male relatives over the age of 45 years, an abnormally high PSA result was detected in 10% (21 men). After biopsies, 3.3% of these (7 cases) were found to have prostate cancer (49). A cohort study following 651 men reported that a DRE and PSA test detected clinically suspicious areas in 5%, whereas 10% had a raised PSA but normal prostate cells (50). The three main treatment options for localised prostate cancer are radical prostatectomy, radiation therapy and watchful waiting, where active treatments are used if symptoms develop. Although the active treatments have the potential to cure, there are a number of adverse effects, such as pain, incontinence, impotence and sometimes death (38). However, the adverse effect of watchful waiting is the risk of the cancer progressing and causing death in a short space of time. Further studies are required to determine the optimal testing measure in men with family history of prostate cancer.

1.3 Epidemiology of Prostate Cancer

The epidemiology of prostate cancer has been extensively studied. There is an increasing burden to public health and the benefit of screening has been debated for some time. Neal *et al* concluded PSA is not a suitable test for prostate cancer as most of the criteria are not met (47). However it could be efficiently used to identify the disease in high risk groups, such as men with first degree relatives with prostate cancer.

1.3.1 Incidence

In Europe, 382, 000 new cases of prostate cancer were estimated to occur in 2008, making it the most common male neoplasm after skin cancer. With almost 90, 000 deaths estimated for the year 2008, it was the third most common cause of cancer death in men, after lung and colorectal cancers (51). The same study reported increasing trends in 24 European countries, with the highest incidence rates in Finland, Sweden and The Netherlands; however rates either stabilized or decreased after 2005. In the USA, prostate cancer accounts for 25% of all new cases of cancer diagnosed. It was estimated that 91% of all new cases would be diagnosed at a local stage, with a 5 year relative survival. Mortality rates for the disease have seen an overall decrease since 1990, by about 25% in the USA (52) and some western countries such as UK, France and The Netherlands (51).

1.3.2 Burden

If the incidence rates remain constant, the impact of prostate cancer in the ageing population of the West will increase. There is a clear impact on the financial burden of treatment of patients, which will only escalate with more patients being diagnosed. There will also be a need for more treatment facilities and more trained specialists. Prevention of this type of cancer would relieve a heavy burden on public health, both in terms of cost and resources.

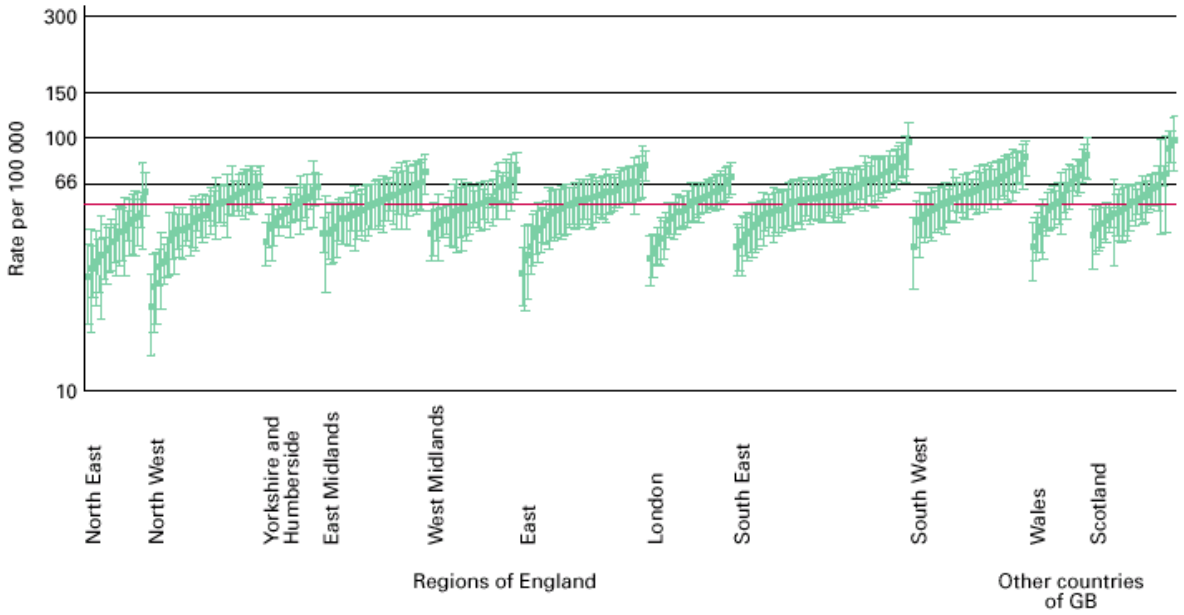
A number of economic evaluations of a testing programme have been conducted, with the estimated cost of a national testing programme for men aged 50-74 years in the USA is between $5.2 billion and $14.1 billion (53), and the UK costs are estimated to be between £500 million and £1.5 billion (38). A study by Chadwick *et al* estimated the cost of testing for

prostate cancer, with the cost of detecting 1 case of prostate cancer, using PSA testing and TRUS, to be £1654.10 (54).

1.3.3 Trends

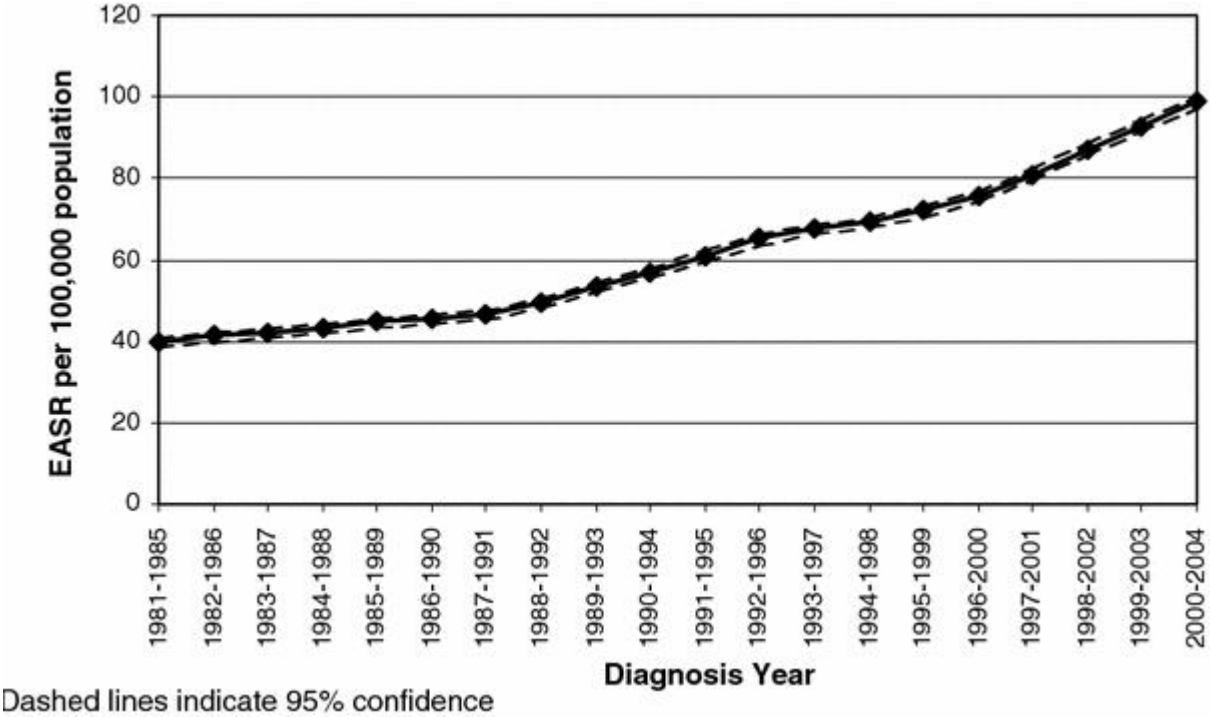
Incidence of prostate cancer has steadily increased since the 1960s worldwide, probably due to better diagnosis methods and a larger ageing population. There were large increases in the 1970s and 1980s in all age groups in England and Wales with a sharp peak in 1994, due to the advent of PSA testing (55). In 2002, a review by Quinn and Babb considered trends within countries and a wide of range of rates were found in Great Britain. The South and South West of England had statistically significantly higher rates of incidence compared to the average incidence for Great Britain. Lower rates were found in the North and North West of England (see Figure 1.2). Prostate cancer was more commonly seen in affluent areas than deprived areas (56). A population based retrospective cohort was recruited in the West Midlands between 1977 and 2004 by the West Midlands Cancer Intelligence Unit (WMCIU). In this cohort, nearly 45, 000 men were diagnosed with prostate cancer. The European age standardized incidence rates (EASR) follow the same pattern as national trends (see Figure 1.3), with a gradually increasing incidence until the early 1990s, after which a slightly sharper rise is observed due to PSA testing (57).

Figure 1.2 Incidence of prostate cancer by local authority within counties of Great Britain and regions of England, 1991–93



Taken from Quinn, M. and P. Babb, *Patterns and trends in prostate cancer incidence, survival, prevalence and mortality. Part II: individual countries.* BJU Int, 2002. **90**(2): p. 174-84.

Figure 1.3 Incidence of prostate cancer in the West Midlands 1977–2004 directly standardized to the European Standard Population



Taken from Cooper, S.C., *et al*., *Patients with prostate cancer are less likely to develop oesophageal adenocarcinoma: could androgens have a role in the aetiology of oesophageal adenocarcinoma?* Cancer Causes Control, 2009. **20**(8): p. 1363-8.

1.4 Risk Factors

In comparison to other common cancers, we know very little on the causes of prostate cancer; only age, race and family history are established risk factors. Previous studies have pointed towards a combination of both genetic and environmental risk factors at play.

1.4.1 Environmental Risk Factors

Age is one of the strongest risk factors for the disease. It is more common in men over the age of 65, representing approximately 85% of all cases diagnosed. It has a much lower incidence in men younger than 50 (less than 0.1% of cases) (58). The introduction of PSA testing in the early nineties caused the incidence of prostate cancer in men aged 50-59 years to rise to 50%, which was a dramatic increase (59). The current knowledge of risk factors possibly associated with the disease is summarized in Table 1.4.

A huge difference in incidence rates of prostate cancer exists in different ethnic groups. The lowest incidence rates are observed in Asian men, namely in India, China and Japan. African American men have the highest rates. In the USA the annual incidence is 272 new cases per 100, 000. It has been previously hypothesized that the higher rates in African Americans are due to social factors, such as poor access to healthcare systems or poor registry of cancers (60). However subsequent studies which have adjusted for such factors have reported consistent results, indicating this is not the case. Migration studies have further reported that Japanese migrants to the USA have a higher risk of the disease compared to their native counterparts, suggesting that there are other unknown environmental risk factors involved.

A number of studies have reported an inverse correlation of prostate cancer mortality and exposure to sunlight (61-62). Ultraviolet radiation (UVR) is a well known causative factor for skin carcinogenesis yet it is also known to decrease the risk of other cancers, such as breast, ovarian and colon cancer (63-65). Although the mechanism is unclear, vitamin D status is likely to play a role, as it is known to inhibit proliferation and promote differentiation of different cell types (66). Vitamin D levels are largely dependent on exposure to sunlight and on diet and supplements to a lesser extent. Pigment characteristics that inhibit vitamin D synthesis are also implicated; dark skin and ability to tan easily have been found to be positively associated with prostate cancer. Some of the known risk factors previously mentioned, such as age and ethnicity, are linked to decreased synthesis of vitamin D (67). There is a vast difference in incidence rates among different ethnic groups. Asia has the lowest rates, especially China, Japan and India and African American men have the highest rates (60). Migrant studies have observed men migrating to Western countries have increased risks compared to their native counterparts, implicating environmental factors, such as UVR exposure, in disease progression.

In 2001, Luscombe *et al* reported a link between increased UVR exposure and reduced risk of advanced stage prostate tumour. This was subsequently replicated by Rukin *et al* in 2007 (68-69). A common weakness of studying an exposure such as UVR is small sample sizes of each exposure category and therefore lack sufficient power to confirm associations. It can be difficult to accurately measure UVR as an exposure; the data are often subject to recall bias when subjects are recruited after diagnosis. There are also few data available on the effect of UVR on stage of disease.

In this thesis, the effect of UVR exposure on prostate cancer risk is assessed. The hypothesis that increased UVR exposure levels are associated with decreased risk of prostate cancer is tested and whether this association is stronger among cases with advanced disease. Instead of the traditional use of prostate cancer cases and population controls, two case groups and two control groups are used. The analysis of between and within group differences allow better understanding of how PSA can be used as a marker for prostate cancer, and whether there is a difference between the use of hospital based controls or the traditional population based controls.

In 2007, the Second Expert Report by the World Cancer Research Fund/American Institute for Cancer Research made no formal judgement for an association between physical activity and prostate cancer overall. It did however, "note" the evidence from two cohort studies (70-71) and suggested that physical activity was associated with a decreased risk of advanced/aggressive prostate cancer (72). This report concluded that physical activity which "promotes a healthy weight would most likely protect against cancers whose risk is increased by weight gain or obesity" (72). A national health and nutrition survey in 2008 was unable to detect a significant difference in prostate specific antigen (PSA) level between men of different levels of physical activity (73). This was consistent with previous studies (74). However a study by Parekh *et al* only reported physical activity levels in participants for the past 30 days. Long term physical activity exposure was not taken into account, which could have a different effect.

Previous research has been able to identify the benefits of physical activity on colon and breast cancer, however, the effect on prostate cancer remains unclear. Earlier studies have produced inconsistent results, with a third of all epidemiological studies observing a protective effect (75-76), others finding no relation (77-81) and one study reporting a positive effect (82). Friedenreich *et al* (83) estimated that the population attributable risk of prostate cancer due to insufficient physical activity by men in Great Britain is 14%.

Apart from regulating the body's energy balance, other potential explanatory mechanisms for a possible association between physical activity and prostate cancer risk include a modifying effect on circulating hormones e.g. testosterone and insulin, reduced inflammation (IL-6) and increased production of superoxide dismutase which protects against oxidative stress (72). Previous epidemiological studies have not been able to control for testosterone levels in their physical activity analysis. With the addition of testosterone data for patients, it might be possible to define a biological mechanism for the associations observed. Also, the method of assessment for physical activity is difficult and previous studies have employed different methods, which could explain the inconsistencies. Epidemiological studies vary in the period of assessment (childhood, adulthood, and lifetime) or frequency, duration and intensity of exposure.

In this thesis, the association of physical activity and prostate cancer is further examined. Using the four distinct comparison groups allows the effect of different types of physical activity at different stages of disease to be considered. The control groups have no prostate symptoms and normal PSA levels, the high PSA groups which could be considered as an

intermediate group for disease and finally the prostate cancer group with confirmed disease. Data on different forms of exercise were used and the duration of exposure was considered and adjusted for testosterone levels. Associations with prostate cancer and its subtypes including advanced and localised disease were examined.

Obesity is a growing epidemic, particularly in the Western world. The USA report some of the highest rates in the world, with over two thirds of adults falling in the category of obese or overweight (84-86). A study by Wang and Beydoun in 2007 predicted that by the year 2015, 75% of adults in the USA will be overweight and over 40% will be obese (87). Another global trend is ageing; people are living longer than ever before. The Office of National Statistics reports that the percentage of people aged 65 and over in the UK has increased from 15% in 1985 to 17% in 2010, with an increase of 1.7 million people (88). If this trend continues, by 2035, the percentage is estimated to be 23% of the population. If the obesity epidemic and ageing trend continue, the Western world is likely to see a huge increase in the incidence of prostate cancer which will further intensify the current burden on public health. Obesity is a strong risk factor for a number of cancers, including cancer of the endometrium, kidney, breast and colon (89-91). The link between obesity and prostate cancer has been extensively researched in recent years; however the reported associations are inconsistent. Gong *et al* compared different body mass indexes (BMIs) and found men with a BMI above 30kg/m^2 had an 18% lower risk of low grade prostate cancer but at the same time had a 29% higher risk of high grade prostate cancer (92). In 2006, a meta analysis of 22 epidemiological studies reported a weak but statistically significant increased risk. After stratifying for severity of tumour, only advanced prostate disease had an association with obesity and not with localised

disease (93). Further studies have replicated the link of obesity with high grade tumours (92, 94-95). Following these studies, it is generally believed that obesity can reduce the risk of being diagnosed with non aggressive tumours while also increasing the risk of developing aggressive tumours (96). A plausible explanation for this is the difference in testosterone levels. Obesity has been linked to lower serum androgen activity, which is due to lower concentrations of circulating total testosterone and higher concentrations of oestrogen. All these factors are known to decrease the risk of prostate cancer (97-99). However, obesity is also linked to higher insulin and free insulin-like growth factor-I and lower sex hormone binding globulin concentrations, which may increase risk (99-100).

A particularly important study by Calle *et al* found the risk of mortality from prostate cancer significantly increased with increasing BMI (98). However other studies have produced inconsistent results, with some reporting increased risks associated with the disease (80, 101-104) and few observe a protective effect (95). BMI is often used as a measure of obesity, as it is an easy measure to collect; only height and weight parameters are required, however it does have limitations (105). Large muscular individuals with little body fat cannot be classified accurately (96). Also, different fat distributions, such as visceral and superficial fat, cannot be distinguished. The distributions of fat can be important in biological mechanisms, such as lipolysis especially in older age (106). Weight very rarely stays stable over time and since prostate cancer has very strong associations with older age, weight over lifetime and change at different life periods could be a very important factor. To date, very little research has been carried out in the area of weight change over time and the disease. Also, most previous studies investigating the association have focused on obesity at the time of diagnosis, and not taken

into account a history of obesity. Of the few that have, most report contrasting risks (107-109).

Some studies have also investigated the effect of obesity on PSA, reporting lower PSA levels in obese men; however such studies are scarce (98-99, 110). A recent study by Werny *et al* in 2011 investigated the association of adiposity with PSA, using BMI and waist circumference as measures of adiposity (111). The results were consistent with previous studies, with negative trends between PSA and the adiposity measures (105, 110). Lower PSA values in obese men could have an impact of the sensitivity of the test when used for diagnosis, as tumours in such men may go undetected leading to poorer prognosis (96). More research on the link between PSA and obesity is required to fully understand the interaction.

In this thesis, the association of obesity and prostate cancer is further examined to disentangle the effect of this factor on the disease. Waist circumference, BMI and weight change over time were used as measures of obesity. Associations with total prostate cancer and its subtypes including advanced and localised disease were examined.

Occupational factors are also believed to play a role in prostate carcinogenesis. A number of studies have investigated occupational exposures to chemical agents, many of which have suggested cadmium, poly aromatic hydrocarbons and pesticides to be responsible for increased risk (112-114). Previous epidemiological studies have reported increased risks for metal workers (115-116) and mechanics (117-118). Other occupations suggested to have increased risks include chemical (119) (118) and rubber industry workers (120). These

industries encompass a wide range of job titles and activities, therefore small sample sizes of each individual job title result in inconsistent findings.

Farming is an important occupational group however extensive association studies investigating the potential risks have reported inconsistent results. A number of meta analyses have found statistically significant elevated risks among farmers (121-123), whereas others have reported no risks or sometimes reduced risks (124-125). Chemical agents used in farming differ greatly depending on the type of farming that is carried out, however no study has yet investigated differences in risk between livestock and crop farming. The main agents thought to affect risk are pesticides and herbicides, which contain organochlorines. Some studies have suggested these compounds can increase or mimic the action of androgenic hormones, however this remains unproven (126).

Parent and Siemiatycki's review on occupation and prostate cancer focused on a few occupations and related exposures and concluded that although some strong evidence exists for the farming, mining, metal and rubber industries, no definitive conclusions could be drawn (127). In 1982, Dost *et al* initiated a large scale cohort of over 8, 000 rubber factory workers to investigate new health problems relating to exposures in this industry. The study was able to show that overall mortality for all cancers was better than the national average, suggesting working conditions in this industry have improved and had a direct effect on health of the workers within it (128). In this thesis, occupations relating to known exposures are investigated. All reported occupations are considered rather than just those related to farming

or rubber or chemical workers. Also, some newer articles, published after Parent and Siemiatycki's review were included for an up to date analysis.

Many previous studies have had inconclusive findings, probably due to small sample sizes for each occupational group. No comprehensive meta-analysis has been conducted to quantify the risks of prostate cancer associated with individual occupations. With a meta-analysis, the unreliability caused by small numbers of cases and controls within each occupational group can be reduced.

A number of epidemiological studies have implicated diet as a potential risk factor, specifically high intake of fat, meat and dairy products. This is consistent with the high incidence rates in Western countries where such diets are common. Although fruit and vegetable intake have not been strongly associated with the disease, a protective effect has been detected for cooked tomatoes. Tomatoes contain high levels of lycopene, a lipophilic carotenoid. It is unclear whether the protective effect is due to lycopene or its metabolic products. It can reverse the effects of the dihydrotestosterone hormone and can induce apoptosis of human prostate cancer cells (129). Studies have investigated high consumption of red meat and found a high risk of disease. It has been suggested that cooking the meat at high temperatures increases the level of carcinogenic substances. It is also thought that such diets are often poor in fruit and vegetable consumption, some of which may protect against cancer. Selenium is a trace mineral found in grains, meat, poultry, fish, eggs and dairy products (130). In the selenomethionine form, it inhibits proliferation and induces cell cycle arrest in prostate cancer cells (131). A number of studies have investigated the association of selenium and

prostate cancer. Some have reported a decreased risk of the disease in men with high levels of selenium (132-133), however, a recent clinical trial has been unable to confirm these (134). It has since been hypothesized that different forms of selenium vary in their biologic effects and may only be beneficial for patients with particular genotypes for the *SOD2* (superoxide dismutase) gene (135). There are inconsistent results for the association of alcohol and prostate cancer. Some studies have found an increased risk in men consuming three or more alcoholic drinks per day yet some have found no increase in risk. Conversely Schoonen *et al* found a protective effect with consumption of 1-3 glasses of wine per week (136).

No clear association exists between smoking and prostate cancer. Some studies have reported smoking to be moderately associated with mortality from the disease. A possible mechanism is the exposure to cadmium. Cadmium is known to increase oxidative stress and cause an increase in androgen levels, which are thought to be mechanisms that promote prostate carcinogenesis. Androgens are responsible for the development and maintenance of the prostate. The two principal androgens in adult men are testosterone and dihydrotestosterone. Androgen deprivation is known to decrease PSA levels and cause apoptosis of prostate cancer cells. Although a number of studies have investigated serum androgens in prostate cancer, the results have been inconsistent. Despite this however, it is generally believed that high levels of testosterone are associated with an increased risk of prostate cancer.

Table 1.4 Possible Risk Factors for Prostate Cancer

Positive Association	**Negative Association**
Red meat consumption	Tomatoes/lycopenes
Smoking	High intake of fruit and vegetables
Farming	Selenium
Exposure to cadmium, polyaromatic hydrocarbons and pesticides	UV exposure
	Physical activity

1.4.2 Genetic Factors

The familial clustering of the disease suggests either an X-linked or recessive mode of inheritance. Twin studies further support this idea, where heritability of the disease is estimated to be between 50 – 60% (137-138). One of the most investigated genes is the androgen receptor gene, known to be implicated in human prostate cancer, as prostate tumours do not occur in dogs or humans castrated before puberty (139). Early studies found that the length of the CAG repeat region is inversely related to its function (140-141). However in 2004, Zeegers *et al* showed that although shorter repeats appeared in prostate cancer cases and were moderately associated with the disease, the absolute difference in number of repeats in cases and controls was less than one (142).

The advent of genome wide association studies have brought to light a number of loci which may harbour prostate cancer susceptibility genes. However the relative importance of variants located within them is questionable. A genomewide scan of 91 prostate cancer families detected a susceptibility locus at chromosome 1q24, now known as Hereditary Prostate Cancer 1 or *HPC1* (143). Further studies have revealed this locus to be linked to younger age and more advanced stage at diagnosis and a higher grade of tumour. It has also been suggested that the *RNASEL* gene on 1q25 might be responsible for the apparent effects. A meta-analysis by Li and Tai in 2006 investigated ten case control studies on *RNASEL* variants E265X, R462Q and D541E and only detected the D541E allele to be associated with the disease; however the effect size was small (144).

Epidemiological studies on family history report a higher risk of prostate cancer in men with an affected brother compared to men with an affected father. These findings point to a potential prostate cancer susceptibility locus on the X chromosome. A study by Xu *et al* resulted in a prostate cancer susceptibility locus being mapped on the X chromosome, known as Hereditary Prostate Cancer X or *HPX*, which accounted for 16% of the disease among the families studied (145).

The recent surge of genome wide association studies (GWAS) has identified a number of loci which may harbour prostate cancer susceptibility genes, however the relative importance of variants located within the regions is uncertain. A number of previous studies have implicated a region on chromosome 8q24 in prostate cancer. In particular, the variant allele -8 of the microsatellite DG8S737 has been shown to have a strong positive signal (OR, 1.79; $P = 3.0 \times 10^{-6}$) (146). This was replicated in Icelandic men (OR, 1.72; $P = 1.8 \times 10^{-3}$) and among Swedish and European American men. Within the same haplotype block, the SNP rs1447295 has also been distinguished; allele A was significantly associated with prostate cancer (OR, 1.72; $P = 1.7 \times 10^{-9}$) (146). This study was replicated in 2007 by Suuriniemi *et al* in European men aged 40-64 years. Although they confirmed the association for the rs1447295-A allele, the results for DG8S737-8 could not be replicated (147). An interesting result from this study was the significant association of the DG8S737-10 allele with high grade tumours. Two further genome wide association studies have also confirmed the association of rs1447295-A allele and prostate cancer risk (148-149). Since few of the studies have stratified by tumour stage, the variability in results could be due to tumour stage heterogeneity, or perhaps genetic heterogeneity within and between populations.

Genome wide association studies are prone to type 1 errors and therefore confirmation of all significant results is required. In this thesis, the association of the rs1447295-A allele, the DG8S737-8 and DG8S737-10 alleles are further examined in a population based sample of Dutch men. The Dutch cohort is potentially a very interesting one as mutations specific for this population have previously been identified, including some predisposing for hereditary breast-ovarian cancer and malignant melanoma (150). In the same study, Zeegers *et al* also detected short chromosomal regions that have remained identical by descent, resulting in relatively limited genetic heterogeneity, which could therefore increase power to discover associations among the Dutch.

It has been hypothesized that other genes involved in testosterone response might play a role, such as genes in the polyamine pathway. Polyamines are present in all mammalian cells and are essential for normal cell growth and differentiation and also play a role in cell death (151). There are three different forms; putrescine, spermidine and spermine, and the prostate has the highest polyamine concentration of any tissue. The polyamines in the prostate are controlled by androgens and evidence is accumulating that the polyamine system is responsible for all final decisions on cell growth, survival and differentiation of cells and eventually death (152). The enzymes involved in polyamine biosynthesis, ornithine decarboxylase (*ODC*), S-adenosylmethionine decarboxylase (*SAMDC*) and spermidine synthase (*SDS*) are also induced by androgens (153-154). Studies of *ODC* activity show that when it is inhibited with difluoromethylornithine, the prostate acini decrease in size (155).

If the rate of polyamine biosynthesis is increased, causing higher levels of putrescine and spermidine in particular, prostate cells will proliferate (156). Prostatic spermine does not promote growth, however it can induce a differentiated prostatic epithelium (157). Other studies have reported inhibited growth of prostate tumours when spermine levels are high (158). In 2002, Rhodes *et al* conducted a meta analysis to identify candidate genes for prostate cancer. Genes involved in the pathway were consistently dysregulated in prostate cancer. The enzymes directing synthesis, such as ODC, SDS, aspartate transaminase (GOT2) and aminoacyclase (ACY1) were over expressed and ornithine aminotransferase (OAT) was under expressed. The overall effect is that polyamines are synthesized at a higher rate, which in turn causes cancer cell proliferation (159).

1.5 Research Questions

Despite the extensive research on risk factors of prostate cancer, only a few have been confirmed. The controversy behind PSA continues as the opinions of it as a suitable tumour marker remain mixed. For this PhD, the Birmingham Prostatic Neoplasms Association Study (BiPAS) was initiated to establish the effect of a wide range of environmental and genetic factors on PSA levels and prostate cancer risk.

The classic case control design for prostate cancer has studied prostate cancer cases and healthy controls from the population. Here four groups were recruited; two types of cases (prostate cancer cases and high PSA cases) and two types of controls (clinical controls and hospital controls). All groups were compared to investigate the effect of risk factors on disease progression and whether PSA is a good marker for prostate cancer.

The only established risk factors are age, ethnicity and family history. The other possible modifiable environmental factors are summarized in Table 1.4. Although information was collected for all of these factors, only some could be investigated for the purpose of this work. The research questions for this thesis therefore are:

1. Is ultraviolet radiation associated with prostate cancer?
2. Is physical activity associated with prostate cancer?
3. Is body mass associated with prostate cancer?
4. Is occupation associated with prostate cancer?
5. Are polymorphisms on chromosome 8q24 associated with prostate cancer?

METHODS

CHAPTER 2 : THE BIRMINGHAM PROSTATIC NEOPLASMS ASSOCIATION STUDY (BiPAS)

2.1 Rationale

Although a large part of the causal pathway to prostate cancer is unknown, a number of epidemiological studies have indicated that both genetic and environmental components exist (138). Previous epidemiological studies have either been subject to bias or obtained inconclusive results due to small sample sizes of exposures. Very few prostate cancer susceptibility genes have been identified to date. The *HPC1* gene is a potential candidate, as is Hereditary Prostate Cancer X or *HPX* and more recently the chromosomal 8q24 region (143, 145, 160). Other possibilities include the vitamin D receptor, polyamine pathway and androgen receptor (142, 152). It has been suggested that risk of disease may be due a number of genes, each conferring a small individual risk.

The Birmingham Prostatic Neoplasms Association Study (BiPAS) was established in order to accumulate a large database for use in prostate cancer research, allowing confirmation of the roles of a number of environmental risk factors. Information on these factors was obtained by means of a questionnaire, which is further discussed in Section 2.5. A second aim was to establish a DNA bank with linked clinical and epidemiological data that can be of use to other collaborators

In this thesis, a case control design was used to investigate risk factors. In this type of study, disease subjects, known as “cases” are compared to non disease subjects, or “controls”. Information on exposure variables that may be implicated in the disease of interest is collected and significant differences are identified. Case control studies are used for uncommon

diseases which require a smaller sample size compared to cohort studies, which follow subjects over a long period of time.

In contrast to previous studies, participants were selected from a number of different groups; prostate cancer patients, patients with high prostate specific antigen (PSA) levels but no clinical symptoms of prostate cancer, hospital based controls and population based controls where patients have no symptoms of prostate disease. By comparing the four groups among each other, the aetiology of prostate cancer can be disentangled and the effect of these factors on PSA levels can be studied.

2.2 Inclusion and Exclusion Criteria

Eligibility criteria were men who were 50 years of age and over and able to provide informed consent. Prostate cancer was defined as ICD-10 code C61, malignant neoplasm of the prostate (31). The study excluded vulnerable groups, such as adults with learning disabilities, those who were unconscious or severely ill, adults with dementia or with other psychological disorders. Men with poor English skills were not automatically excluded; they were recruited with help of interpreter services where possible.

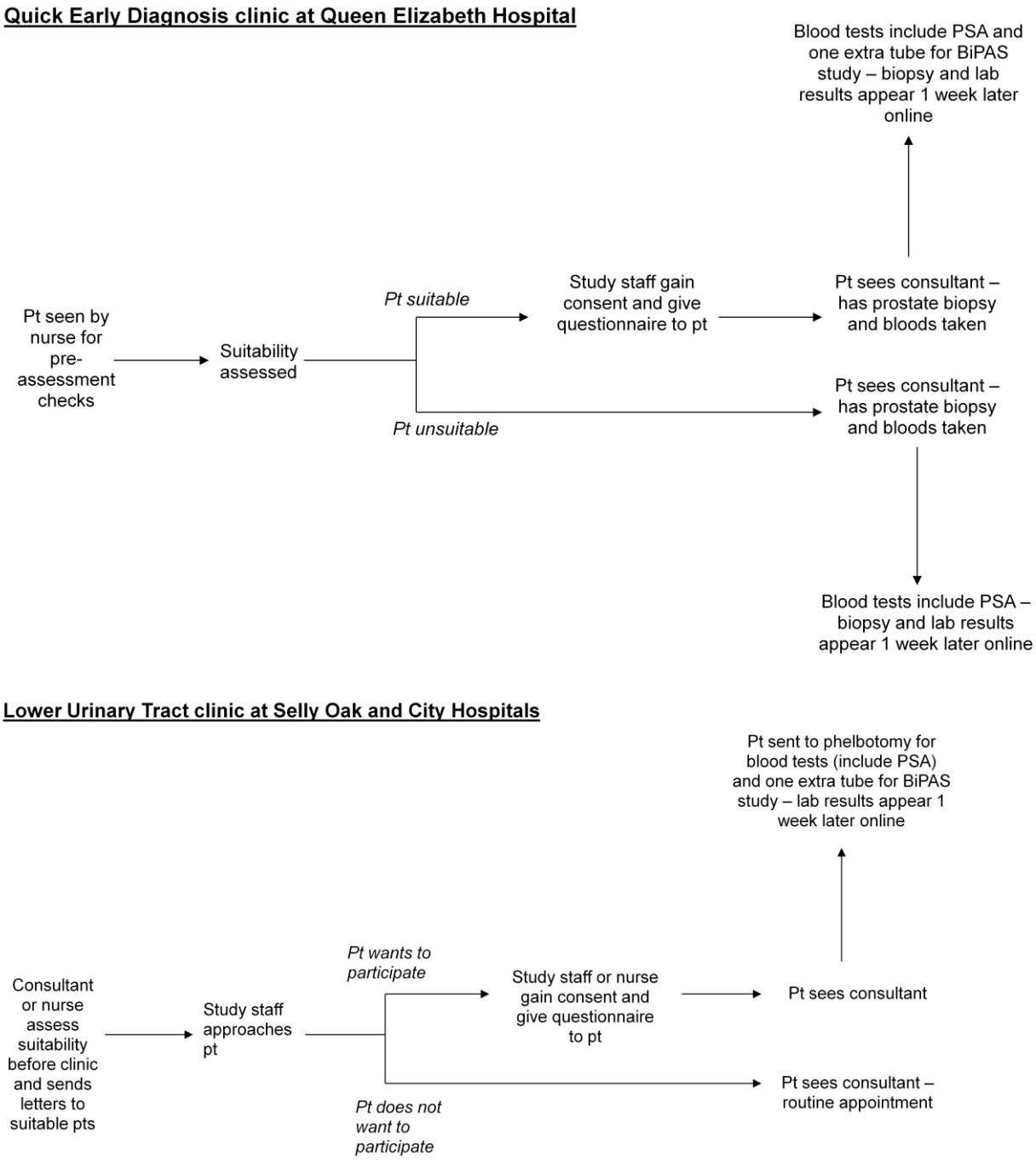
2.3 Recruitment

The study recruited eligible men living in Birmingham (UK) attending clinics. Patients with suspected prostate abnormalities and/or high serum PSA levels are routinely referred to the Urology Department at The Queen Elizabeth Hospital, Birmingham. After a repeat PSA test,

prostate biopsies are carried out where appropriate. All patients were recruited between March 2007 and October 2010.

The main recruitment strategy can be found in Figure 2.1. Recruitment strategies were adapted depending on the recruitment centre. For the Quick Early Diagnosis (QED) clinic at the Queen Elizabeth Hospital, the recruitment responsibility was divided between the clinic nurse and a member of the BiPAS team. Study information sheets were posted to all subjects attending biopsy and urodynamics clinics, along with their appointment letters and biopsy information booklets, two weeks before their attendance in accordance to Centre of Research Ethical Campaign (COREC) guidelines. The document explained the purpose of the study and the requirements, risks of participating and a complaints procedure. After the patient's arrival at the clinic, a pre-assessment with the nurse was carried out. Unsuitable or ineligible patients would continue with a routine appointment. If the nurse or BiPAS researcher concluded that the eligibility criteria had been met, informed consent was obtained and the questionnaire was completed independently by the participant. The patient would then see the consultant for biopsy and an extra blood sample was obtained for the study. Patients with histologically confirmed adenocarcinoma of the prostate formed the prostate cancer case group. High PSA cases were defined as patients with a high repeat PSA and negative or no biopsy and were identified in both the prostate and lower urinary tract symptoms (LUTS) clinics.

Figure 2.1 Recruitment strategies for BiPAS



Pt: patient *PSA: prostate specific antigen*

BiPAS: Birmingham Prostatic Neoplasms Association Study

Hospital control patients were recruited through urodynamics clinics at the Queen Elizabeth Hospital, Selly Oak Hospital and City Hospital, Birmingham. These recruitment centres see a vast number of patients, most of whom would be ineligible therefore a blanket mailing of the information sheet would be unsuitable. For these subjects, the consultant or nurse would assess the suitability of patients prior to the clinic and send out information letters to eligible subjects only.

Population controls were invited to take part by their general practitioner (GP) surgeries. A computer search was carried out at four GP practices for men aged 50 years or over with no known prostate cancer symptoms. After suitability was assessed by the GP, invitation letters were sent out to a random sample of patients and home visits or clinic appointments were arranged for positive replies. No genetic sample was obtained for this group. The recruitment target for this group was 160 patients (40 patients per practice), however, a high response rate meant the target was exceeded (45 patients were recruited at Quinton Medical Practice, 44 patients at The Old Priory, 44 patients at Quinborne and 42 patients at Grange Hill).

All recruitment documents given to the patients received favourable ethical opinion by the South Staffordshire Ethics Committee. The study was conducted in accordance with the principles of the International Conference of Humanisation guidelines on Good Clinical Practice (ICH-GCP). The study protocol containing the questionnaire, study information sheet and consent form can be found in Appendices 1 – 3.

2.4 Data collection and handling

The environmental data were collected in the form of a questionnaire and DNA formed the genetic sample. The blood sample for each patient was obtained by either a doctor or a phlebotomist using standard venipuncture methods. The blood was contained in a 5ml tube containing EDTA and stored at 2^{o}C for the duration of the clinic. The samples were then transported in a cool bag with cool packs to the BiPAS freezer and stored at -20^{o}C until DNA extraction took place. DNA was extracted using QIAGEN maxi blood kit. For population controls, a blood sample was obtained for PSA testing and transported to Selly Oak Hospital. For all participants, the Roche E170 assay was used to determine PSA level. For population controls with a raised PSA, a standard operating procedure (SOP) was developed to inform the general practitioner for further management.

The genetic data from BiPAS have contributed towards the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium. DNA samples for prostate cancer cases and hospital based controls were sent to the consortium for a follow up of a previous genome wide association study, which led to a publication in Nature Genetics (161). Selected SNPs were investigated in 3, 650 cases and 3, 940 controls. All the previously identified loci were confirmed and an additional seven new prostate cancer susceptibility loci on chromosomes 8, 4, 8, 11 and 22 were identified (161-162).

2.5 Measurement of environmental exposures

The questionnaire was divided into various sections investigating different environmental factors. The first section related to background information, such as address, marital status, ethnicity, educational qualifications received and past history of cancer. Section two asked of occupational history, starting with most recent and all other past jobs. Subjects were asked to record their occupational activities, duration of employment, whether it was shift work and if the job involved indoor or outdoor work (Figure 2.2). Section three was dietary information, with 39 different food items classified into a number of categories; staple foods (bread, potatoes, pasta etc...), meat products, fish, vegetables, fruit, dairy products, foods of interest (fast food, restaurant etc...) and other foods (pulses, nuts and seeds etc...). A similar approach was used for fluid intake, with categories including alcoholic drinks, hot and cold drinks. For each product, patients were asked to indicate how frequently it was consumed over the last 12 months. The options were "never or less than once a month", "1-3 times per month", "once a week", "2-4 times per week", "5-6 times per week" and "at least once a day". A small section of the table used to record dietary intake can be found in Figure 2.3. Section four was ultraviolet (UV) light exposure; patients were asked to describe their skin type, hair and eye colour. They also recorded average times spent in the sun and different life periods (age 60 onwards, age 40-59 and 20-39 years) during the weekdays and weekends (Figure 2.4). Section five was based on physical activity; patients were asked how often they do walking, cycling, gardening, housework, competitive and non competitive sport at different periods in life. Tobacco use was investigated in section six, patients were asked the maximum number they currently or used to smoke and duration of smoking. Section seven investigated body dimensions at current age and at younger ages (Figure 2.5). Section eight was based on family

history of cancer, including details of parents, siblings and children (Figure 2.6). The final section was on testosterone levels, based on the androgen deficiency in ageing males (ADAM) scale (163). There were ten questions within this section relating to different androgen related parameters (Figure 2.7).

The data were entered into a password protected Microsoft Access database and later converted to a STATA data file. The paper questionnaires were electronically scanned and archived according to the original ethics application.

Figure 2.2 Table to record occupational history for BiPAS participants

Job Title	Time period that you worked there		Description of Activities	Name of Organisation	Location (Town/City)	Type of Business	Mainly indoor activity	Mainly outdoor activity	Shift work
	From (y)	To (y)					**Please tick one**		
EXAMPLE: DRIVER	1972	1996	HGV DRIVER	JO BLOGGS BUILDERS	BIRMINGHAM	BUILDING CONTRACTORS	√		YES

Figure 2.3 Table to record dietary intake for BiPAS participants

	Average Use Last Year					
	Never or less than once per month	1-3 per month	Once a week	2-4 per week	5-6 per week	At least once per day
STAPLE FOODS						
Bread ……	☐	☐	☐	☐	☐	☐
Potatoes…….	☐	☐	☐	☐	☐	☐
Pasta…… (eg. Macaroni, Spaghetti)	☐	☐	☐	☐	☐	☐
Rice ……	☐	☐	☐	☐	☐	☐
Noodles……	☐	☐	☐	☐	☐	☐
Wheat ……. (eg. Whole grain bread)	☐	☐	☐	☐	☐	☐
Cereal…….. (eg. Oats, bran, corn)	☐	☐	☐	☐	☐	☐

Figure 2.4 Table to record UVR exposure for BiPAS participants

On average how many **hours per day** did you spend outdoors during early adult life (**20-39**)?

Weekdays	never	☐	**Weekends**	never	☐
	0 – 4 hours	☐		0 – 4 hours	☐
	5 – 9 hours	☐		5 – 9 hours	☐
	10 – 14 hours	☐		10 – 14 hours	☐
	More than 15 hours	☐		More than 15 hours	☐

Figure 2.5 Table to record body dimensions for BiPAS participants

Please answer the following questions using **either** metric (cm, kg) or imperial (feet, inches, stones, pounds) measurements

What is your current height (in bare feet)?	feet	inches	***Or***	cm
What is your current weight (in light clothing)?	stone	pounds	***Or***	kg
How much did you weigh at age 20?	stone	pounds	***Or***	kg
How much did you weigh at age 30?	stone	pounds	***Or***	kg
How much did you weigh at age 40?	stone	pounds	***Or***	kg
What is your current waist size?		inches	***Or***	cm

Figure 2.6 Table to record family history of cancer for BiPAS participants

SIBLING'S MEDICAL HISTORY

We would now like you to provide some more information about your **brothers** and **sisters** including any **medical history of cancer**. You **DO NOT** need to tell us about any **adopted** or **step-relations.**

	Gender *(Please tick)*		Year of Birth	Have they ever had cancer? *(Please tick)*		If Yes, which type of cancer?	Age at which cancer was diagnosed (if known)
	Male	Female		Yes	No		
1							
2							
3							
4							
5							
6							

2.6 Assessment of Exposures

A validated UVR exposure section recorded the amount of hours patients spent in the sun during different time periods (61). Life course was split into three different periods; early adult life (20-39 years) and middle age (40-60 years) and late adult life (60+ years). This allowed for the wide range of age at diagnosis for both cancer and control groups. Time spent outside was calculated by summing the weighted numbers of hours spent outdoors during weekdays and weekends (weights: never = 0, 0 – 4 hours/day = 1, 5 – 9 hours/day = 2, 10 – 14 hours/day = 3). Missing answers were removed from the dataset. The distribution of this score was then further classified into tertiles based upon the distribution of the total control population: "low", "medium" and "high" UVR exposure. A separate score for lifetime sun exposure was derived by summing all three life periods using the same method. Further detail on the number of times men were sun burnt as children, how often they use sun beds per year and if they have ever lived abroad in a sunny country were also analyzed. Subjects were also asked for pigmentation information (skin reaction to the sun and hair colour) and behaviours which affect UVR exposure such as details of sun protection used.

The Physical Activity questions were based on the standardized questions from the short version of the International Physical Activity Questionnaire (IPAQ) (164). The IPAQ is a recall assessment method designed by physical activity experts in 2000 (165). The reliability of the test was reported to be $p = 0.76$ (166). Participants were asked questions on different types of physical activities e.g. mode of transport (walking, cycling) household activity (housework, gardening) and recreational activity (non-

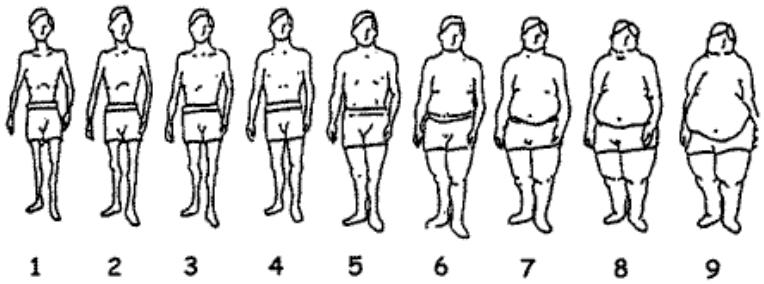
competitive/social and competitive sport). Levels of physical activities were reported for three distinct periods in life, (12-19 years, 20-39 years and from the age of 40 to diagnosis/entry in the study). Participants were asked to indicate the level of physical activity in terms of hours per week they undertook each of the various activities at each age-group according to five predefined categories: never, 0-4 hours, 5-9 hours, 10-14 hours and more than 15 hours per week. The main focus of the analyses was the level of physical activity since the age of 40. As the average age for men in all groups were mid 60s, this covered approximately 25 years of physical activity behaviour. Testosterone summary scores were obtained using the androgen deficiency in ageing males (ADAM) scale (163). There were ten questions within this section (see Figure 2.7) on different androgen related parameters. A positive result was defined as an affirmative answer for question 1 or 7, or for any other 3 questions.

Figure 2.7 Androgen deficiencies in ageing males (ADAM) scale

1.	Do you have a decrease in libido (sex drive)?	○ Yes ○ No
2.	Do you have a lack of energy?	○ Yes ○ No
3.	Do you have a decrease in strength and/or endurance?	○ Yes ○ No
4.	Have you lost height?	○ Yes ○ No
5.	Have you noticed a decreased "enjoyment of life"	○ Yes ○ No
6.	Are you sad and/or grumpy?	○ Yes ○ No
7.	Are your erections less strong?	○ Yes ○ No
8.	Have you noticed a recent deterioration in your ability to play sports?	○ Yes ○ No
9.	Are you falling asleep after dinner?	○ Yes ○ No
10.	Has there been a recent deterioration in your work performance?	○ Yes ○ No

Obesity was measured as BMI using self reported measures of height and weight at diagnosis. The anthropometry section included questions on body size at time of diagnosis and at different periods in life, including questions regarding current height in feet/inches or cm, current weight in stones/pounds or kilograms and current waist circumference in inches or cm. Weight in stones/pounds or kilograms at age 20, 30 and 40 years was also obtained. Finally, patients were given a pictogram (Figure 2.8) with 9 different options and asked to mark the one they thought corresponded to their body size at ages 20, 30, 40 and their current age. These figures were obtained from the standardized visual stimuli set designed by Bulik and colleagues (167). Since BMI is questionable as a measure of obesity, it could be that self perceived body size could be a better measure for fat distribution. The figures take into account different central fat distributions, which is common in men (167). All reported weights were converted from stones and pounds into kg and height was converted from feet and inches into metres. BMI was derived from weight and height measurements at time of diagnosis as kg/m^2.

Figure 2.8 Pictogram for self perceived body size



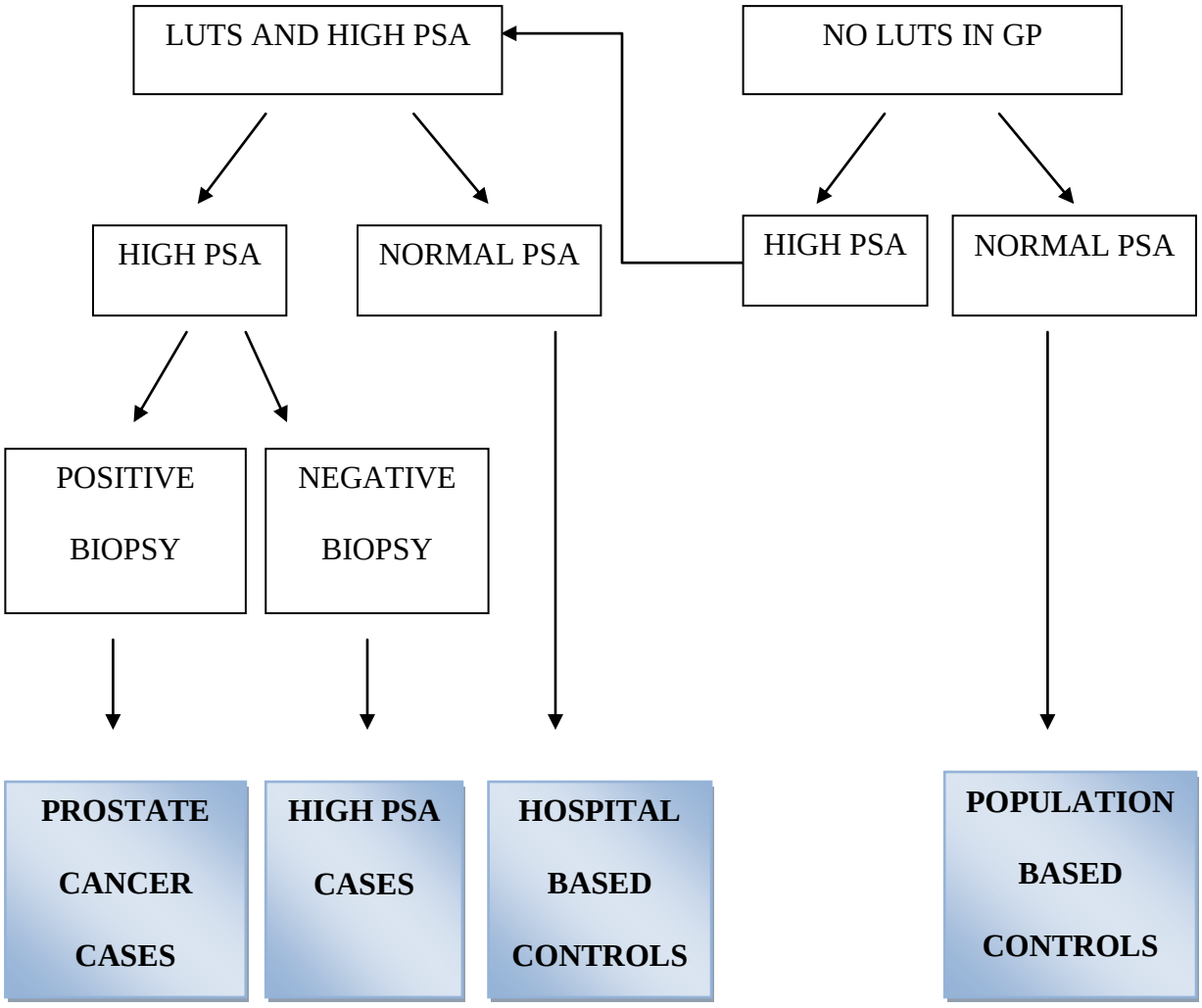
At around age 20	○	○	○	○	○	○	○	○	○
At around age 30	○	○	○	○	○	○	○	○	○
At around age 40	○	○	○	○	○	○	○	○	○
Now	○	○	○	○	○	○	○	○	○

2.7 Statistical Analysis

Statistical Analysis for Chapter 3 – 5: BiPAS

The Birmingham Prostatic Neoplasms Association Study recruited 314 prostate cancer cases, 381 cases with high PSA but no prostate cancer, 201 hospital based controls and 175 population based controls between 2007 and 2010. For the analysis, men were categorized into two different case groups and two control groups. The case groups consisted of biopsy confirmed prostate cancer cases and other patients with high PSA, in which cancer could not be confirmed (high PSA cases). The control group also had two further groups; hospital based controls and population based controls (see figure 2.9). The different cases and control groups allowed comparison of PSA level, suggesting differing disease stage rather than the traditional disease vs. non disease approach.

Figure 2.9 Case Control Strategies



LUTS: Lower urinary tract symptoms *PSA: Prostate specific antigen*

For each analysis chapter, the following comparisons were analyzed, however not all tables are presented:

1. Combined cases vs. hospital based controls
2. Combined cases vs. population based controls
3. Combined controls vs. prostate cancer cases
4. Combined controls vs. high PSA cases
5. Combined cases vs. combined controls

All additional analyses can be found in appendices 4 – 7. Odds ratios were used to measure the association between exposure variables and prostate disease. This is the ratio of the odds of exposure in cases and odds of the exposure in controls. If an odds ratio is above 1, it is said that the variable is more likely to be exposed to cases compared to controls. Since the case control design only includes a sample of the population, the odds ratios are only estimates of association; 95% confidence intervals are therefore calculated for accuracy. These indicate the limits in which the true value of association will lie. A confidence interval that does not include the value 1.00 is considered statistically significant. Logistic regression was used to calculate odds ratios and corresponding 95% confidence intervals for all associations. All hypothesis tests were two-sided and p-values less than 0.05 were considered to be statistically significant. All analyses were performed in STATA 11.0 (StataCorp, 2009).

Both crude and multivariable (age, ethnicity and family history of prostate cancer) adjusted analyses were performed. Confounding factors were adjusted for according to

the variable of interest. In chapter three, pigmentation can affect behaviours when exposed to sunlight. For example, usual reaction to sunlight and hair colour can affect vitamin D absorption; therefore adjustment was made for these variables where applicable. In chapter four, additional adjustment included regular smoking (never vs. ever), education level and BMI (kg/m^2) and testosterone summary scores. In chapter five, testosterone summary score adjusted analyses were performed. Associations with the localised tumour stage (Gleason score between 2 and 6) or advanced tumours (Gleason score between 7 and 10) were also evaluated. For this analysis, the standard clinical classification for risk (D'Amico scale) was adapted for larger sample sizes.

Due to the small number of participants in some of the five categories for the different physical activities, some groups were combined to create new categories. These categories were either low, medium and high (e.g. walking, gardening and housework; highest vs. lowest level) or they were dichotomized into ever vs. never (e.g. cycling, non-competitive/social sport and competitive sport). Social sport (or non competitive) was defined as aerobics, golf, sailing and skiing. Competitive sport was defined as cricket, football, badminton, squash, athletics and dance.

Duration of physical activity across lifetime was also assessed by comparing the levels of non-occupational physical activity between participants for each of the three age groups: 12-19 years, 20-39 years and from 40 years to diagnosis/entry in the study and calculating ORs and 95% CIs for the physical activities at each of these age categories.

The intensity of physical activity was assessed by multiplying the weekly hours performing each activity (mid value) by its metabolic equivalent (MET) value. METs can be defined as the "ratio of associated metabolic rate for a specific activity compared to the resting metabolic rate" (12). To assign METs to each of the particular physical activities, values from the Compendium of Physical Activities were used, specifically 3.0 for walking, 8.0 for cycling, 3.5 for housework, 4.0 for gardening, 4.8 for non-competitive sport (based on value for golf) and 8.0 for competitive sport (168). METs for total physical activities were calculated as continuous variables and were subsequently categorized as low, medium and high intensity levels of physical activity based on cut-off points determined from the distribution among the controls.

BMI was categorised into quartiles according to the WHO guidelines for evaluation of obesity; <18.5 (underweight), 18.5 – 24.99 (normal), 25.0 – 29.9 (overweight) and ≥ 30.0 (obese). Where numbers were small, overweight and obese categories were combined into one overweight category. Waist circumference was categorised into tertiles based on the distribution amongst controls; <34.0, 34.1 – 37.0, ≥ 37.1 inches. Weight data for each decade were converted into a BMI category. Weight change over lifetime was obtained by calculating the difference between body weight in kg for current age and body weight at age 20. The weight changes were then converted into categorical variables, with a stable weight classed as gain or loss of ≤5 kg over lifetime as the reference group. Relative weight change was also examined, defined as weight change over lifetime divided by weight at age 20.

Statistical Analysis for Chapter 6: Meta analysis

Search Strategy

The Medline/PubMed and EMBASE databases were searched to identify all relevant literature up until December 2010. The search terms used were combinations of the following keywords: *prostate cancer*, *prostatic neoplasms*, *urologic*, *occupation*, *work*, *industry*, *meta-analysis*, *review*, *epidemiology*, *cohort study*, *case control* and *nested case control studies*. Titles and abstracts were systematically reviewed against the original inclusion.

Inclusion criteria

Bibliographies of all studies included were checked for earlier publications until no new studies were found. All case-control and cohort studies measuring prostate cancer as the outcome of interest and occupation as an exposure variable were included. A measure of strength of association must have been reported as odds ratio (OR) or risk ratio (RR), or sufficient data available to calculate this and 95% confidence intervals (95% CI) from raw data. Mortality studies were not included in this meta analysis as cancer incidence was the outcome of interest. If standard errors for odds ratios were not reported, they were calculated. A standardized reporting form was used to extract the following data: author, country, year of publication, sample size, mean age or age range of cases and controls, study design, assessment method, covariates for multivariable models, risk estimates and 95% confidence intervals. Occupations were coded using the Standard Occupation Classification (SOC2000) devised by the Office for National Statistics, UK (169). To examine associations, each category of classification was compared according

to the SOC2000 guide; major groups (9 occupational groups), sub-major groups (19 occupational groups) and minor groups (24 occupational groups) (169).

All risk estimates from each individual study were grouped together by their classification codes and pooled to produce a single estimate using a fixed effects model. For example, where a risk estimate for nurses and another for midwives existed in the same study, the two were pooled into a single estimate for health associate professionals. These occupational estimators or odds ratios (OR) were then pooled by each occupational classification using a random effects model to produce a summary odds ratio (SOR) and corresponding 95% confidence intervals were calculated. Meta-regression models were used to examine whether the country where the study was conducted and study design had an effect on the SORs for study comparability. Publication bias was investigated by the use of funnel plots and also statistically by the Begg and Mazumdar test (170).

Statistical Analysis for Chapter 7: NLCS

Study Population

The study population for this chapter was obtained from the Netherlands Cohort Study (NLCS) (171). In brief, the study recruited over 58, 000 men between the ages of 55 and 69 years at baseline. This chapter reports from the dataset after 8.3 years of follow up, using a case cohort approach. After the follow up period, prostate cancer cases were identified using computerized record linkage with all nine cancer registries in the Netherlands. Controls were selected from a random sub cohort sample of 2,411 men and followed up for information on vital status. The epidemiological dataset for analysis was

provided by Dutch collaborators at Maastricht University (MP Zeegers, LJ Schouten, BAC van Dijk, RA Goldbohm, J Schalken and PA van den Brandt).

Biological Samples

Paraffin blocks of tumour and normal tissue samples were obtained from cases. After exclusions of insufficient non tumour tissue, 300 cases were available for analysis. Buccal swab samples were obtained from 300 controls from the NLCS sub cohort. Genotyping was performed by collaborators at New York University (A Pearlman, S Shajahan, C Oddoux and H Ostrer) using Genemapper software version 4.0 and the Taqman SNP Genotyping Assay (Applied Biosystems, CA) for the rs1447295 SNP marker.

Statistical Analysis

Linkage disequilibrium between marker alleles and deviation from Hardy Weinberg equilibrium were tested by χ^2 tests. Associations for the SNP and microsatellite were tested by both allelic and genotypic analysis. Odds ratios and corresponding 95% confidence intervals were calculated using logistic regression. For allelic analyses, robust standard errors were calculated to model potential clustering of alleles within individuals.

In microsatellite analyses, alleles -8 and -10 were compared to all other alleles, using the most common allele (-14) as the reference group. The microsatellite was further tested at different breakpoints to test association between groups of alleles. Both crude and multivariable (age, alcohol intake from wine, body mass index (BMI), energy intake,

family history of prostate cancer and level of education) adjusted analyses were performed. Differences in associations with the localised tumour stage (T0-2, M0) and advanced (T3-4, M0 and T0-4, M1) were also investigated. Stage was reported by the cancer registries and coded according to the UICC TNM (172). All statistical analysis was carried out by H Khan.

RESULTS

CHAPTER 3

IS UV RADIATION ASSOCIATED WITH PROSTATE CANCER?

By October 2010, a total of 1,071 men had been recruited to the BiPAS project. The questionnaire was completed by 314 prostate cancer cases, 381 high PSA cases, 201 hospital based controls and 175 population based controls. The study sample characteristics can be found in Table 3.1. The mean age within each group was 68 years, 65.2 years, 66.6 years and 64.8 years respectively. The percentage of family history within each group also differed slightly, 12.8%, 8.6%, 11.4% and 4.7% respectively. In the prostate cancer case group, 133 subjects had localised disease and 178 were described as advanced tumour stage. In all groups, more than 85% of patients were Caucasian.

There was very little difference in results when the combined high PSA group were compared to either the hospital controls or the population controls (see appendix 4). Both analyses reported an increase in risk of high PSA level with low exposure to sunlight.

When the two control groups were compared to a high PSA control group, again there was little difference when compared to prostate cancer cases and other high PSA cases separately (see Table 3.2). Both analyses detected trends indicating decreasing risks for more time spent outside in the sun. Significant results were observed for early adulthood in particular, with an increased risk of prostate cancer associated with lower sun exposure, an OR of 4.92 was observed (95% CI 1.63 – 14.89; p trend 0.02). This was consistent for the high PSA cases, however it was not quite statistically significant; an OR of 3.71 was observed (95% CI 1.20 – 11.46; p trend 0.07). A statistically significant protective effect was observed in men who rarely or never burn in the sun, (OR 0.65; 95% CI 0.47 – 0.90; p trend 0.01).

All other analyses also pointed to the same direction. For example, in prostate cancer cases, a moderately high exposure to sun during the life course in prostate cancer cases resulted in an OR of 1.07 (95% CI 0.56 – 2.05) compared to low exposure, where the risk increases with an OR of 1.59 (95% CI 0.70 – 3.60). A similar pattern was observed in high PSA cases, where the OR for moderate exposure was 0.65 (95% CI 0.31 – 1.36) and increased to 1.55 (95% CI 0.68 – 3.53).

Table 3.3 shows the results for the combined case group compared to the combined control group. Again, a statistically significant protective effect for higher UVR exposure was observed in early adulthood, OR 4.28; 95% CI 1.49 – 12.3; p trend 0.03. This was consistent for the life course analysis however it was not statistically significant.

The results of the advanced (Gleason score above 7) and localised (Gleason score $\geq$ 10) analysis can be found in the appendix. Although no statistically significant results were seen, some effect estimates were in the same direction as previous literature, such higher risk of advanced disease for men who rarely or never burn in the sun (OR 1.36; 95% CI 0.82 – 2.28). Interestingly, there was very little difference in risk for different sun exposures in early adulthood, for moderate exposure an OR of 1.34 (95% CI 0.65 – 2.73) compared to low exposure, with an OR of 1.35 (95% CI 0.43 – 1.20).

Table 3.1: Study Characteristics for UV – Results from the Birmingham Prostatic Neoplasms Association Study

	Prostate cancer cases n (%)	High PSA cases n (%)	Hospital controls n (%)	Population controls n (%)
Age group (years)				
40 - 50	0	7 (1.8)	3 (1.5)	3 (1.7)
51 - 60	57 (18.2)	111 (29.1)	57 (28.4)	61 (34.9)
61 - 70	134 (42.7)	171 (44.9)	69 (34.2)	65 (37.1)
71 - 80	104 (33.1)	78 (20.5)	55 (27.4)	37 (21.2)
≥ 81	19 (7.0)	14 (3.7)	17 (8.5)	9 (5.1)
Ethnicity				
Caucasian	274 (94.2)	298 (89.0)	155 (86.6)	163 (94.2)
Non Caucasian	17 (5.8)	37 (11.0)	24 (13.4)	10 (5.8)
Family history of prostate cancer				
Yes	37 (12.8)	28 (8.6)	20 (11.4)	8 (4.7)
No	253 (87.2)	297 (91.4)	156 (88.6)	162 (95.3)
Smoking status				
Never smoked	126 (44.7)	148 (46.3)	76 (45.0)	51 (29.8)
Used to/still smokes	156 (55.3)	172 (53.7)	93 (55.0)	120 (70.2)
Education history				
Low	50 (27.8)	66 (27.2)	28 (21.5)	38 (31.9)
Medium	64 (35.6)	77 (31.7)	49 (37.7)	50 (42.0)
High	66 (36.6)	100 (41.1)	53 (40.8)	31 (26.1)
Tumour Grade				
Localised*	133 (42.8)			
Advanced**	178 (57.2)			

*Gleason score between 2 and 6 **Gleason score between 7 and 10

Table 3.2: Odds Ratios and 95% Confidence intervals for UVR comparing combined controls with prostate cancer cases and high PSA cases - Results from The Birmingham Prostatic Neoplasms Association Study

		Combined Controls n (%)	Prostate cancer cases n (%)	Adjusted OR (95% CI)	p value/ p trend	High PSA cases n (%)	Adjusted OR (95% CI)	p value/ p trend
Usual reaction to sunlight								
Always/easily burns		109 (31.4)	97 (33.0)			135 (41.2)		
Rarely/never burns		238 (68.6)	197 (67.0)	0.94 (0.67 - 1.33)*	0.74	193 (58.8)	0.65* (0.47 - 0.90)	0.01
Number of times severely sunburnt as a child	1	29 (8.8)	21 (7.5)			26 (8.2)		
	≥ 2	299 (91.2)	258 (92.5)	1.14 (0.61 - 2.10)*	0.69	290 (91.8)	1.18 (0.67 - 2.07)*	0.57
Ever lived abroad in a sunny country	No	276 (82.9)	220 (81.8)			235 (75.1)		
	Yes	57 (17.1)	49 (18.2)	0.95 (0.62 - 1.47)*	0.83	78 (24.9)	1.61 (1.10 - 2.36)*	0.02
Time spent outside								
Early adulthood (20-39 years)	High	4 (1.4)	14 (6.4)			12 (4.7)		
	Medium	54 (18.4)	46 (21)	1.22 (0.78 - 1.93)**		44 (17.1)	0.94 (0.60 - 1.47)§	
	Low	236 (80.2)	159 (72.6)	4.92 (1.63 - 14.89)**	0.02§	201 (78.2)	3.71 (1.20 - 11.46)§	0.07§
Middle age (40-60 years)	High	20 (7.0)	21 (9.8)			17(6.6)		
	Medium	79 (27.8)	63 (29.4)	1.13 (0.75 - 1.70)**		61 (23.6)	0.79 (0.53 - 1.18)§	
	Low	185 (65.2)	130 (60.8)	1.36 (0.70 - 2.65)**	0.62§	180 (69.8)	0.88 (0.44 - 1.77)§	0.5§
Late adulthood (60+ years)	High	6 (30.0)	9 (34.6)			4 (22.2)		
	Medium	5 (25.0)	12 (46.2)	6.70 (1.20 - 37.39)**		5 (27.8)	1.68 (0.31 - 9.20)§	
	Low	9 (45.0)	5 (19.2)	4.19 (0.67 - 26.18)**	0.09§	9 (50.0)	0.89 (0.16 - 4.92)§	0.76
Life course	High	12 (7.7)	15 (10.7)			15 (10.1)		
	Medium	23 (14.8)	22 (15.7)	1.07 (0.56 - 2.05)**		16 (10.8)	0.65 (0.31 - 1.36)§	
	Low	120 (77.5)	103 (73.6)	1.59 (0.70 - 3.60)**	0.84§	117 (79.1)	1.55 (0.68 - 3.53)§	0.26§
Sunbathing in life course	High	57 (24.4)	49 (24.1)			69 (27.8)		
	Medium	89 (38.0)	83 (40.9)	1.21 (0.78 - 1.87)**		77 (31.0)	0.82 (0.53 - 1.27)§	
	Low	88 (37.6)	71 (35.0)	1.14 (0.68 - 1.91)**	0.7§	102 (41.2)	1.21 (0.74 - 1.99)§	0.29§

* Adjusted for age at diagnosis, ethnicity and family history of prostate cancer

** Adjusted for age at diagnosis, family history of prostate cancer, skin type and hair colour

§ P trend

Combined controls: hospital + population controls

Table 3.3 Odds Ratios and 95% Confidence intervals for UVR comparing combined controls and combined case groups - Results from The Birmingham Prostatic Neoplasms Association Study

		Combined Controls n (%)	Combined Cases n (%)	Crude OR	Adjusted OR* (95% CI)	P value or p trend §
Usual reaction to sunlight						
Always/easily burns		109 (31.4)	232 (37.3)			
Rarely/never burns		238 (68.6)	390 (62.7)	0.77	0.78 (0.59 - 1.03)*	0.08
Number of times severely sunburnt as a child						
1		29 (8.8)	47 (7.9)			
≥ 2		299 (91.2)	548 (92.1)	1.13	1.18 (0.71 - 1.92)*	0.52
Ever lived abroad in a sunny country	No	276 (82.9)	455 (78.2)			
	Yes	57 (17.1)	127 (21.8)	1.35	1.30 (0.92 - 1.85)*	0.14
Time spent outside						
Early adulthood (20-39 years)	High	4 (1.4)	26 (5.5)			
	Medium	54 (18.4)	90 (18.9)	1.08	1.07 (0.73 - 1.57)**	
	Low	236 (80.2)	360 (75.6)	4.22	4.28 (1.49 - 12.3)**	0.03§
Middle age (40-60 years)	High	20 (7.0)	38 (8.1)			
	Medium	79 (27.8)	124 (26.3)	0.93	0.94 (0.67 - 1.33)**	
	Low	185 (65.2)	310 (65.6)	1.12	1.08 (0.61 - 1.03)**	0.89§
Late adulthood (60+ years)	High	6 (30.0)	13 (29.5)			
	Medium	5 (25.0)	17 (38.6)	2.19	3.01 (0.77 - 11.73)**	
	Low	9 (45.0)	14 (31.9)	1.39	1.94 (0.46 - 8.16)**	0.28§
Life course	High	12 (7.7)	30 (10.4)			
	Medium	23 (14.8)	38 (13.2)	0.91	0.85 (0.48 - 1.53)**	
	Low	120 (77.5)	220 (76.4)	1.37	1.56 (0.76 - 3.23)**	0.38§
Sunbathing in life course	High	57 (24.4)	118 (26.2)			
	Medium	89 (38.0)	160 (35.5)	0.92	0.99 (0.68 - 1.45)**	
	Low	88 (37.6)	173 (38.3)	1.07	1.16 (0.75 - 1.80)**	0.74§

* Adjusted for age at diagnosis, ethnicity and family history of prostate cancer

** Adjusted for age at diagnosis, family history of prostate cancer, skin type and hair colour

§ P trend

Combined controls: hospital + population controls

Combined cases: prostate cancer cases + high PSA cases

Conclusion

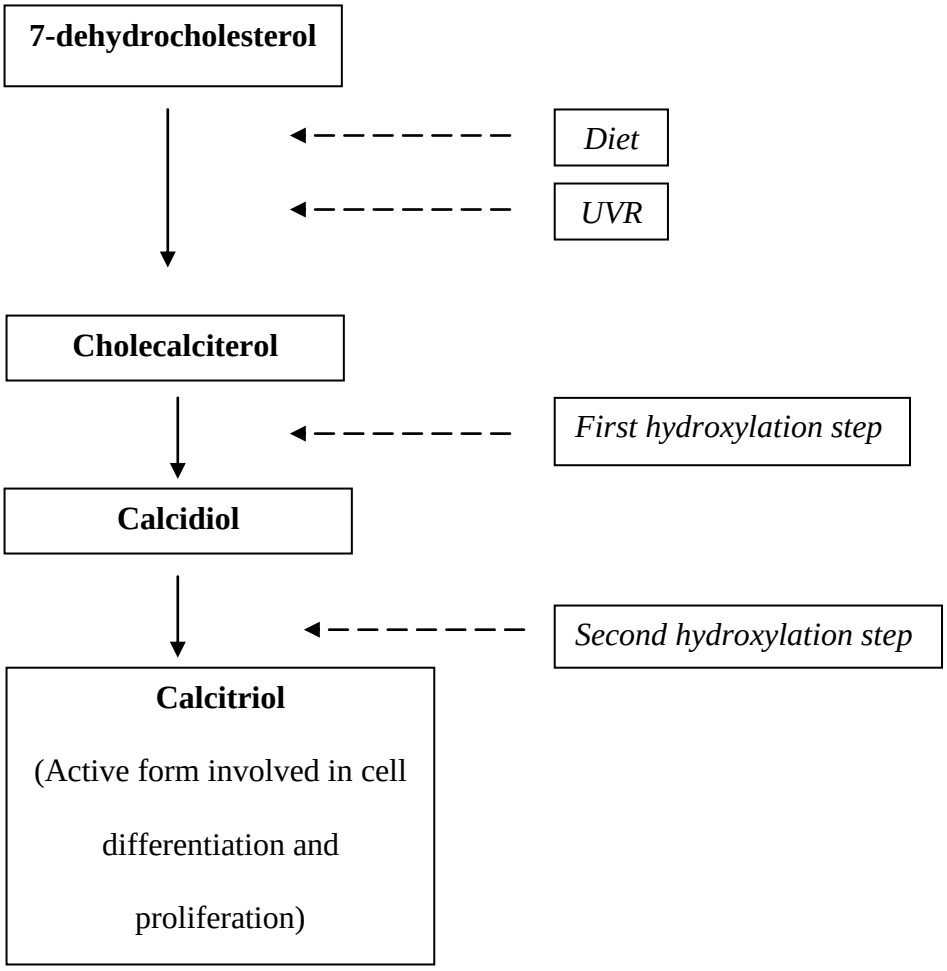
The results show a marked decreased risk associated with high levels of sun exposure in early adulthood in particular, consistent with previous studies. A study of 210 prostate cancer cases and 155 benign prostatic hyperplasia (BPH) controls in North Staffordshire, England compared acute and chronic sun exposures (173). High sunbathing scores, regular foreign holidays and sunburn in childhood had a protective effect. In the case group, men with lowest exposures developed prostate cancer earlier; the median age of diagnosis was 67.7 years compared to 72.1 years of age for all other patients. Although these results were in the same direction as other UVR studies, further confirmation was required. A further 212 prostate cancer cases and 135 BPH controls were subsequently recruited between 2001 and 2002 (61). This study confirmed all the previous results; men with the lowest quartile of UVR exposure had almost a threefold increased risk of prostate cancer compared to men in the highest quartile. These replicated findings show the original associations were not spurious and that UVR exposure may have a protective role in prostate cancer.

The significant results for early adulthood suggest that acute exposures may be more important in prostate carcinogenesis than chronic exposures over time. Also, in early adulthood, more time is generally spent outdoors, for example in recreational activities, holidays abroad or working hours. Younger adults may also be more health conscious and take vitamin supplements, which will include vitamin D or eat more vitamin D rich foods.

Although the exact mechanism for this protective effect is unclear, vitamin D synthesis has been implicated. Figure 3.2 shows the current understanding of the role

of vitamin D on prostate cancer. After exposure to UVR, 7-dehydrocholesterol in the skin is converted to vitamin D_3. Vitamin D_3 can also be obtained by diet; this is especially true for USA, where small quantities are added to milk, cereals and orange juice. In order to become biologically active, the D_3 form then undergoes two hydroxylation processes, firstly in the liver and then again in the kidney, colon and prostate cells. The active form, known as calcitriol is involved in cell differentiation and proliferation.

Figure 3.2 Role of Vitamin D on cancer



It is known that vitamin D deficiency leads to diseases such as rickets or osteomalacia; however levels sufficient for a healthy skeleton may be inadequate for a healthy prostate. Well established risk factors can also be related to the vitamin D hypothesis; prostate cancer risk is known to increase with age, as does vitamin D deficiency. Older people have less exposure to UVR, especially if they are housebound or have limited mobility. They also have a thinner epidermis which contains less 7-dehydrocholesterol than younger people, resulting in lower levels of active form of the vitamin.

Another well studied risk factor is ethnicity; African American men have between 1.3 – 2 times higher incidence than Caucasian men (174). Dark skinned individuals absorb UV rays, which will inhibit vitamin D synthesis. In contrast, a traditional Asian diet in Japan contains oily fish, which is a rich source of vitamin D. This could cause the low risk of prostate cancer in Japanese men living in Japan. There is also evidence for an inverse relationship between UVR exposure and vitamin D levels and other diseases, such as type 1 diabetes, multiple sclerosis and arthritis (175-177).

This study is a novel approach in the current methods of case control analyses, due to the comparison of four distinct groups. The results showed little difference when the case group was prostate cancer or the high PSA cases. Following these results, PSA could potentially be used as an intermediate marker for prostate cancer, and where cancer cases are difficult to recruit, high PSA cases could be used as an alternative.

The data are mostly consistent with previous findings, and are therefore compatible with the hypothesis that low levels of active vitamin D increase prostate cancer risk.

However the public health implications of UVR exposure are important therefore these data should be interpreted with caution. Although high levels of sun exposure have a consistently protective effect, the risk of skin cancer will also increase (178). A controlled level or pattern of UVR exposure or perhaps increased dietary intake that results in sufficient synthesis of vitamin D is required that does not increase risks for skin cancer.

CHAPTER 4

IS PHYSICAL ACTIVITY ASSOCIATED WITH PROSTATE CANCER?

Table 4.1 presents key study characteristics and physical activity behaviour (for the oldest age category; from 40 years to diagnosis/entry into the study) for the prostate cancer cases, high PSA cases, hospital controls and healthy population controls. Overall, prostate cancer cases were older than the two control groups ($p<0.001$). They also had a statistically significant higher percentage of family history of prostate compared to population controls but not for the hospital controls. In terms of the number of hours engaged in the different types of physical activity only participation in social sport was significantly different overall (highest non-participation in social sport) ($p=0.02$). This trend was consistent across all the three age groups (data not shown).

The ORs and 95% CIs for the number of hours per week engaged in the different physical activities from the age of 40 onwards for prostate cancer cases compared with combined control groups are shown in Table 4.2. Although an inverse association was observed for participants who had ever cycled, ever taken part in social sport and higher levels of housework, they were not statistically significant. Increased risks were observed for high levels (>10 hours per week) of walking (OR 1.42; 95% CI 0.69 – 1.65; p trend = 0.63), medium levels (5 – 9 hours per week) of gardening (OR 1.29; 95% CI 0.68 – 2.43; p trend = 0.72) and participation of competitive sport (OR 1.41; 95% CI 0.95 – 2.09; p trend = 0.09).

Table 4.3 shows the results of the combined cases and controls when a low versus high PSA comparison is made. Although no statistically significant associations were observed, similar results were observed for cycling (OR 0.90; 95% CI 0.64 – 1.27; p value = 0.55), medium levels of housework (OR 0.84; 95% CI 0.40 – 1.77; p trend =

0.83). Increased risks were again associated with walking (OR 1.57; 95% CI 0.87 – 2.83; p trend = 0. 23), medium levels (5 – 9 hours per week) of gardening (OR 1.29; 95% CI 0.76 – 2.19 p trend = 0.58) and participation of competitive sport (OR 1.29; 95% CI 0.93 – 1.78; p trend = 0.13). These effects remained when controlled for testosterone levels.

Table 4.4 presents the results after stratification by severity of disease. Although statistical significance was not achieved, some of the effect estimates were in the same direction. The associations for participants who had ever cycled, level of housework and participation in competitive sport produced consistent results with table 4.3. In general, more similar associations were observed for advanced tumours, indicating physical activity has a similar effect on advanced carcinogenesis as normal disease progression.

When the results were repeated for the younger two age groups (combined cases vs. combined controls; 12-19 years and 20-39 years) a statistically significant inverse association for participating in social sport between the ages of 20-39 years (p=0.02). This association also gave a borderline significant result in 12-19 years (p=0.07) (see appendix table).

No potential association or trend was observed when the intensity of the combined activities was assessed in terms of METs for this age category (low: 6-30; medium 31-51, high ≥51.5 METs) (data not shown).

Table 4.1 Study sample characteristics for physical activity – Results from the Birmingham Prostatic Neoplasms Association Study

Variable	Prostate Cancer Cases	High PSA cases	Hospital Controls	Population Controls
Age	67.9 (8.0)	64.7 (7.6)	65.7 (8.5)	64.6 (9.3)
Ethnicity				
White	238 (96%)	275 (91%)	132 (89%)	161 (94%)
Non-White	9 (4%)	27 (9%)	16 (11%)	10 (6%)
Ever-smoker				
No	109 (44%)	141 (47%)	67 (45%)	50 (30%)
Yes	136 (56%)	162 (53%)	82 (55%)	119 (70%)
Family History Prostate Cancer				
No	214 (86%)	277 (91%)	132 (87%)	160 (95%)
Yes	34 (14%)	26 (9%)	20 (13%)	8 (5%)
Education				
Early leaver	88 (35%)	74 (24%)	44 (29%)	53 (31%)
High School Cert	44 (18%)	61 (20%)	18 (12%)	38 (22%)
Trade/Diploma	57 (23%)	73 (24%)	45 (29%)	49 (29%)
University degree	61 (24%)	98 (32%)	47 (31%)	31 (18%)
BMI	27.0 (4.1)	26.8 (3.8)	26.8 (4.3)	27.5 (4.6)

Table 4.2: Odds Ratios and 95% Confidence intervals for physical activity comparing combined controls and prostate cancer case group: Results from the Birmingham Prostatic Neoplasms Association Study

	Combined Controls	Prostate cancer Cases	Non Adjusted	Adjusted	Adjusted	p value or
	n (%)	n (%)	OR	OR*	95%CI	p trend§
Walking hours per week						
Low (Never – 4)	225 (67.8)	173 (65.3)	1	1		
Medium (5-9)	80 (24.1)	69(26.0)	1.12	1.07	0.69 - 1.65	
High (≥10)	27 (8.1)	23 (8.7)	1.11	1.42	0.69 - 2.90	0.63§
Cycling hours per week						
Never	203 (66.8)	168 (72.1)	1	1		
Ever	101 (33.2)	65 (27.9)	0.78	0.85	0.55 - 1.30	0.45
Gardening hours per week						
Low (Never – 4)	292 (89.3)	227 (88.0)	1	1		
Medium (5-9)	32 (9.8)	28 (10.9)	1.13	1.29	0.68 - 2.43	
High (≥10)	3 (0.9)	3 (1.1)	1.29	0.9	0.18 - 4.59	0.72§
Housework hours per week						
Low (Never – 4)	291 (93.3)	232 (95.1)	1	1		
Medium (5-9)	19 (6.1)	11 (4.5)	0.73	0.63	0.23 - 1.73	
High (≥10)	5 (0.6)	1 (0.4)	0.25	0.4	0.06 - 2.50	0.42§
Social Sport hours per week						
Never	191 (62.6)	151 (66.8)	1	1		
Ever	114 (37.4)	75 (33.2)	0.83	0.75	0.49 - 1.15	0.18
Competitive Sport hours per week						
Never	204 (66.2)	133 (57.1)	1	1		
Ever	104 (33.8)	100 (42.9)	1.47	1.41	0.95 - 2.09	0.09

* Adjusted for age at diagnosis, family history of prostate cancer, ethnicity, education, ever smoker and BMI § P trend

Combined controls = hospital controls + population controls

Table 4.3: Odds Ratios and 95% Confidence intervals for physical activity comparing combined controls and combined case group: Results from the Birmingham Prostatic Neoplasms Association Study

	Combined Controls	Combined Cases	Crude	Adjusted	p value or	Adjusted	p value or
	n (%)	n (%)	OR	OR* (95% CI)	p trend§	OR**95%CI	p trend§
Walking hours per week							
Low (Never – 4)	225 (67.8)	387 (66.8)	1	1		1	
Medium (5-9)	80 (24.1)	140 (24.2)	1.02	1.09 (0.75 - 1.57)		0.85 (0.54 – 1.32	
High (≥10)	27 (8.1)	52 (9.0)	1.12	1.57 (0.87 - 2.83)	0.32§	1.73 (0.86 – 3.51)	0.19§
Cycling hours per week							
Never	203 (66.8)	356 (69.3)	1	1		1	
Ever	101 (33.2)	158 (30.7)	0.89	0.9 (0.64 - 1.27)	0.55	0.88 (0.59 – 1.31)	0.53
Gardening hours per week							
Low (Never – 4)	292 (89.3)	501 (88.4)	1	1		1	
Medium (5-9)	32 (9.8)	57 (10.1)	1.04	1.29 (0.76 - 2.19)		1.31 (0.67 – 2.56)	
High (≥10)	3 (0.9)	9 (1.5)	1.75	1.4 (0.35 - 5.57)	0.58§	1.60 (0.33 – 7.91)	0.63§
Housework hours per week							
Low (Never – 4)	291 (93.3)	505 (93.5)	1	1		1	
Medium (5-9)	19 (6.1)	27 (5.0)	0.82	0.84 (0.40 - 1.77)		1.13 (0.45 – 2.83)	
High (≥10)	5 (0.6)	8 (1.5)	0.92	1.26 (0.40 - 3.94)	0.83§	1.43 (0.41 – 5.02)	0.83§
Social Sport hours per week							
Never	191 (62.6)	307 (61.8)	1	1		1	
Ever	114 (37.4)	190 (38.2)	1.04	0.96 (0.69 - 1.34)	0.82	0.84 (0.55 – 1.28)	0.43
Competitive Sport hours per week							
Never	204 (66.2)	201 (66.3)	1	1		1	
Ever	104 (33.8)	102 (33.7)	1.32	1.29 (0.93 - 1.78)	0.13	1.08 (0.73 – 1.59)	0.70

* Adjusted for age at diagnosis, family history of prostate cancer, ethnicity, education, ever smoker and BMI

** Adjusted for age at diagnosis, family history of prostate cancer, ethnicity, education, ever smoker, BMI and testosterone summary score

Combined cases = prostate cancer cases + high PSA cases

Combined controls = hospital controls + population controls § P trend

Table 4.4: Odds Ratios and 95% Confidence intervals for physical activity after stratification for severity of disease - Results from the Birmingham Prostatic Neoplasms Association Study

	Localised		Advanced	
	Adjusted OR* (95% CI)	p value	Adjusted OR* (95% CI)	p value
	Localised		Advanced	
Walking hours per week				
Low (Never – 4)	1		1	
Medium (5-9)	0.98 (0.69 - 1.38)	0.9	1.10 (0.77 - 1.94)	0.63
High (≥10)	1.14 (0.77 - 1.94)	0.64	1.70 (0.92 - 3.13)	0.09
Cycling hours per week				
Never	1		1	
Ever	0.96 (0.69 - 1.32)	0.79	0.93 (0.65 - 1.33)	0.7
Gardening hours per week				
Low (Never – 4)	1		1	
Medium (5-9)	0.99 (0.60 - 1.62)	0.79	1.35 (0.79 - 2.33)	0.28
High (≥10)	2.11 (0.55 - 8.06)	0.27	1.30 (0.28 - 5.96)	0.74
Housework hours per week				
Low (Never – 4)	1		1	
Medium (5-9)	0.86 (0.45 - 1.63)	0.64	0.86 (0.45 - 1.63)	0.64
High (≥10)	1.08 (0.34 - 3.42)	0.9	1.08 (0.34 - 3.42)	0.9
Social Sport hours per week				
Never	1		1	
Ever	1.04 (0.76 - 1.43)	0.79	1.11 (0.78 - 1.57)	0.57
Competitive Sport hours per week				
Never	1		1	
Ever	1.20 (0.88 - 1.65)	0.26	1.33 (0.95 -1.87)	0.1

* Adjusted for age at diagnosis, family history of prostate cancer, ethnicity, education, ever smoker and BMI

Localised Gleason score between 2 and 6 Advanced Gleason between 7 and 10

Conclusion

Although no strong significant associations were detected, there are indications for either direction. A consistent reduced risk of prostate cancer was observed for men who ever cycled in all analyses. This was also true for associations in the 12-19 and the 20-39 years age category.

Conversely, ever having played competitive sports was associated with increased risk of prostate cancer among all comparison groups, including for both localised and advanced tumours and when adjusted for testosterone levels, therefore the results cannot be explained by testosterone. Five or more hours of housework per week appeared to be protective. High levels or ever participating in lower intensity type activities, such as walking or gardening appeared to be inversely associated with the odds of prostate cancer in most comparison groups. Although housework would be considered to be a low intensity type of physical activity, men who were classified in the highest category for this (i.e. 10 or more hours per week) were potentially at increased risk of prostate cancer.

The results remain similar for all case control comparisons (see appendix tables), therefore it can be concluded that physical activity has the same effect on prostate cancer and men with high PSA. This serves as further proof, along with previous chapters, that this group of men with high PSA levels could be used instead of or in conjunction with confirmed prostate cancer cases.

The results do not fully support the hypothesis that high levels of all physical activity protect against prostate cancer. The type of activity was distinguished into two

categories; low intensity (including activities such as walking, gardening and housework) and high intensity (such as cycling, social sport and competitive sport). When categorized, high levels of low intensity activity were generally found to have an increased risk and participants who had ever taken part in high intensity activity generally had a decreased risk.

In this context, the results are consistent with previous studies. Vigorous activity is known to be associated with reduced risk of advanced disease (70, 179). When corrected for testosterone, the results were not altered; therefore other biological pathways could be involved. The main mechanism of physical activity is via insulin and insulin-like growth factor (IGF). IGF and insulin binding is involved in cell proliferation, differentiation, apoptosis and angiogenesis (180). Vigorous exercise also increases sensitivity of insulin and lowers inflammatory factors and increases anti-inflammatory cytokines (181-183). It is difficult to pinpoint a specific biological mechanism as exercise can simultaneously affect multiple biological pathways. The potential biomarkers that are currently known include insulin and IGF-1, mentioned previously, also leptin, tumour necrosis factor-alpha (TNF-α) and vitamin D. Their role in increasing cell proliferation and decreasing apoptosis is known; however they may be involved in several other pathways causing an additional indirect effect on prostate carcinogenesis.

A limitation to this study is selection bias. Men who are healthier and are more physically active may be more likely to attend prostate cancer testing than less active men. Also, the slow growing nature of a prostatic tumour means more men will die

with undiagnosed prostate cancer, and these patients may be incorrectly categorized as control patients.

In conclusion, physical activity is an important risk factor in reducing prostate cancer risk. There is increasing evidence emerging for underlying biological mechanisms involved. The study reported a protective effect associated with high intensity physical activity levels. However a contrasting increased risk associated with low intensity exercise, such as walking and gardening is observed. The associations were similar in all case control comparisons, further supporting the use of high PSA cases. These results warrant further study on the effect of physical activity, especially at different intensities. Future studies should also investigate the multi faceted effect on biomarkers, for example how low insulin levels *and* inflammatory markers are related to prostate cancer. Genetic subtypes could also be analysed, to investigate individual responses to biomarkers at different levels of physical activity.

CHAPTER 5

IS BODY SIZE ASSOCIATED WITH PROSTATE CANCER?

The study sample characteristics in relation to obesity can be found in Table 5.1 including waist circumference, BMI and self perceived body size categories. The numbers for self perceived obese men at age 20 and 30 were very small; these were later combined into one overweight group for analysis.

Table 5.2 shows the results for waist circumference and adult weight change. Interestingly, a statistically significant protective effect was observed for a large waist circumference; however the risk did not differ as circumference increased. An increasing risk was detected as adult weight change increased, however none of the estimates was statistically significant. Interestingly an increased risk was also associated with adult weight loss (OR 1.44; 95%CI 0.57 – 3.63). This trend continued when weight change from different ages (such as weight change from age 20 to age 30 and from age 20 to age 40) was calculated (data not shown).

Table 5.3 shows results for actual and self reported body size at different ages over lifetime. A protective effect was associated with men defined as overweight by the WHO guidelines, however an increased risk was observed in obese men. Although this result was in contrast with previous studies, it is consistent with the results for self perceived body size at different ages. In these analyses, a lower risk is associated with a BMI of between 25.0 and 29.99 and an increased risk is observed for a BMI of 30 or over. The most interesting result was detected for men at age 20, a highly significant increased risk was detected for obese men, using the BMI measures (OR 1.84; 95% CI 1.23 – 2.75).

Table 5.4 shows the results stratified by stage of disease. Most effect estimates were in the same direction as previous analyses, there was a consistent decreased risk associated with waist circumference for both overweight and obese men, and this was statistically significant. There was an interesting observation for the current BMI. Instead of a decreased risk associated in overweight men and an increased risk associated with the obese, the risk of localised disease was decreased for both overweight and obese groups (OR 0.90/0.98; 95%CI 0.63 – 1.28/0.68 – 1.41). The risk of advanced disease had an increased risk for both groups, although this was only marginal in overweight men (OR 1.01/1.20; 95% CI 0.71 – 1.45/0.84 – 1.72).

Table 5.1 Study Sample Characteristics from The Birmingham Prostatic Neoplasms Association Study

Variable		Prostate Cancer Cases (n 314)	High PSA Cases (n 381)	Hospital Controls (n 201)	Population Controls (n 175)
BMI age 20	Underweight	85	108	52	23
	Normal	61	63	30	35
	Overweight	16	22	12	13
	Obese	45	72	37	8
BMI age 30	Underweight	51	57	33	18
	Normal	164	181	86	99
	Overweight	53	64	41	45
	Obese	46	79	41	13
BMI age 40	Underweight	174	189	88	80
	Normal	73	68	45	55
	Overweight	25	52	28	30
	Obese	42	72	40	10
BMI current age	Underweight	3	2	2	0
	Normal	77	86	44	49
	Overweight	97	131	63	78
	Obese	137	162	92	48
Self perceived body size age 20	Underweight	44	63	25	32
	Normal	152	157	99	84
	Overweight	68	82	42	56
	Obese	6	3	0	1
Self perceived body size age 30	Underweight	6	14	4	7
	Normal	119	133	74	71
	Overweight	139	150	87	91
	Obese	4	7	2	4
Self perceived body size age 40	Underweight	1	2	2	3
	Normal	62	90	36	36
	Overweight	194	188	116	126
	Obese	13	26	13	8
Self perceived body size current age	Underweight	4	7	4	1
	Normal	25	39	18	21
	Overweight	166	197	105	104
	Obese	77	63	41	44

Table 5.2 Odds Ratios and 95% Confidence intervals for waist circumference and weight loss over time - Results from The Birmingham Prostatic Neoplasms Association Study

Variable	Combined controls n	Prostate cancer cases n	Crude OR	Adjusted OR*	Adjusted* 95%CI	P value
Waist Circumference (cm)						
<34.0	147	158	1	1		
34.1 - 37.0	85	57	0.62	0.6	0.40 - 0.92	
≥37.1	128	91	0.66	0.64	0.44 - 0.93	0.02[§]
Adult weight change categorical (kg)						
≥ 5 kg weight loss	13	12	1.46	1.44	0.57 – 3.63	0.44
≤5 kg Weight loss and ≤5 kg weight gain	41	26	1	1		
≤15 kg weight gain	109	72	1.04	1.04	0.58 – 1.88	0.89
≤25 kg weight gain	75	49	1.03	1.12	0.61 – 2.09	0.70
≤45 kg weight gain	37	30	1.28	1.31	0.65 – 2.65	0.45

* Adjusted for age at diagnosis, ethnicity, family history of prostate cancer and testosterone summary score
[§] P trend Combined controls = hospital controls + population controls

Table 5.3: Odds Ratios and 95% Confidence intervals for actual and self reported BMI for different age categories - Results from The Birmingham Prostatic Neoplasms Association Study

Variable	OR* (95% CI)	P trend	OR* (95% CI)	P trend	OR* (95% CI)	P trend	OR* (95% CI)	P trend
Age (years)	**20**		**30**		**40**		**50**	
BMI								
Underweight (<18.4)	0.51 (0.19 – 1.39)		1.09 (0.75 – 1.60)		1.49 (1.09 – 2.05)		1.24 (0.24 – 6.48)	
Normal (18.5 – 24.99)	1.00		1.00		1.00		1.00	
Overweight (25.0 – 29.99)	0.91 (0.54 – 1.53)		0.73 (0.52 – 1.02)		0.95 (0.62 – 1.46)		0.94 (0.67 – 1.30)	
Obese (≥30)	1.84 (1.23 – 2.75)	0.001	0.96 (0.58 – 1.59)	0.24	1.11 (0.62 – 1.99)	0.03	1.13 (0.80 – 1.60)	0.68
Self perceived body size								
Underweight (<18.4)	0.89 (0.56 – 1.41)		0.56 (0.19 – 1.59)		0.28 (0.07 – 1.19)		1.29 (0.41 – 4.09)	
Normal (18.5 – 24.99)	1.00		1.00		1.00		1.00	
Overweight (25.0 – 29.99)	0.93 (0.64 – 1.36)	0.85	0.96 (0.69 – 1.35)	0.57	0.76 (0.55 – 1.06)		1.09 (0.71 – 1.69)	
Obese (≥30)					0.93 (0.51 – 1.71)	0.17	1.04 (0.64 – 1.68)	0.95

*Adjusted for age at diagnosis, ethnicity, family history of prostate cancer and testosterone summary score

Table 5.4: Odds Ratios and 95% Confidence intervals for obesity stratified by severity of disease - Results from The Birmingham Prostatic Neoplasms Association Study

Variable	Localised		Advanced	
Waist Circumference (cm)	**Adjusted OR* (95% CI)**	**p value**	**Adjusted OR* (95% CI)**	**p value**
<34.0	1		1	
34.1 - 37.0	0.65 (0.45 - 0.94)	0.02	0.66 (0.46 - 0.96)	0.03
≥37.1	0.62 (0.44 - 0.86)	0.005	0.63 (0.46 - 0.88)	0.006
Self perceived body size age 20				
18.5 - 24.99	1		1	
25.0 - 29.99	0.87 (0.63 - 1.20)	0.4	0.89 (0.65 - 1.22)	0.48
≥30.0	4.66 (0.55 - 39.67)	0.16	4.45 (0.55 - 35.86)	0.16
Self perceived body size age 30				
18.5 - 24.99	1		1	
25.0 - 29.99	0.85 (0.64 - 1.14)	0.29	0.97 (0.73 - 1.30)	0.86
≥30.0	1.08 (0.38 - 3.11)	0.88	1.17 (0.41 - 3.38)	0.77
Self perceived body size age 40				
18.5 - 24.99	1		1	
25.0 - 29.99	0.68 (0.49 - 0.95)	0.02	0.80 (0.57 - 1.12)	0.2
≥30.0	0.90 (0.48 - 1.69)	0.75	1.03 (0.55 - 1.92)	0.94
Self perceived body size current age				
18.5 - 24.99	1		1	
25.0 - 29.99	0.94 (0.62 - 1.45)	0.79	1.03 (0.67 - 1.58)	0.91
≥30.0	0.80 (0.49 - 1.31)	0.38	0.94 (0.58 - 1.53)	0.81
Current BMI				
18.5 - 24.99	1		1	
25.0 - 29.99	0.90 (0.63 - 1.28)	0.56	1.01 (0.71 - 1.43)	0.95
≥30.0	0.98 (0.68 - 1.41)	0.92	1.20 (0.84 - 1.72)	0.32

*Adjusted for age at diagnosis, ethnicity, family history of prostate cancer and testosterone summary score
Localised Gleason scores between 2 and 6
Advanced Gleason score between 7 and 10

Conclusion

A statistically significant increased risk was associated with a BMI classified as obese at age 20; however this effect seemed to disappear with age. This suggests that obesity at an early age has a real impact on the risk of prostate cancer later in life. The effect of body size in early life could either be on prostate carcinogenesis, or even PSA levels. Risk of prostate cancer increased with increasing adult weight change; however this was also true for adult weight loss. This was one of the few studies looking at a lifetime history of obesity so it can be concluded that in this study sample, obesity at an early age has a significant effect on the risk of prostate cancer and high PSA. Different associations for waist circumferences and BMI were observed; the risk of disease actually decreases with an increasing circumference. A recent review by Browning *et al* reported waist circumference to be a better predictor for cardiovascular disease more often that BMI (184). Larger waist circumference is used as a measure of central fat distribution, which is known to be more common in men. A further study by Pi-Sunyer reports this type of fat distribution to have a biological mechanism relating to increased lipolysis, which could have a direct effect on the risk of type 2 diabetes mellitus and cardiovascular disease (106). It could be that the proposed mechanism has a protective effect in prostate carcinogenesis in some way; however this has not been further investigated. It can therefore be concluded that waist circumference cannot be used instead of BMI as a measure for obesity in prostate studies. The problems of BMI as a measure are limited to high lean body mass and large framed subjects. However these individuals only constitute a relatively small proportion of most populations, therefore we can assume BMI is a relatively accurate measure (96). Our inclusion of the pictogram (Figure 5.1) allows

the differentiation of fat distributions. Where BMI is lacking as a measure of obesity, the pictogram compensates by differentiating distribution of body fat.

Generally a decreased risk was associated with overweight men and a positive association was observed in obese men. This was true for both BMI and self perceived body size and in all other analyses (see appendix 6), therefore it can be concluded that obesity has the same effect on prostate cancer and men with high PSA. The self perceived body image was used as a tool for estimating BMI at different ages. Although the results for current self perceived body image were similar to actual BMI, the t test gave a statistically significant p value, meaning the two measures are not comparable. This result supports the use of the self perceived visual stimuli in addition to actual measures, which takes into account fat distributions.

The actual BMI was calculated from the height and weight measure reported by the patient. Although this is still self reported, patients recruited in the hospital had their weight and height measured by the clinic nurse just minutes before they completed the questionnaire, therefore it can be assumed that the measures are fairly accurate, with perhaps just a small degree of error. Adult weight gain did not have a significant association with prostate cancer in this sample. A recent study in Japan by Mori *et al* reported a highly significant increased risk associated with adult weight gain and prostate cancer in men, specifically for a weight gain of 10 – 14.9 kg (185). Incidence patterns vary greatly between the UK and Japan, so this could be an important factor to explain the differences. Another study by Hernandez *et al* actually observed a decreased risk associated in Japanese men in Hawaii and California, but an increased risk for weight gain in African American men in the same cohort (186). Differences in

ethnicity cause differences in accumulation of body fat, particularly in adipose tissue around the abdominal region (187-189). This could explain the conflicting results for adult weight gain in the various studies, including this one.

After stratification according to severity of disease the results support the general idea that obesity could reduce the risk of localised disease, yet at the same time, increase the risk of advanced disease. Previous studies have confirmed obesity can play a role in aggressiveness of tumours, causing higher stage and grade of tumours and sometimes increase the risk of recurrence (190-191). As previously discussed, obesity can reduce PSA levels, causing early stages of cancer to go undetected. Also the other hormonal factors affected by obesity, such as steroid hormones, adipokines and inflammatory mediators could have a biological mechanism on prostate carcinogenesis (190).

In summary, waist circumference was negatively associated with prostate cancer in this sample and obesity at a younger age increased the risk of disease. When obesity over lifetime was investigated, the risk was usually lower for men described as overweight and higher for obese men. The associations were similar in all case control comparisons, further supporting the use of high PSA cases. Although the results confirm the findings of most previous studies, further research into the complex association is required to fully elucidate the biological action of obesity on prostate carcinogenesis.

CHAPTER 6

IS OCCUPATION ASOCIATED WITH

PROSTATE CANCER?

After exclusion of non original results (such as editorials, comments and reviews) 43 abstracts remained. A further 10 abstracts were identified from references lists of publications. Of the 53 publications retrieved, 17 met the inclusion criteria and were extracted for meta-analysis (Table 6.1). Most of the studies were conducted in Western countries; ten in the USA (78, 119, 121, 123-124, 192-196), five in Europe (117, 197-200), one in Japan (201) and one in New Zealand (202). In total, 7, 762 cases and 20, 634 controls were analysed.

None of the associations in the meta analysis was statistically significant. When analysing the occupations by their major classification (Table 6.2), decreased risks (SOR below 1) were found for personal service occupations (SOR 0.85; *95%CI 0.33 – 2.20*), process, plant and machine operatives (SOR 0.94; *95%CI 0.72 – 1.23*) and elementary occupations (SOR 0.91; *95%CI 0.59 – 1.39*). When classifying by sub major occupations (Table 6.3), an increased risk was associated (SOR above 1.2) with agriculture managers (SOR 1.23; *95%CI 0.88 – 1.79*), teaching and research professionals (SOR 3.18; *95%CI 0.51 – 19.82*), secretarial and related occupations (SOR 1.33; *95%CI 0.74 – 2.39*), customer service occupations (SOR 1.39; *95%CI 0.82 – 2.38*) and elementary administration and service occupations (SOR 1.35; *95%CI 0.31 – 5.83*). A decreased risk was associated with corporate managers (SOR 0.74; *95%CI 0.49 – 1.13*), health and social welfare professionals (SOR 0.78; *95%CI 0.24 – 2.58*), process, plant and machine operatives (SOR 0.83; *95%CI 0.57 – 1.21*) and elementary trades, plant and storage related occupations (SOR 0.91; *95%CI 0.56 – 1.5*). The minor occupational group analysis (Table 6.4) showed increased risks associated with secretarial and related occupations (SOR 1.33; *95%CI 0.74 – 2.39*), construction trades (SOR 1.27; *95%CI 0.69 – 2.35*), customer service occupations

(SOR 1.39; *95%CI 0.82 – 2.38*) and elementary personal service occupations (SOR 1.35; *95%CI 0.31 – 5.83*). A decreased risk was associated with functional managers (SOR 0.79; *95%CI 0.56 – 1.12*), government related administrative occupations (SOR 0.89; *95%CI 0.58 – 1.36*), electrical trades (SOR 0.66; *95%CI 0.29 – 1.51*), sales assistant and retail cashiers (SOR 0.88; *95%CI 0.42 – 1.80*), elementary agricultural occupations (SOR 0.67; *95%CI 0.29 – 1.51*) and elementary construction occupations (SOR 0.76; *95%CI 0.35 – 1.65*).

Study design and country were examined as potential causes of heterogeneity however no significantly decreased *P* values for interaction were observed. There was no strong evidence for publication bias as funnel plots of the relative risk estimates showed no clear asymmetry for studies.

Table 6.1: Publications included for meta-analysis on occupation and prostate cancer

AUTHOR	REFERENCE	LOCATION	PUBLICATION DATE	STUDY DESIGN
ERNSTER	(119)	USA	1979	CASE CONTROL
PEARCE	(202)	NEW ZEALAND	1987	CASE CONTROL
BROWNSON	(192)	USA	1988	CASE CONTROL
YU	(193)	USA	1988	CASE CONTROL
OISHI	(201)	JAPAN	1989	CASE CONTROL
LE MARCHAND	(78)	USA	1990	CASE CONTROL
VAN DER GULDEN	(197)	NETHERLANDS	1992	CASE CONTROL
HIATT	(194)	USA	1994	NESTED CASE CONTROL
VAN DER GULDEN	(117)	NETHERLANDS	1995	CASE CONTROL
ANDERSSON	(198)	SWEDEN	1996	CASE CONTROL
ARONSON	(195)	USA	1996	CASE CONTROL
EWINGS	(199)	UK	1996	CASE CONTROL
KRSTEV	(203)	USA	1998	CASE CONTROL
BAND	(123)	USA	1999	CASE CONTROL
PARKER	(121)	USA	1999	COHORT
SANDERSON	(196)	USA	2004	CASE CONTROL
ZEEGERS et al	(200)	NETHERLANDS	2004	NESTED CASE CONTROL

Table 6.2: Summary odds ratios for prostate cancer associated with major group classifications

Major Group	SOC 2000 Code [20]	SOR (95% CI)	Number of studies (reference)
Managers and senior officials	1	1.01 (0.82 – 1.23)	4 (78, 117, 123, 202)
Professional occupations	2	1.06 (0.84 – 1.03)	4 (117, 123, 194, 200)
Associate professional and technical occupations	3	1.02 (0.88 – 1.19)	5 (123, 195, 200, 202-203)
Administrative and secretarial occupations	4	1.11 (0.92 – 1.34)	6 (78, 117, 123, 195, 200, 203)
Skilled trades occupations	5	1.09 (0.95 – 1.25)	5 (117, 123, 194-195, 200)
Personal service occupations	6	0.85 (0.33 – 2.20)	2 (194, 202)
Sales and customer service occupations	7	1.01 (0.79 – 1.29)	5 (78, 123, 200, 202-203)
Process, plant and machine operatives	8	0.94 (0.72 – 1.23)	4 (78, 117, 123, 200)
Elementary occupations	9	0.91 (0.59 – 1.39)	5 (78, 117, 194, 200, 202)

SOR = summary odds ratio for major group occupation ever held
95%CI = 95% confidence interval

Table 6.3: Summary odds ratios for prostate cancer associated with sub major group classifications

Sub major group	SOC 2000 Code (169)	SOR (95% CI)	Number of studies (reference)
Corporate managers	11	0.74 (0.49 – 1.13)	2 (123, 202)
Managers and proprietors in agriculture and services	12	1.25 (0.88 – 1.79)	2 (123, 202)
Science and technology professionals	21	1.03 (0.70 – 1.51)	3 (123, 194, 200)
Teaching and research professionals	23	3.18 (0.51 – 19.82)	3 (117, 123, 200)
Business and public service professionals	24	1.08 (0.69 – 1.71)	2 (117, 123)
Health and social welfare associate professionals	32	0.78 (0.24 – 2.58)	2 (123, 203)
Protective service occupations	33	1.05 (0.84 – 1.31)	4 (123, 195, 200, 202)
Business and public service associate professionals	35	0.94 (0.76 – 1.16)	2 (123, 202)
Administrative occupations	41	1.07 (0.88 – 1.31)	4 (117, 123, 200, 203)
Secretarial and related occupations	42	1.33 (0.74 – 2.39)	2 (123, 195)
Skilled agricultural trades	51	1.17 (0.97 – 1.41)	4 (117, 123, 195, 200)
Skilled metal and electrical trades	52	1.02 (0.77 – 1.34)	4 (117, 123, 195, 200)
Skilled construction and building trades	53	1.09 (0.85 – 1.378)	4(117, 123, 195, 200)
Textiles, printing and other skilled trades	54	1.01 (0.73 – 1.78)	4 (117, 123, 195, 200)
Sales occupation	71	1.04 (0.79 – 1.36)	4 (123, 200, 202-203)
Customer service occupations	72	1.39[§] (0.82 – 2.38)	2 (78, 198)
Process, plant and machine operatives	81	0.83 (0.57 – 1.21)	2 (123, 200)
Elementary trades, plant and storage related occupations	91	0.91 (0.56 – 1.47)	4 (117, 194, 200, 202)
Elementary administration and service occupations	92	1.35 (0.31 – 5.83)	2 (117, 202)

SOR = summary odds ratio for sub major occupation ever held 95%CI = 95% confidence interval
SOR[§] = summary odds ratio for longest held occupation (when ever held occupation was not available)

Table 6.4: Summary odds ratios for prostate cancer associated with minor group classifications

Minor Group	SOC 2000 Code (169)	SOR (95% CI)	Number of studies (reference)
Functional Managers	113	0.79 (0.56 – 1.12)	2 (123, 202)
Managers in farming, horticulture, forestry and fishing	121	1.01[§] (0.50 – 2.06)	2 (78, 198)
Science professionals	211	0.99 (0.55 – 1.77)	2 (123, 200)
Engineering professionals	212	0.95[§] (0.19 – 4.87)	2 (123, 201)
Teaching professionals	231	1.02 (0.69 – 1.50)	3 (117, 123, 200)
Protective service occupations	331	1.05 (0.84 – 1.31)	4 (123, 195, 200, 202)
Sales and related associate professionals	354	0.94 (0.80 – 1.12)	2 (123, 202)
Administrative: government and related	411	0.89 (0.58 – 1.36)	2 (117, 200)
Administrative: general	415	1.05 (0.80 – 1.39)	2 (117, 203)
Secretarial and related occupations	421	1.33 (0.74 – 2.39)	2 (123, 195)
Agricultural trades	511	1.17 (0.97 – 1.41)	4 (117, 123, 195, 200)
Metal forming, welding and related trades	521	0.97 (0.42 – 2.26)	3 (117, 123, 200)
Vehicle trades	523	1.06 (0.86 – 1.30)	4 (117, 123, 195, 200)
Electrical trades	524	0.66 (0.29 – 1.51)	4 (117, 123, 195, 200)
Construction trades	531	1.27 (0.69 – 2.35)	3 (117, 123, 195)
Building trades	532	1.11 (0.57 – 2.15)	3 (117, 123, 200)
Textiles and garments trades	541	0.90 (0.45 – 1.81)	3 (123, 195, 200)
Food preparation trades	543	1.05 (0.77 – 1.53)	4 (117, 123, 195, 200)
Sales assistants and retail cashiers	711	0.88 (0.42 – 1.82)	3 (123, 200, 202)
Sales related occupations	712	0.96 (0.64 – 1.43)	3 (123, 202-203)
Customer service occupations	721	1.39[§] (0.82 – 2.38)	2 (78, 198)
Elementary agricultural occupations	911	0.67 (0.29 – 1.52)	2 (117, 202)
Elementary construction occupations	912	0.76 (0.35 – 1.65)	2 (117, 200)
Elementary personal service occupations	922	1.35 (0.31 – 5.83)	2 (117, 202)

SOR = summary odds ratio for minor group occupation ever held 95%CI = 95% confidence interval
SOR[§] = summary odds ratio for longest held occupation (when ever held occupation was not available)

Conclusion

This is the first meta-analysis on occupational groups which is based on a large total number of cases and controls. Although sufficient power exists to detect associations, no statistically significant associations for any job title were found.

Despite the high number of studies and therefore large case control base, there are some limitations in the study. Some occupations were associated with a slightly increased risk, although not statistically significant. These small excess risks could be due to an unknown or known but unmeasured confounding factor. Publication bias was tested for in this meta analysis; however other types of bias could exist, such as selection bias or an incomplete search for publications. Pooling individual risk estimates into one summary odds ratio is also limiting, as heterogeneity of exposures is not taken into account. For example, subjects with the same job title might be exposed to different agents in different countries. In the pooling process, we assume the same job title will equate to the same type, duration and intensity of exposures. Some occupations with a previously identified increased risk, such as farmers and mechanics also showed an increased risk in the meta analysis. However metal workers were shown to have a decreased risk associated with the disease, in contrast to previous studies. This is perhaps due to the associations being a result of early studies which may not be representative of recent working conditions.

The results suggested moderately increased and decreased risks associated for some occupations however none was statistically significant, despite the large sample size of this meta-analysis, therefore prostate cancer is most likely not an occupational disease.

CHAPTER 7

IS 8q24 ASSOCIATED WITH PROSTATE CANCER?

After exclusion of subjects with missing data, 277 cases and 282 controls remained. There were no significant differences for age (P_{cases}=0.50, $P_{controls}$=0.50) and family history of prostate cancer (P_{cases}=0.95, $P_{controls}$=0.38). The mean age at baseline of cases was 63 years, which was higher than the mean age of the sub cohort (60 years). Only 4.13% of the combined sample had positive family history of prostate cancer. There was strong evidence for linkage disequilibrium between allele -10 of DG8S737 and the A allele of the SNP (χ^2=61.2, P<0.001). No deviations from Hardy Weinberg equilibrium were detected in controls for the frequency of alleles and genotypes for the microsatellite marker DG8S737 and the SNP rs1447295 (P=0.14).

The main results for the genotypic and allelic associations are presented in Table 7.1. The DG8S737-8 allele was unexpectedly associated with a statistically significant decreased risk for prostate cancer (OR, 0.62; 95%CI, 0.40 – 0.96; P = 0.03). The increased frequency of the -10 allele among cases suggested an increased risk of disease, however it was not statistically significant (OR, 1.50; 95% CI, 0.88 – 2.55). The results of all alleles can be found in the appendix. The SNP analysis suggested an increase in the crude OR with the presence of allele A, (OR, 1.38; 95%CI, 0.94 – 2.20), although this was also not statistically significant (P = 0.10). This remained the case for either one or two copies of allele A (OR, 1.40; P = 0.14 and OR, 1.54; P = 0.48 respectively). The results were not considerably different after multivariable adjustment which could suggest an assumption of Mendelian randomisation can be made in this context (204). No influence was observed for TNM stage (data not shown).

Table 7.1 Crude and Multivariable adjusted odds ratios for rs1447295 and DG8S737

	Alleles	Cases (n)	Controls (n)	OR (95% CI)	P	Adjusted OR (95% CI)	P
rs1447295							
Allelic Association	Allele A absent	318	346	1		1	
	Allele A present	142	114	1.38 (0.94 – 2.02)	0.10	1.39 (0.94 – 2.04)	0.10
Genotypic Association	C/C	224	196	1		1	
	A/C	53	64	1.40 (0.90 – 2.18)	0.14	1.41 (0.90 – 2.21)	0.14
	A/A	4	7	1.54 (0.46 – 5.09)	0.48	1.56 (0.46 – 5.34)	0.48
DG8S737							
Allelic Association	Allele -8 absent	417	394	1		1	
	Allele -8 present	43	66	0.61 (0.39 – 0.94)	0.03	0.62 (0.40 – 0.96)	0.03
	Allele -10 absent	417	430	1		1	
	Allele -10 present	43	30	1.50 (0.89 – 2.53)	0.13	1.50 (0.88 – 2.55)	0.13

Adjusted for age, alcohol intake from wine, body mass index (BMI), energy intake, family history of prostate cancer and level of education

Conclusion

This analysis provided an interestingly significant decreased risk associated with allele -8 of the microsatellite marker. This is unlikely to be a result of population structure, due to the matching of cases and controls, the absence of apparent outliers and the relative genetic homogeneity of the Dutch population (205-206). The apparent protective effect is in contrast with the Amundadottir study (146). In the latter analysis, the associations of the -8 allele and the A allele of rs1447295 were detected in all of the Caucasian populations studied, however, they were not replicated in African American men. Since this population is more genetically diverse, Amundadottir et al concluded that the variant responsible for conferring the increased prostate cancer risk must be the DG8S737 -8 allele itself or be extremely close to it.

However, the results of this analysis and those of Suuriniemi *et al* do not detect an association, which indicate that the allele itself may not be directly responsible for conferring increased prostate cancer risk (147). It is possible that DG8S737 and rs1447295 are tightly linked markers flanking the actual causative variant and that there may be potentially more than one high risk haplotype present in the Caucasian population. Wang *et al* observed a stronger association with the 8/A haplotype and familial prostate cancer, whereas the 10/A haplotype was most strongly associated with aggressive prostate cancer, which supports the Suuriniemi study (207). Future studies comparing more detailed haplotypes in this region could help to narrow this interval and enable identification of the actual causative variant.

DISCUSSION

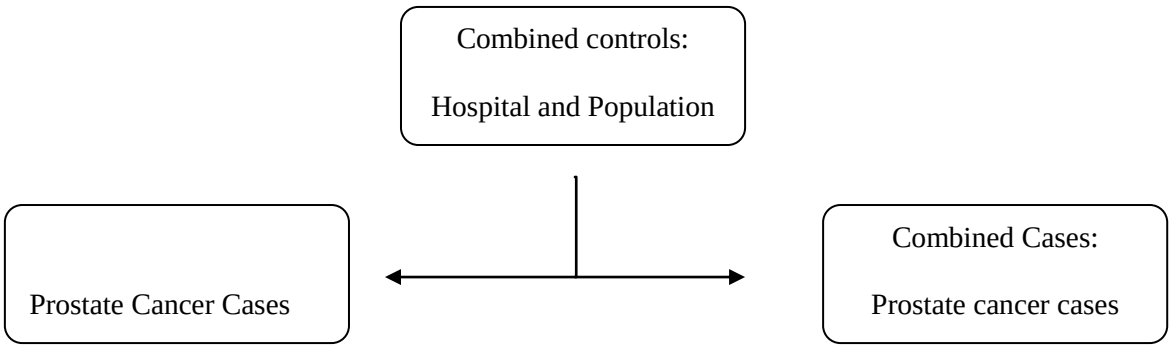
CHAPTER 8

8.1 General discussion

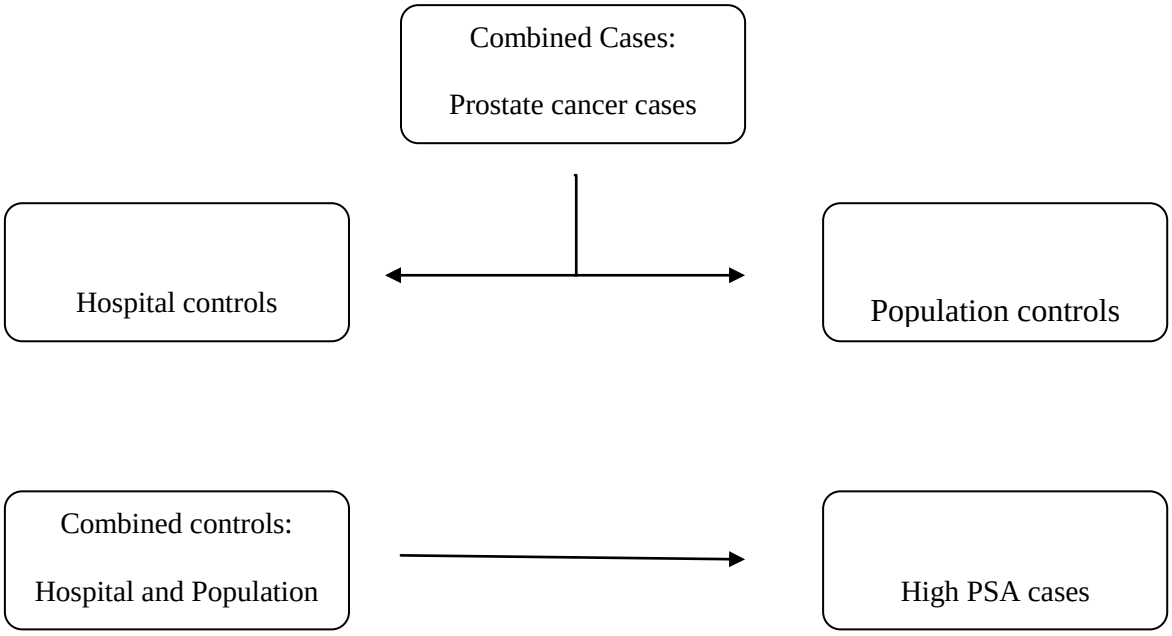
This case control study was a novel approach for investigating prostate cancer, due to the categorization of groups. Instead of the traditional case control study design of prostate cancer cases versus healthy controls from the population, the associations at varying risk of the disease were investigated by comparing cancer cases, elevated PSA cases, hospital controls and healthy population controls. By analyzing between and within group differences, the use of PSA as a marker for prostate cancer could be assessed, and also whether there is a difference between the use of hospital based controls and the traditional population based controls. For each chapter, the same case control strategy was used for comparison (see Figure 8.1), however not all tables were presented. All additional analyses can be found in appendices 4 – 8. The effect of the variable of interest remained consistent when either prostate cancer cases or high PSA cases were used. It can therefore be concluded that for the factors studied in this thesis, the use of high PSA cases in epidemiological studies on prostate cancer could be useful, especially when prostate cancer cases are difficult to recruit. This also serves as further evidence for the use of PSA as marker for prostate cancer. The same was true for hospital based controls and population based controls. These groups were combined for final analyses to increase statistical power.

Figure 8.1 Case Control Comparisons

Reported associations:



Appendix associations:



Little is known about the carcinogenesis of prostate cancer; age, family history and ethnicity are the only well established lifestyle risk factors associated with the disease (208-209). The risk of disease increases after the age of 55 years. It is also up to 60% higher in African American men compared to white men. A first degree relative increases the risk up to threefold and can increase with more relatives. These risk factors are not modifiable; therefore further epidemiological studies are still required in order to prevent the disease and to gain more insight into its pathogenesis. An obvious factor under investigation is obesity (see chapters four and five), which translates to diet, physical activity and body size. A Western diet usually involves high intake of fat and calories. Studies on prostate cancer incidence and a diet of high saturated fat report elevated risks (210). Previous studies have been unable to show substantial association between obesity and prostate cancer (211). Some studies have reported a reduced risk of prostate cancer associated with physical activity, however others report the opposite. The difficulties of measuring this exposure accurately at different life periods have been previously discussed in chapter four. Occupation has also been studied, specifically occupational exposures, and although there is some strong evidence for association with the farming, mining, metal and rubber industries, no definitive conclusions have been drawn. This is probably due to small sample sizes for each occupational group (see chapter six). A number of epidemiological studies have suggested sunlight deprivation increases incidence of prostate cancer and a high level of cumulative UV exposure has a protective effect against the disease. This has also been previously discussed in chapter three.

The advent of genome wide association studies (GWAS) have brought to light a number of loci which may harbour prostate cancer susceptibility genes. However the relative

importance of variants located within them is questionable. Hereditary prostate cancer is known to be caused by a number of genes interacting with environmental factors. It is believed that interaction among the genes themselves may also be involved. Genome wide association studies have detected different loci and different genetic variants; however these only explain a small increase in prostate cancer risk. The different genes are also likely to be associated with different population frequencies in different ethnic groups. More recently, the variant allele -8 (22 repeats) of the microsatellite DG8S737 has been extensively studied, along with additional variants in the 8q24 region (160). Despite the promising results, this region has no known protein encoding genes. The closest cancer related gene to the region is *C-MYC* which has been associated with prostate cancer risk. It has been hypothesized that other genes involved in testosterone response might also play a role, such as genes in the polyamine pathway (152). Further work is being carried out on genes involved in the polyamine biosynthesis pathway and prostate cancer, using the data from BiPAS. Tagging SNPs were identified for the following genes: ornithine decarboxylase (*ODC*), S-adenosylmethionine decarboxylase (*SAMDC*), spermidine synthase (*SDS*), aspartate transaminase (*GOT2*), aminoacyclase (*ACY1*) and ornithine aminotransferase (*OAT*). Genotyping is being performed using Taqman SNP Genotyping Assays (Applied Biosystems, CA) for the markers.

The data from BiPAS have contributed to collaborations with the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium. DNA samples for prostate cancer cases and hospital based controls were sent to the consortium for a follow up of a previous genome wide association study, which led to a paper in Nature Genetics (161). A further international collaboration

was achieved with researchers from Maastricht University and New York University, with the analysis of the genetic data in chapter seven, which resulted in a publication in the European Journal of Human Genetics (212).

8.2 Summary and implication of findings

Chapter three investigated the association of ultraviolet radiation exposure and prostate cancer using results from the Birmingham Prostatic Neoplasms Association Study. A statistically significant increased risk of prostate cancer was observed with lower exposure to sunlight during early adulthood. A statistically significant protective effect for higher sun exposure was also observed in early adulthood. The results show that instead of analysing chronic exposures over time, acute exposures may be more important in prostate disease. It is biologically plausible that exposure to vitamin D in early life can contribute to a reduced risk. Exposure to high levels of the hormonal form of vitamin D causes alterations on the cellular composition of the prostate in rats (213). The normal ratio of epithelial cells to stromal cells in rodents is 5:1 (214). However when exposed to high levels of the vitamin, the prostate gland is mostly composed of stromal cells. Carcinogenesis is a type of cancer specifically arising from epithelial cells, therefore a reduction in the epithelial cell population will cause a reduction in prostate cancer risk. Future studies should take this into account, and gather information from participants regarding exposures in younger life, especially in UVR studies, as more time is generally spent outdoors in this life period compared to later years.

Chapter four examined the association of physical activity and prostate cancer in BiPAS. A consistent reduced risk of prostate cancer was observed for men who ever cycled in all

analyses. Conversely, ever having played competitive sports was associated with increased risk of prostate cancer among all comparison groups, including for both localized and advanced tumours and when adjusted for testosterone levels. In summary, high levels of low intensity activity were generally found to have an increased risk and participants who had ever taken part in high intensity activity generally had a decreased risk. In this context, the results were consistent with previous studies and warrant further studies on different intensities of exercise.

Chapter five studied the effect of different measures of body size on the risk of prostate cancer. A protective effect was observed for waist circumference of over 37cm. A decreased risk was associated with overweight men (BMI 25.0 – 29.9) and an increased association was observed in obese men (BMI of 30.0 or over). No significant associations were detected for adult weight change. Interestingly, a highly significant increased risk was associated with a BMI classified as obese at age 20; however this effect seems to disappear with age. Very few previous studies have examined body size in early life; the main limitation of such studies is the small number of obese subjects at a young age. The data available on early adulthood obesity suggest it is inversely associated with advanced prostate cancer but not with localized cancer. Further research into the complex association is required to fully elucidate the biological action of obesity on prostate carcinogenesis.

Chapter six was a large meta analysis of 7, 762 cases and 20, 634 controls investigating the association between occupational groups and prostate cancer incidence. Despite the large case control base, we were unable to detect any statistically significant associations. Increased risks were observed in farmers and mechanics, which are consistent with

previous literature, however the results were not statistically significant. In future studies, more detail on exposures should be taken into account rather than job title or industry. In particular, type of exposures and the duration would be most interesting, better methods for assessing such factors need to be developed.

Chapter seven was a replication study of a whole genome wide association study on markers on chromosome 8q24. Allele -8 of microsatellite DG8S737 and allele A of single nucleotide polymorphism rs1447295 were investigated in a nested case control study from a Dutch population. The effect estimate for rs1447295-A allele was in the same direction as previous studies however it was not statistically significant. Interestingly a statistically significant decreased risk was detected for DG8S737–8 allele, in contrast with previous studies. It is more likely that DG8S737 and rs1447295 are tightly linked markers flanking the actual causative variant and that there may be potentially more than one high risk haplotype present in the Caucasian population. Future studies should try to compare more detailed haplotypes in this locus among populations in order to further narrow this interval and assisting with identification of the causative variant.

8.3 Limitations of study

Self administered questionnaires are one the cheapest methods of collecting information in research; however a major limitation of them is that they are limited to literate populations. In BiPAS, the questionnaires were largely completed by the subjects; however some were completed by a family member acting as an interpreter. Another disadvantage is the researcher has little control over the quality of data collected. In the questionnaire, closed ended questions were used where possible to reduce the bias arising from interpretation

bias. The study was piloted with a small number of patients and some questions were adapted to allow ease of completion and data entry. The main changes were adapting open questions to closed questions, such as in the case of UVR exposure. Patients were originally asked to recall the number of hours per day they spent in the sun; this was subsequently changed to options of a range of hours. All questions were worded in simple language to avoid ambiguity or inference of a preferred answer. The questions order was also adapted to a logical sequence.

Although some parts of the questionnaire were validated, such as the UVR sections and anthropometry questions, some sections were not, which is another limitation of the study. Validity is defined as the extent to which an instrument measures what it intends to measure. This can only be evaluated when there is a reference procedure or gold standard. It is usually difficult to carry out, expensive and sometimes impossible. Also validity in one population may not guarantee validity in another.

8.4 Prostate cancer: current status and future research

8.4.1 Research on environmental exposures

Risk factors for prostate cancer have been extensively studied in the past yet few definitive causal associations have been identified. A popular risk factor under study is obesity due to its prevalence in the western world. However fat distributions and alternative measurements to BMI are yet to be investigated fully. Within this theme body size, physical activity and diet are also common factors under study. All of these factors form part of the body's natural energy balance, suggesting an imbalance of any or all of these factors is important in disease progression. For prostate cancer, higher levels of physical

activity have been shown to reduce mortality (215). The population attributable risks (PAR) associated with risk factors investigated are quite high. Friedenreich and colleagues calculated gender specific PARs for a number of cancer sites associated with physical activity (83). They calculated approximately 14% or 52, 464 new cases of prostate cancer could be prevented if sufficient levels of physical activity were achieved by all European men. A study by Aronson *et al* detected strong associations for four environmental exposures (metallic dust, liquid fuel combustion products, lubricating oils and greases and polyaromatic hydrocarbons from coal) (195). When all four exposures were combined, they were estimated to cause 12% of prostate cancer in the study population. Such high PARs represent an important public health issue. Few studies have evaluated the time course of risk factors, defining when a risk factor has a real impact on disease will better inform clinical interventions. Understanding when a factor impacts on progression is particularly important in a disease such as prostate cancer which occurs in later in life. In prostate cancer studies, measures at time of recruitment are often used for analysis, whereas in fact when time course of exposures are used, a more accurate insight could be gained. Such analyses allow preventative measures to be focused to allow behaviour change which could reduce risk or even prevent recurrence. It also provides insight to when treatments would be most beneficial. Early life exposures, such as the ones investigated in this thesis are important. Advice could be offered to family members of affected men to allow behaviour change in offspring and reduce risk.

8.4.2 Research on genetics

Genome wide association studies on various types of cancers first appeared in representative journals in 2005. Since then, a number of common DNA sequence differences have been shown to influence genetic susceptibility for over 40 different

common diseases. The current standard of over 1, 000 subjects for case and control groups provide much more power to detect small effect sizes and identify new unsuspected candidate genes (216). However this ability has identified multiple genomic regions with no known protein-coding genes, as in the case of 8q24 and prostate cancer. Despite the limitations, collaborative GWAS are considered to be an essential strategy for identifying genetic susceptibility for major human diseases. A number of GWAS have been successful at identifying variants associated with prostate cancer, some of the regions include 3p12, 3q21, 8q24, 11q13, 17q24, 19q13 and 22q13.2 (217). An interesting study by Al Olama *et al* in 2009 reported many of the variants mentioned above are localized to blocks in high linkage disequilibrium with 8q24 (218). It is fair to say that more positive hits have been found for prostate cancer than any other type of cancer. This supports the hypothesis that prostate cancer is caused by a number of common variants with small effects (219). The variants detected have little effect on severity of disease, suggesting that they have more influence on tumour initiation than progression. This is discussed in more detail in chapter seven. Also, the correlation of these variants with non genetic factors is unknown, due to the large sample sizes needed to investigate such effects. In a study by Lindstrom *et al*, 39 SNPs identified by GWAS were examined for gene environment interactions with known or proposed risk factors for prostate cancer. No statistically significant association were detected for interactions with family history of prostate cancer, BMI, smoking and alcohol consumption (220). There is a large amount of evidence of the genetic basis for the disease; however it has proved to be extremely difficult to pinpoint specific inherited susceptibility genes. As previously mentioned, it is believed that a number of genes could be interacting together, possibly even with environmental factors to cause the disease. Future candidate gene studies should take into account the effects of multiple genes instead

of concentrating on single genes, and their interaction with environmental factors. In order to study such small effects, larger sample sizes would be required. The results of this thesis show the traditional prostate cancer cases and population based controls could be supplemented with high PSA cases and hospital based controls to improve on power. More sophisticated analysis techniques are still required to study such large sample sizes and to model the complex genetic pathways. Collaborative efforts could also achieve the larger sample sizes, as shown in the worldwide consortiums such as the International Consortium for Prostate Cancer Genetics (ICPCG), Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) and the Breast and Prostate Cancer Cohort Consortium (BPC3) (162, 221-222).

Prostate cancers can also arise from somatic epigenetic alterations (223). In normal cell DNA, an asymmetric methylation of cytosine bases occurs in the nucleotide sequence CpG. Somatic changes in DNA methylation at these CpG islands are associated with gene silencing, often affecting activity of tumour suppressor genes (224). Chromatin protein marks work alongside DNA methylation to regulate gene activity. This process is also altered in cancer cells. The role of GSTP1 in prostate cancer has been extensively studied, particularly its activity after methylation. Hypermethylation of the gene is consistently detected in over 90% of prostate cancers (225). Over the next few years, more research in DNA methylation and chromatin protein marks will provide better insight in prostate cancer phentotypes, providing more opportunities for biomarker discovery and targeted treatment options.

8.4.3 Future use of PSA in testing

The long latency period of prostate cancer makes it an excellent candidate for a testing programme for detection. The digital rectal examination (DRE) and PSA testing are routinely performed worldwide as a means of diagnosing prostate cancer, despite the controversy surrounding its effectiveness. The DRE was introduced in the USA in the 1970s and PSA blood testing was approved by the Food and Drug Administration (FDA) in 1986. The two combined caused a huge improvement in the treatment and overall survival rates for prostate cancer patients. Although the DRE is the most efficient at detecting large prostate tumours, they are often advanced and may have metastasized. Guidelines for the use of PSA exist to enable efficient screening. The National Comprehensive Cancer Network (NCCN) guidelines for the USA state high risk patients include men of African American origin or a family history of prostate cancer (226). High risk men with a baseline PSA $\geq$ 1.0 ng/mL are recommended to have a follow up PSA test and DRE one year later. If after a year the PSA result is still $\geq$ 1.0 ng/mL, annual PSA testing and DREs are recommended. If an abnormal result is detected on the DRE at any stage, patients should be referred for biopsy and further tests. The NCCN guidelines also take into account PSA velocity. The NICE clinical guidelines used in the UK stratify men with localised prostate cancer into three groups (see Table 8.1). Clinicians treating men in the low risk group are advised to use watchful waiting and active surveillance (227). Intermediate groups should be offered radical prostatectomy or radical radiotherapy. Men in the high risk category are offered systemic treatment such as adjuvant hormonal therapy combined with radiotherapy. The PSA values used in the UK are much higher than the NCCN guideline, which is likely to be a reflection of the different healthcare systems.

Table 8.1 Risk stratification for men with localised prostate cancer

Risk	**PSA ng/ml**		**Gleason Score**		**Clinical Stage**
Low	<10	And	≤6	And	T1 – T2a
Intermediate	10 – 20	Or	7	Or	T2b – T2c
High	>20	Or	8 – 10	Or	T3 – T4

Taken from the National Institute for Health and Clinical Excellence (2008) *Prostate cancer treatment and diagnosis*. (58). London: National Institute for Health and Clinical Excellence.

Although PSA has some clear advantages as a marker, it also has some limitations. For example, a PSA test alone cannot differentiate between lethal and non lethal prostate cancer. Another disadvantage is no clear benefit for treatment; a patient will receive a cancer diagnosis that they would not have otherwise had due to screening, but they often do not require or benefit from treatment. Patients that are treated could suffer from adverse effects of unnecessary treatment. A recent study by Strope and Andriole proposed two opposing mechanisms were occurring in prostate cancer diagnosis; although PSA screening was detecting higher numbers of localized tumours, pathologists were less likely to grade cancers as well differentiated and therefore tumours were being assigned to higher Gleason scores (228). Side effects of radiation therapy include diarrhoea, urinary frequency, dysuria and skin changes. Long term effects include urinary incontinence and sexual dysfunction. Side effects of hormonal therapy include impotence, decreased libido and weight gain. The main risk associated with active surveillance as a treatment option is anxiety. However this choice is still a better overall option to reduce overtreatment. Etzioni *et al* describe the widespread use of PSA testing as one of the greatest uncontrolled experiments in modern medical history (229). Despite the lack of substantial evidence that PSA testing is beneficial, it is routinely used in the USA and is used widely in many other countries. The study reported PSA testing is likely to explain half or more of mortality reduction in the USA since the early 1990s, and therefore a testing programme is beneficial to prostate cancer control (229).

Two recent clinical trials were carried out to resolve controversies regarding PSA testing. The Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial (PLCO) was set up in the USA to assess the difference in mortality from testing for the specified diseases (230).

The study reported that higher levels of testing make little difference to mortality from prostate cancer. The European Randomized Study of Screening for Prostate Cancer (ERSPC) compared testing versus no testing and reported that prostate cancer mortality was reduced in the tested group (25). This study used a PSA level of 3.0 ng/ml as an indicator for biopsy. After further investigation, it was reported that 80% of men between the ages of 55 and 74 years have a PSA lower than this cut off value and therefore do not receive biopsies, which could lead to tumours in this group being missed. However taking biopsies of the whole study population would identify seven times more cancers than those who were actually at risk of dying from the disease. The researchers therefore concluded that although cut off values in a testing programme could miss cancers, the overall benefits of outweigh the risks of over diagnosis and over treatment. Although testing for the disease has been shown to reduce mortality, the poor diagnostic performance of PSA contributes to the risk of over diagnosis and over treatment. PSA testing could be improved by using a combination of different biomarkers. A number of urinary markers are currently being developed; one promising test is the PCA3 test. A review by Ploussard and de la Taille showed a vast improvement of prostate cancer detection by using a combination of PSA and PCA3 (231). PCA3 is a prostate specific gene and is known to be over expressed in prostate cancer (232). It is a non-coding RNA and though the exact function is unknown, one potential mechanism is in gene regulation, in a similar way to PCGEM1, another prostate specific gene which is expressed in the androgen receptor. A number of molecular techniques have consistently reported PCA3 outperforms serum PSA in cancer diagnosis. Since PSA is not specific to prostate cancer, elevated levels could be due to benign prostatic hyperplasia or other prostatic conditions. However if PSA is used in conjunction with PCA3, the accuracy of diagnosis could be vastly improved (232).

The main burden to public health is the costs associated with treatment. Any strategy to reduce prevalence of the disease or delay the need for biopsy and/or treatment will therefore reduce the number of men at risk for adverse symptoms associated with treatment. An accurate testing programme to reduce the burden would be advantageous. As discussed above, PSA testing cannot distinguish between aggressive and non aggressive disease and cannot solve problems associated with over treatment and over diagnosis. However the use of a combination of different markers could be implemented in a detection programme, where the risks and benefits associated with testing were clearly explained to the population. Some limitations of PSA testing have been previously mentioned, such as overdiagnosis and overtreatment. A number of other risks exist for PSA testing and the DRE itself. The PLCO study reported harmful effects of phlebotomy for PSA testing include dizziness, bruising and fainting (230). The most frequently reported harmful side effect is anxiety, especially following an elevated result which could turn out to be a false positive (233-234). Complications associated with DRE include discomfort and sometimes bleeding (226).

Better methods for detection and treatment of early stage tumours are required. The current research is aimed at developing new biomarkers to complement PSA and compensate for its limitations as a prostate cancer biomarker. Since prostate cancer is a slow growing tumour which can be asymptomatic for a number of years, not all men would benefit from early detection. The American Cancer Society Early Detection Guidelines for Prostate Cancer recommend testing should be limited to men with at least a 10-year life expectancy. Men with serious co-morbidities which affect life expectancy, such as severe

chronic pulmonary obstructive disease end stage renal disease and life limiting cancer, are unlikely to benefit from screening as are men of advanced age.

8.4.4 Future use of PSA in research

As previously discussed, the use of high PSA cases could be advantageous in case control studies. When considering results for chapters 3-5, similar results were detected for both prostate cancer cases and high PSA cases, therefore it can be inferred that the high PSA case groups could be combined with case groups to increase statistical power. Another potential use for PSA would be as a surrogate endpoint for prostate cancer. Changes in PSA level could be used to measure how well a treatment works. A clinical trial by Petrylak and colleagues noted changes in the PSA level of patients were reflective of outcomes of the trial (235). For men in the treatment arm of the study, serum PSA level decreased by 30% in the first three months of entering the study. The risk of death in this group of men was also reduced by 50%. More studies on the use of PSA as an endpoint are required to validate these findings. PSA testing is a useful tool for the analysis of severity of disease. With the widespread use of screening, a much higher number of patients with early localized disease are identified. PSA levels can be used to categorize cases into severity of tumours for studies on disease progression.

8.4.5 Future studies on prostate cancer

With the four distinct groups of patients within BiPAS, the effect of different exposures on disease progression was analysed, which help to pinpoint the effect of certain factors. Demographic differences of all groups were reported in each chapter. Prostate cancer patients were older than both control groups and had a significantly higher percentage of

family history of prostate cancer compared to population controls (but not hospital controls). For the factors investigated in this thesis, there was little difference in associations to prostate cancer patients or high PSA cases. The results for population controls and hospital based controls were also mostly consistent. The two categories within the case control groups were combined for all chapters to give more statistical power. The strengths and weaknesses of different types of control groups have rarely been evaluated (236-238). It is generally believed that population controls are the gold standard for a control base, as they normally arise from the same primary base as cases, which is a methodological advantage. However, this can be a challenge in some studies as there is not always a suitable resource from which to draw such controls. With this study, we were able to show similar associations for hospital based controls, therefore in future case control studies; benign prostatic hyperplasia or patients with similar prostatic diseases could be used as an effective control group. A study on occupational risk factors by Aronson *et al* in 1996 also pooled hospital and population based controls after testing for heterogeneity between the groups. They did not detect conflicting results for either groups and therefore advocate the idea of combining the groups for more statistical power.

Epidemiological studies on lifestyle factors continue to report conflicting results for exposures, suggesting a highly complex disease process. Therefore study designs need to be refined in order to investigate the effect on different stages of cancer as well as different types of exposure. In addition, more research into biological mechanisms of the known variants and their interaction with other pathways is required in order to converge the knowledge of genetic and environmental causes of prostate cancer.

We are still in the infancy of analysis and follow up of GWAS, therefore statistical methods need to be refined to complement the emerging high throughput techniques. The new phase of next generation sequencing and consortium collaborations for both genetic and environmental studies will yield many more promising tools for the diagnosis and prognosis of urological cancers.

8.5 Concluding advice

8.5.1 Advice to prostate cancer patients

The main findings of this thesis can be split into lifestyle factors and genetic predisposition; however it may not be appropriate to advise cancer patients of all such findings. The main adverse consequence of awareness of a genetic predisposition for a disease is anxiety. However, knowledge of genetic information can prompt beneficial actions or avoidance of harmful ones. Although no risk factor is established to reverse disease progression, patients should be made aware of plausible protective risk factors such as dietary intake of vitamin D and a sufficient level of physical activity, although it should be clear that these are not guaranteed to treat the cancer. For the risk factors investigated in this thesis, many of the exposures have an effect in early adulthood. Once these risks are fully established, they should also be reported to the general public to promote healthy behaviours for prostate cancer prevention.

8.5.2 Advice to clinicians

The American Cancer Society Early Detection Guidelines for Prostate Cancer advise a set of core information should be provided to patients before testing. Clinicians are advised to point out that prostate cancer is an important concern for men and that testing by PSA tests

and DREs can detect tumours earlier. Patients should also be told however that there is no established evidence that prostate cancer testing can reduce the risk of dying from the disease and there is no guaranteed benefit from treatment. The side effects of all treatments should be discussed. It should be made clear that there is a possibility for false positive and false negative results. Finally, the side effects and complications of biopsy should also be discussed (226). In conclusion, there is a great deal of controversy surrounding the use of current detection methods. Clinicians are also advised not to test asymptomatic men over the age of 75 or men with serious co-morbidities, which results in a life expectancy of less than 10 years.

For men in high risk groups for cancer, advice on modifiable lifestyle factors should be provided. Physical activity is widely recognized as an important component of cancer prevention programmes. Current guidelines from the US department of Health and Human Services and the World Cancer Research Fund recommend 30-60 minutes of moderate intensity activity for at least five days a week. Although the exact details on the optimum type, dose and intensity of activity are not known, this guideline is a useful reference standard. The protective effect of UVR could also be discussed; however clinicians should be cautious due to the very strong association of sunlight to skin cancers. Despite the significant findings in this thesis, when put into perspective with all other literature in this area, more firm recommendations cannot be given to clinicians.

8.5.3 Advice to researchers

One of the main findings of the novel study design of BiPAS is the similar associations for the cases and controls groups. This design could be used in future epidemiological studies

to either increase statistical power if the effect of factors is similar, or to look at differences at different stages of disease if they are not similar. Another interesting feature was the inclusion of testosterone summary scores. With this information, it was possible to exclude the effects of testosterone for the associations observed. For some of the factors under investigation, exposure in early adulthood was important. Future studies should record data for different life periods where possible, especially for the period of between 20 years and 30 years of age. Further studies on physical activity are required with more detail on activity types and specific duration. In studies examining obesity, different measures of obesity are important. Although BMI is quite reliable as a measure for obesity, it does not take into account different fat distributions. For this thesis, a pictogram was used which proved to be an effective tool for this. For future studies on occupation, new methods to assess exposure type and duration need to be developed. Finally, more replication studies are required to elucidate the role of genetic variants identified by GWAS. Where the variant is not the actual causative factor, gene environment interactions and more detailed haplotype analysis should be applied.

8.6 Conclusion

Despite the extensive research on prostate cancer, the complexity of the disease means it remains difficult to pinpoint causal factors. Multiple genetic variants have been implicated and few environmental factors have been fully established. We are yet discover a recognized causal factor, as in the case of smoking and lung cancer or the *BRCA1* gene and breast cancer. It could be argued that such a factor does not exist for prostate cancer. Although this thesis was unable to establish definitive causal factors, it does add to the current knowledge by highlighting the importance of environmental exposures in early

adulthood. The answers are more likely to lie in the more complex gene-environment or even gene-gene interactions. Future studies on prostate cancer, and indeed other complex cancers and chronic diseases should concentrate on these effects. The traditional case control design could also be modified by the inclusion of different types of cases and controls, as shown by this thesis.

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Appendices

Appendix 1: Participant information sheet

UNIVERSITY OF BIRMINGHAM

Birmingham Prostatic Neoplasms Association Study (BiPAS)

Chief Investigator: Professor Maurice Zeegers
The University of Birmingham
Name of your doctor:

We would like to invite you to take part in a clinical research study.

Taking part in the study is entirely voluntary. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish and please ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

The following information sheet is in two parts:
Part 1 tells you the purpose of this study and what will happen if you take part.
Part 2 gives you more detailed information about the conduct of the study.

Part 1 – Purpose and your role in the study

1. What is the purpose of the study?
Prostate cancer is an important health problem. It is the most common cancer in the UK. However, the cause of prostate cancer is still not known. In this research we aim to identify lifestyle or genetic causes of this disease. With this knowledge we hope to be able to find clues for the prevention or cure of prostate cancer.

2. Why have I been chosen?
You have been referred to a prostate or flow clinic based on a suspicion of a prostate abnormality. We are hoping to collect information on patients before a formal diagnosis is made. We intend to study at least 2000 patients over the next 2 years.

3. Do I have to take part?
No. it is up to you to decide whether or not to take part and you do not have to decide straight away. If you do agree, you will be given this information sheet to keep and asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time or a decision not to take part, will not affect the standard of care you receive or your relationship with your doctor.

4. What will happen to me if I take part?
If you agree to take part and have signed a consent form, the study will run alongside your standard treatment. The study will be conducted over 5 years however; your actual involvement will take approximately one hour of your time. When you come

to the clinic, our research nurse (this will change after one year) will talk to you about the study and will ask you to fill in a questionnaire. The questions will be on your background, medical history and lifestyle. We would need you to give us about 45 minutes of your time to fill in this questionnaire. We will also ask you to give one extra tube of blood in addition to the blood that you would normally give. In addition we will ask you permission to look through your medical notes.

5. What do I have to do?

Other than your normal treatment, we would need you to complete our questionnaire and let us have a sample of your blood. Blood will be drawn from a vein usually inside the elbow or from the back of the hand. The site is first cleaned with an antiseptic, a tourniquet is placed around the upper arm to temporarily restrict blood flow and a sterile needle is inserted into a vein. Blood is collected in an air tight vial or syringe.

6. What will happen to the blood samples taken as part of this study?

The blood samples that will be collected as part of the research study will be stored centrally at a laboratory at The University of Birmingham. The blood sample will be used to investigate potential prostate cancer genes. In addition we would also like to store your DNA and later use the samples donated as part of this study for future research, although such research projects have not yet been planned and could occur many years in the future. These future research projects may involve studies of your genes and DNA. By giving your consent for your blood to be stored you will be offering your samples as a gift.

The blood and DNA samples will be stored under strict security and are given a code, so that the researchers receiving the samples do not know your name or any other personal details. Researchers who wish to use the samples that are stored will only be given access to the samples after their research has been approved by an Independent Research Ethics Committee who makes sure that the research is in the interest of the patients and is carried out safely.

7. What are the possible disadvantages and risks of taking part?

There are no foreseeable risks in taking part. This study will run alongside your routine treatment and follow up; it will not influence this process.

8. What are the possible benefits of taking part?

There is no intended immediate clinical benefit from taking part in this study. However, the information obtained from this study may result in changes in the future prevention,
diagnosis, treatment and follow up of patients with prostate cancer or benign prostate hypertrophy. These changes may also benefit you.

9. What happens when the research study stops?

When the study stops your routine treatment and follow up will continue in the normal way, although it may incorporate new discoveries or information generated by this study.

10. What if there is a problem?

Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed. More detailed information regarding this is given in part 2 (please refer to question 15). However if you have any problems concerning the study, then please do not hesitate to contact the local investigator (01214721311) or the Chief Investigator, Professor Zeegers (01214146721).

11. Will my taking part in the study be kept confidential?

Yes. All information which is collected about you during the course of the research will be kept strictly confidential. The details are included in Part 2.

12. Contact Details about the study

If you have any concerns or other questions about this study or the way it has been carried out, you should contact the urologist listed below:

Contact Details:
Urologist: Telephone:

This completes Part 1 of the information sheet. If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

Part 2 – Information about the study

13. What if relevant information becomes available?

Sometimes during the course of a research project, new information becomes available about the disease that is being studied. If this happens, your research doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your urologist will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information, your research doctor might consider it to be in your best interest to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue. If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

14. What will happen if I don't want to carry on with the study?

As mentioned in Part 1 of this form your participation in this study is entirely voluntary and you are free to withdraw from it at any time. If you withdraw from the study, we will destroy all you identifiable samples, but we may still need to use the data collected up to your withdrawal. With your permission we would like to collect follow up information about you from the NHS Central Register (NHSCR).

15. What if there is a problem?

If you have a concern about any aspect of the study; you should ask to speak to the researchers who will do their best to answer your question. If you remain unhappy

and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the research sponsor (University of Birmingham) but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

16. Will my taking part in this study be kept confidential?

All information, which is collected about you during the course of the research, will be kept strictly confidential. If you agree to take part in this study we will need you to sign a consent form. You will be given a copy of the consent form and this information sheet to keep.

We would like to collect some contact details from you including your current address and telephone number. We would like to collect these details so that we contact you again in the future. Your contact details will be kept strictly confidential and only members of the BiPAS research team would be allowed access to them.

Information on all patients entered into this study will be sent to the BiPAS Study Office which is located at the University of Birmingham where it will be retained in secure storage and handled according to the 1998 Data Protection Act. No personally identifiable information will be released from the BiPAS study office. Limited clinical information may be passed on to researchers within the UK. It would not be possible to identify any patient from this information and any information provided will be handled according to the normal standard of medical confidentiality and data protection. With your consent we will also be informing your GP of your participation in the study.

17. What will happen to the samples I give?

The blood samples that will be collected as part of this research study will be stored centrally at a laboratory at The University of Birmingham. The blood samples will be used to investigate prostate specific antigen (PSA) levels and potential prostate cancer genes. In addition, we would also like to store your DNA and later use the samples donated as part of the study for future research, although such research projects have not yet been planned and could occur for many years in the future. These future research projects may involve studies of your genes and DNA. By giving your consent for your blood to be stored you will be offering your samples as a gift.

The blood and DNA samples will be stored under strict security and are given a code, so that researchers receiving the samples do not know your name or any other personal details. Researchers who wish to use the samples that are stored will only be given access to the samples after their research has been approved by an Independent Research Ethics Committee who makes sure that the research is in the interest of the patients and is carried out safely.

18. Will any genetic tests be done?

Participants who have been recruited from hospitals will be tested for specific genes which have been linked to possible increased risk of prostate cancer. The samples will be fully anonymised and the results from these tests are not expected to be meaningful and available to individuals at this stage. The research may inform future testing programmes which would make information available later through the NHS. Samples and information may be retained for future genetic studies in which case additional consent will be sought from the participants or the study will be presented to an ethics committee for consideration.

19. What will happen to the results of the research study?

Important results from the study will be published as they become available, which may be during the course of the study or after the study has finished, and this could possibly take several years. We intend that any results will be published in peer reviewed journals or will be presented at meetings involved with this field of cancer research, and these publications will be available upon request from your specialist doctor. You will not be identified in any report or publication.

20. Who is organising and funding the research?

The research is being organised by The Departments of Public Health and Epidemiology, Primary Care and General Practice, Institute of Biomedical Research and the Cancer Research UK Institute for Cancer Studies at The University of Birmingham in collaboration with the Department of Urology at The Queen Elizabeth Hospital, Birmingham. The research is funded by Cancer Research UK. The doctors conducting this study are not being paid for including and looking after you within this study.

21. Who has reviewed this study?

This study has been reviewed by the Multi-centre Research Ethics Committee and by scientific experts at Cancer Research UK.

Should you wish to participate in this study you will be given a copy of this patient information sheet and a signed consent form to keep. Thank you for taking the time to read this sheet and should you require further information please do not hesitate to contact us.

Yours sincerely,
Urologist.

Appendix 2: Participant consent form

UNIVERSITY OF BIRMINGHAM

Birmingham Prostatic Neoplasms Association Study (BiPAS)

Centre Name:
Study Number:
Patient Identification Number:

Name of Researcher:

1. I confirm that I have read and understood the information sheets version 1.1 of the above study and have had the opportunity to ask questions. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that my medical notes may be looked at by members of the BiPAS research team and regulatory authority representatives, but understand that strict confidentiality will be maintained. I give permission for these individuals to have access to my records. ☐

4. I agree for blood to be taken, stored and used for current and future biological research projects which WILL receive appropriate scientific and ethical approval. ☐

5. I agree to my GP being informed of my participation in the study. ☐

6. I agree for my contact details to be stored and used to contact me about aspects of the study either by telephone or post. ☐

7. I agree to take part in the above Birmingham Prostate Cancer Association Study (BiPAS). ☐

_____	_____	_____
Name of patient	Date	Signature
_____	_____	_____
Name of person taking consent	Date	Signature

Appendix 3: Questionnaire

THE BIRMINGHAM PROSTATIC NEOPLASMS ASSOCIATION STUDY

Patient Identifier: ☐☐☐☐☐1☐☐☐
First 3 letters from surname followed by first 2 letters of forename followed by [site identified] and number eg: John Smith = SMIJO[site identifier]001

Date of Birth: ☐☐/☐☐/☐☐☐☐

Patient/Hospital number: ☐☐☐/☐☐☐/☐☐☐☐☐

Hospital:

Clinic:

Date of Interview: ☐☐/☐☐/☐☐☐☐

Will the interview take place with the support of a translator? Yes ☐ No ☐

Name of Translator: …….

INTRODUCTION

In order to provide better health care for prostate cancer patients, we hope to discover more about how prostate cancer is related to people's circumstances. In order to do this we are asking a range of people to answer these questions including some people with prostate problems and others with no such problems.

The questions that you will be asked will include questions about your lifestyle, your behaviours, your health and the help and support you receive from the people around you.

There may be some questions that you think are unusual. The questions are not used to test you in any way and the responses you give will not be used to make any judgements about you. There are no right or wrong answers. All of your responses will be treated as strictly confidential and will be used only for medical research.

Some of the questions will ask for personal information. If you feel that any of the questions are too personal, do not answer them. However, by answering these questions, you will help us to discover links between lifestyle and health.

There is no time limit, so if you want a little time to think about any of the questions please do so. Try to answer every question, even if the answer is 'I can't remember' or 'I don't know'.

GENERAL INFORMATION

CONTACT DETAILS:

What is your postal address:

House Number and Street
……..

Area
……..

Location (Town/City)
……..

Postcode (if known) …….

What is your home telephone number? ☐☐☐☐☐/☐☐☐☐☐☐☐☐

MARITAL STATUS

What is your current marital status? (tick appropriate box)

Single ☐ Married/living with partner ☐ Widowed ☐ Separated ☐ Divorced ☐

ETHNIC ORIGIN

To which of these groups do you consider you belong to ? (tick appropriate box)

White ☐ Black, Caribbean ☐ Black, other ☐ Chinese ☐
Pakistani ☐ Indian ☐ Bangladeshi ☐ Other ☐
'Other' specify ……..
Mixed ☐ (specify)…… If 'white' please specify origin eg. Irish etc

EDUCATION AND QUALIFICATIONS

How old were you when you left school? ☐☐

Do you have any of the following qualifications? *(Tick all applicable*)

School Leaving Certificate	☐ CSE	☐
GCE "O" Level or GCSE	☐Technical College Exams / City and Guilds	☐
Completed Apprenticeship	☐Higher National Diploma (HND)	☐
"A" Level, Highers	☐Matriculation (University entry exam)	☐
Trade Certificates	☐Teaching Diploma, NHC	☐
Secretarial College Exam	☐University Degree	☐

PAST HISTORY OF CANCER

Have you ever been diagnosed as suffering from any of the following cancers?

If YES, please give date of diagnosis:

			Year
Prostate	No ☐	Yes ☐	☐☐☐☐
Kidney	No ☐	Yes ☐	☐☐☐☐
Bladder	No ☐	Yes ☐	☐☐☐☐
Liver	No ☐	Yes ☐	☐☐☐☐
Melanoma	No ☐	Yes ☐	☐☐☐☐
Non-melanoma Skin Cancer	No ☐	Yes ☐	☐☐☐☐
Lung	No ☐	Yes ☐	☐☐☐☐
Colorectal/Bowel cancer	No ☐	Yes ☐	☐☐☐☐
Upper GI (Stomach/pancreas)	No ☐	Yes ☐	☐☐☐☐
Haematological (e.g. Leukemia)	No ☐	Yes ☐	☐☐☐☐
Other …….....	No ☐	Yes ☐	☐☐☐☐

HAVE YOU HAD A VASECTOMY?

Yes ☐ Date…….. No ☐ *Please turn over*

Have you had a reversal of a vasectomy?

Yes ☐ Date…… No ☐

If you have had a reversal of a vasectomy have you subsequently had children?
Yes ☐ Details No ☐

OCCUPATION HISTORY

SECTION 5: OCCUPATIONAL HISTORY

In this section we would like you to tell us about your **most recent job** and all of your **previous jobs** that you have had for one year or more.

We have allowed enough sections for **up to 6** jobs. If you have had more than 6 jobs, complete all sections of this part of the questionnaire and then continue on a **separate piece of paper. THE FIRST LINE HAS BEEN FILLED IN AND USED AS AN EXAMPLE.**

Job Title	Time period that you worked there		Description of Activities	Name of Organisation	Location (Town/City)	Type of Business	Mainly indoor activity	Mainly outdoor activity	Shift work
	From (y)	To (y)					**Please tick one**		
EXAMPLE: DRIVER	1972	1996	HGV DRIVER	JO BLOGGS BUILDERS	BIRMINGHAM	BUILDING CONTRACTORS	√		YES

DIETARY BEHAVIOURS

The questions in this section ask about your normal diet during the last year

Please indicate how often on average, **during the past year**, you have eaten each of the food types that are listed below

	Average Use Last Year					
	Never or less than once per month	1-3 per month	Once a week	2-4 per week	5-6 per week	At least once per day
STAPLE FOODS						
Bread ……	☐	☐	☐	☐	☐	☐
Potatoes……	☐	☐	☐	☐	☐	☐
Pasta…… (eg. Macaroni, Spaghetti)	☐	☐	☐	☐	☐	☐
Rice ……	☐	☐	☐	☐	☐	☐
Noodles……	☐	☐	☐	☐	☐	☐
Wheat ……. (eg. Whole grain bread)	☐	☐	☐	☐	☐	☐
Cereal…….. (eg. Oats, bran, corn)	☐	☐	☐	☐	☐	☐
MEAT						
Meat (no organs)…….. (eg. Pork, Steak, Beef, Lamb)	☐	☐	☐	☐	☐	☐
Organ Meat…… (eg. Liver, Heart, Kidney)	☐	☐	☐	☐	☐	☐
Chicken……..	☐	☐	☐	☐	☐	☐
Other Poultry……. (eg. Goose, Duck)	☐	☐	☐	☐	☐	☐
FISH						
Dark Fleshed Fish…… (eg. Mackerel, Salmon, Anchovies, Sardines, Trout, Tuna, Herring)	☐	☐	☐	☐	☐	☐
White fleshed fish ……. (eg. Cod, Haddock, Hake, Halibut, Plaice, Seabass, Skate, Sole)	☐	☐	☐	☐	☐	☐
Seafood…… (eg. Prawn, Crab, Lobster, Cockles, Winkles, Squid, Octopus, Mussels)	☐	☐	☐	☐	☐	☐

DIETARY BEHAVIOURS

	Average Use Last Year					
	Never or less than once per month	**1-3 per month**	**Once a week**	**2-4 per week**	**5-6 per week**	**At least once per day**
VEGETABLES						
Fruit Vegetables…… (eg. Tomato, Cucumber, Aubergine)	☐	☐	☐	☐	☐	☐
Flower vegetables…… (eg. Broccoli, Cauliflower)	☐	☐	☐	☐	☐	☐
Leafy vegetables…… (eg. Spinach, Cabbage, Lettuce)	☐	☐	☐	☐	☐	☐
Stem vegetables…… (eg. Asparagus, Celery, Fennel)	☐	☐	☐	☐	☐	☐
Mushrooms……	☐	☐	☐	☐	☐	☐
Bulbs…… (eg. Onion, Garlic, Leek, Shallot)	☐	☐	☐	☐	☐	☐
Roots…… (eg. Beetroot, Swede, Carrot, Parsnip)	☐	☐	☐	☐	☐	☐
FRUIT						
Citrus Fruits…… (eg. Orange, Lemon, Lime, Grapefruit)	☐	☐	☐	☐	☐	☐
Stone Fruits…… (eg. Plum, Apricot, Peach, Cherry)	☐	☐	☐	☐	☐	☐
Soft Fruits…… (eg. Strawberry, Rasberry)	☐	☐	☐	☐	☐	☐
Fleshy Fruits…… (eg. Apple, Pear, Banana, Pineapple)	☐	☐	☐	☐	☐	☐
Vine Fruits…… (eg. Grape, Melon, Cantalope)	☐	☐	☐	☐	☐	☐
DAIRY						
Cream……	☐	☐	☐	☐	☐	☐
Butter / Margarine……	☐	☐	☐	☐	☐	☐
Yogurt…….....	☐	☐	☐	☐	☐	☐
Cheese ……	☐	☐	☐	☐	☐	☐
Egg ……	☐	☐	☐	☐	☐	☐
OTHER FOODS						
Pulses…… (eg. Pea, Bean, Lentil)	☐	☐	☐	☐	☐	☐
Nuts and Seeds……	☐	☐	☐	☐	☐	☐
Soy/Tofu products……. (eg. Soy milk, Tofu, Soya meat)	☐	☐	☐	☐	☐	☐
Sweets and snacks…… (eg. Crisps, cakes, chocolate)	☐	☐	☐	☐	☐	☐

DIETARY BEHAVIOURS

FLUID INTAKE

Please indicate how often, during the past year, you have drunk one measure each of the types of drinks that are listed below.

		Average Use Last Year						
	Measure	Never or less than one measure per month	1-3 per month	One a week	2-4 per week	5-6 per week	At least one per day	How many per day?
ALCOHOLIC DRINKS								
Wine or champagne……	1 small glass	☐	☐	☐	☐	☐	☐ →	☐☐
Fortified Wine…… (eg. Port, Sherry, Cinzano)	1 small glass	☐	☐	☐	☐	☐	☐ →	☐☐
Beer…… (eg. Beer, Lager, Stout)	1 Pint	☐	☐	☐	☐	☐	☐ →	☐☐
Cider……	1 Pint	☐	☐	☐	☐	☐	☐ →	☐☐
Spirits…… (eg. Gin, Brandy, Rum, Vodka, Whisky)	1 pub measure (25cl)	☐	☐	☐	☐	☐	☐ →	☐☐
Liqueurs…… (eg. Tia Maria, Cointreau, Baileys, Grand Marnier, etc)	1 pub measure (25cl)	☐	☐	☐	☐	☐	☐ →	☐☐
HOT DRINKS								
Coffee……	1 cup	☐	☐	☐	☐	☐	☐ →	☐☐
Tea……	1 cup	☐	☐	☐	☐	☐	☐ →	☐☐
Hot Chocolate……	1 cup	☐	☐	☐	☐	☐	☐ →	☐☐
Ovaltine / Horlicks……	1 cup	☐	☐	☐	☐	☐	☐ →	☐☐
Soup……	1Cup/ bowl	☐	☐	☐	☐	☐	☐ →	☐☐
COLD DRINKS								
Fizzy pop…… (eg. Lemonade, Cola)	½ pint glass	☐	☐	☐	☐	☐	☐ →	☐☐
Pure fruit juice…… (eg. Orange, Apple, etc)	½ pint glass	☐	☐	☐	☐	☐	☐ →	☐☐
Fruit squash or cordial …….	½ pint glass	☐	☐	☐	☐	☐	☐ →	☐☐

Milk ……	½ pint glass	☐	☐	☐	☐	☐	☐ → ⌊⌊
Water ……	½ pint glass	☐	☐	☐	☐	☐	☐ → ⌊⌊

DIETARY BEHAVIOURS

	Average Use Last Year					
	Never or less than once per month	1-3 per month	Once a week	2-4 per week	5-6 per week	At least once per day
FOODS OF INTEREST						
Fast Foods e.g. McDonalds, Fish 'n Chips……..	☐	☐	☐	☐	☐	☐
Takeaway/Pub Meal/ Restaurant ……	☐	☐	☐	☐	☐	☐
Ketchup or Tomato Sauces……	☐	☐	☐	☐	☐	☐
Tomato based foods e.g. Pastas, soups pizzas ……	☐	☐	☐	☐	☐	☐

Could you kindly indicate which of the following non prescribed **treatments/supplements** you are currently taking IN ADDITION to your diet?

Name	Brand	Date Started	Dose
Prostabrit	……	……	……
Saw Palmetto	……	……	……
Selenium	……	……	……
Vitamin E	……	…….	……..
Vitamin D	……	…….	……..
Multivitamin	…….	…….	……
Other (please specify) …….		……..	……

Ultra violet light exposure risk

Skin Type: How would you describe your skins reaction to sun exposure?

Always burns and never tans ☐
Usually burns and tans a little ☐
Burns rarely and tans gradually ☐
Never burns and always tans ☐

Adult Hair colour (Before grey):

Red ☐
Auburn ☐
Blonde ☐
Brown ☐
Black ☐

Adult Eyes colour:

Blue ☐ Grey ☐
Green ☐ Hazel ☐
Brown ☐

With regard to your **jobs** over the years, please say how many **years** you have spent working :

Outdoors =	never	☐	**Indoors** =	never	☐
	0 – 9 years	☐		0 – 9 years	☐
	10 –19 years	☐		10 –19 years	☐
	20 – 29 years	☐		20 – 29 years	☐
	Over 30 years	☐		Over 30 years	☐

Travel

Have you ever lived abroad?

☐ No ☐ Yes Time lived aboard: ☐☐ years ☐☐ months

Where did you live abroad? ……

Was it a sunny country? ☐ Yes ☐ No

Do you remember being badly sunburnt as a child (blistering or sunburn lasting more than 48 hours)?

☐ Yes How many times? ☐☐☐
☐ No

Ultra violet exposure clarification

We are interested to learn about your **history of sun exposure/baking**. The following questions relate to the amount of time you have spent outside in the sun since you were a young adult.

From Age **60+**, on average how many **hours per day** did you spend outdoors on:

Weekdays	never	☐	**Weekends**	never	☐
	0 – 4 hours	☐		0 – 4 hours	☐
	5 – 9 hours	☐		5 – 9 hours	☐
	10 – 14 hours	☐		10 – 14 hours`	☐
	More than 15 hours	☐		More than 15 hours	☐

During middle age (**40-59**)

Weekdays	never	☐	**Weekends**	never	☐
	0 – 4 hours	☐		0 – 4 hours	☐
	5 – 9 hours	☐		5 – 9 hours	☐
	10 – 14 hours	☐		10 – 14 hours	☐
	More than 15 hours	☐		More than 15 hours	☐

During early adult life (**20-39**)

Weekdays	never	☐	**Weekends**	never	☐
	0 – 4 hours	☐		0 – 4 hours	☐
	5 – 9 hours	☐		5 – 9 hours	☐
	10 – 14 hours	☐		10 – 14 hours	☐
	More than 15 hours	☐		More than 15 hours	☐

On average how many **weeks** do you spend abroad in a hot country **per year**? ☐

What Sun Protection Factor (SPF) do you usually use? ☐☐

Extent of sunbathing / Lying / Sitting in the sun at the following ages:

	Frequently	Occasionally	Rarely	Never
60+				
40-59				
0-39				

(Participants have to answer whether they are someone who sunbathes (and how often) or not for each age category)

On average, during your lifetime, how many sunbed sessions (electric) have you had per year?

☐☐☐

PHYSICAL ACTIVITY

How would you classify yourself?

Type of activity

Walking (including to school, work, shopping or a as a leisure activity)

From **12 – 19 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

From **20 – 39 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Since **40** (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Cycling (including to school, work, shopping or a as a leisure activity)

From **12 – 19 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

From **20 – 39 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Since **40** (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Gardening

From **12 – 19** years of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

From **20 – 39 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Since **40** (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Housework (cooking, cleaning, childcare)

From **12 – 19 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

From **20 – 39 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Since **40** (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Non-competitive sport (aerobics, skiing, etc)

Please specify which sport ……..

From **12 – 19 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

From **20 – 39 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Since 40 (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Competitive sport or dance

Please specify which sport ……..

From **12 – 19 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

From **20 – 39 years** of age (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

Since **40** (number of **hours per week**):

Never ☐
0 – 4 hours per week ☐
5 – 9 hours per week ☐
10 – 14 hours per week ☐
More than 15 hours ☐

These questions are about the time you spend sitting while at work, at home and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

During an average week, how much time do you usually spend **sitting on a weekday**?

Never ☐
0 – 4 hours per day ☐
5 – 9 hours per day ☐
10 – 14 hours per day ☐
All day ☐

During an average week, how much time do you usually spend **sitting at the weekend**?

Never ☐
0 – 4 hours per day ☐
5 – 9 hours per day ☐
10 – 14 hours per day ☐
All day ☐

TOBACCO

Have you ever **regularly** smoked cigarettes for **at least a year**? By regularly we mean: **at least 1 cigarette per day** OR **at least 5 cigarettes per week** OR **at least 1 pack per month.**

Yes ☐
No ☐
Don't know ☐

At what age did you **start to smoke** regularly (*in years)?* ☐☐

Do you still smoke regularly?

Yes ☐
No ☐ At what age did you stop ☐☐
Don't know ☐

On average, how many cigarettes do/did you smoke per day? ☐☐

What was the **maximum** number you ever smoked per day **if different from above**?
☐☐

For how long did you smoke **this many**? ☐☐ months *or* ☐☐ years

Body Dimensions

Please answer the following questions using **either** metric (cm, kg) or imperial (feet, inches, stones, pounds) measurements

What is your current height (in bare feet)?	feet	inches	***Or***	cm

What is your current weight (in light clothing)?	stone	pounds	***Or***	kg
How much did you weigh at age 20?	stone	pounds	***Or***	kg
How much did you weigh at age 30?	stone	pounds	***Or***	kg
How much did you weigh at age 40?	stone	pounds	***Or***	kg

What is your current waist size?		inches	***Or***	cm

Have you ever taken supplements to lose weight? Yes ☐ No ☐

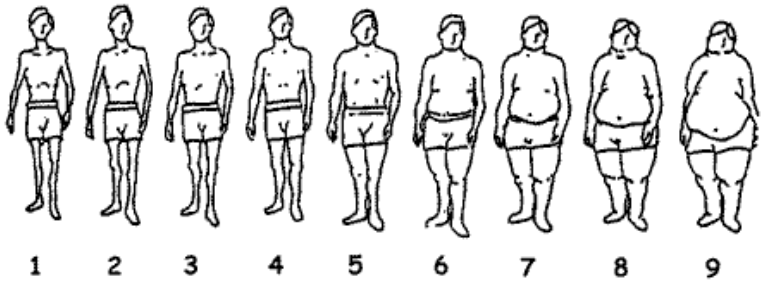
If 'yes' please specify……

Have you ever taken supplements to gain weight/muscle? Yes ☐ No ☐

If 'yes' please specify……

Body Dimensions

Body size in different periods of life (pictogram): please mark how **you think you looked** at different ages:



At around age 20	○	○	○	○	○	○	○	○	○
At around age 30	○	○	○	○	○	○	○	○	○
At around age 40	○	○	○	○	○	○	○	○	○
Now	○	○	○	○	○	○	○	○	○

FAMILY HISTORY

1. Have 3 or more **first-degree** relatives (e.g. father, brother, son) had prostate cancer?

Yes ☐ No ☐ Don't Know ☐

2. Has there been prostate cancer in **3 successive generations** on either your maternal or paternal side?

Yes ☐ No ☐ Don't Know ☐

3. Have at least **2 relatives been affected** by prostate cancer at either 55 years or younger?

Yes ☐ No ☐ Don't Know ☐

In this section we would like you to tell us about your PARENTS. Please fill in the table below using **CAPITAL LETTERS** and **completing ONE section** for **EACH parent.**

Relation	Year of Birth	Have they ever had cancer?		If Yes, which type of cancer?	Age at which cancer was diagnosed (if known)
		Yes	No		
FATHER					
MOTHER					

FAMILY HISTORY

COULD YOU PLEASE TELL US THE NUMBER OF SIBLINGS YOU HAVE?

Please **ONLY** include siblings which are you are **related to by blood**. You **DO NOT** need to tell us about any **adopted** or **step-relations.**

BROTHERS ☐☐ **SISTERS** ☐☐

SIBLING'S MEDICAL HISTORY

We would now like you to provide some more information about your **brothers** and **sisters** including any **medical history of cancer**. You **DO NOT** need to tell us about any **adopted** or **step-relations.**

	Gender *(Please tick)*		Year of Birth	Have they ever had cancer? *(Please tick)*		If Yes, which type of cancer?	Age at which cancer was diagnosed (if known)
	Male	Female		Yes	No		
1							
2							
3							
4							
5							
6							

If you have more than 6 siblings, complete all 6 sections of this part of the questionnaire and then continue on a **separate piece of paper**.

FAMILY HISTORY

COULD YOU PLEASE TELL US THE NUMBER OF CHILDREN YOU HAVE?

You **DO NOT** need to tell us about any **adopted** or **step-relations.**

SONS ☐☐ **DAUGHTERS** ☐☐

CHILDREN'S MEDICAL HISTORY

We would now like you to provide some more information about your **children** including any **medical history of cancer**. You **DO NOT** need to tell us about any **adopted** or **step-relations.**

	Gender *(Please tick)*		Year of Birth	Have they ever had cancer? *(Please tick)*		If Yes, which type of cancer?	Age at which cancer was diagnosed (if known)
	Male	Female		Yes	No		
1							
2							
3							
4							
5							
6							

If you have more than 6 children, complete all 6 sections of this part of the questionnaire and then continue on a **separate piece of paper**.

TESTOSTERONE QUESTIONS

We would like to obtain more information about the link between testosterone and prostate disease.

1. Do you have a decrease in libido (sex drive)?	Yes	No
2. Do you have a lack of energy?	Yes	No
3. Do you have a decrease in strength and/or endurance?	Yes	No
4. Have you lost height?	Yes	No
5. Have you noticed a decreased "enjoyment of life"	Yes	No
6. Are you sad and/or grumpy?	Yes	No
7. Are your erections less strong?	Yes	No
8. Have you noticed a recent deterioration in your ability to play sports?	Yes	No
9. Are you falling asleep after dinner?	Yes	No
10. Has there been a recent deterioration in your work performance?	Yes	No

Finally, in this study we are asking participants to give blood, however, in the future we may be using saliva samples for analysis.

Could you kindly indicate if you were asked in the future for research purposes which sample you would prefer to provide.

Blood ☐ Saliva ☐ Don't care ☐

Thank you for taking the time to complete this questionnaire.

Appendix 4: Additional Analysis for Chapter 3

Table 3.4: Odds Ratios and 95% Confidence intervals for UVR exposure comparing hospital controls and combined case group - Results from The Birmingham Prostatic Neoplasms Association Study

	Hospital Controls N	Combined Cases N	Non Adjusted OR	Adjusted OR*	Adjusted 95%CI	p value or p trend§
Usual reaction to sunlight						
Always/easily burns	59	232	1	1		
Rarely/never burns	118	390	0.84	0.89*	0.62 - 1.29	0.54
No of times sunburnt as a child						
1	13	47	1	1		
≥ 2	154	548	0.98	1.06*	0.55 - 2.04	0.87
Ever lived abroad in a sunny country						
No	136	455	1	1		
Yes	30	127	1.27	1.20*	0.76 - 1.89	0.44
Time spent outside						
Early adulthood (20-39 years)						
High	2	26	1	1		
Medium	30	90	0.87	1.06**	0.63 - 1.76	
Low	104	360	3.77	3.38**	0.79 - 14.49	0.26§
Middle age (40-60 years)						
High	10	38	1	1		
Medium	34	124	1.01	1.00**	0.62 - 1.61	
Low	85	310	1.05	0.79**	0.37 - 1.70	0.83§
Late adulthood (60+ years)						
High	5	13	1	1		
Medium	3	17	2.02	2.61**	0.55 - 12.42	
Low	5	14	0.93	1.09**	0.21 - 5.76	0.41§
Life course						
High	6	30	1	1		
Medium	13	38	0.73	0.57**	0.27 - 1.20	
Low	55	220	1.26	1.59**	0.59 - 4.24	0.17§
Sunbathing in life course						
High	23	118	1	1		
Medium	40	160	1.34	1.48**	0.90 - 2.42	
Low	58	173	1.77	2.31**	1.24 - 4.32	0.03§

*Adjusted for age of diagnosis, ethnicity and family history of prostate cancer **Adjusted for age of diagnosis, ethnicity, skin type and hair colour § p trend

Table 3.5: Odds Ratios and 95% Confidence intervals for UVR exposure comparing population controls and combined case group - Results from The Birmingham Prostatic Neoplasms Association Study

	Population Controls N	Combined Cases N	Non Adjusted OR	Adjusted OR*	Adjusted 95%CI	p value or p trend§
Usual reaction to sunlight						
Always/easily burns	50	232	1	1		
Rarely/never burns	120	390	0.7	0.74*	0.51 - 1.07	0.11
No of times sunburnt as a child						
1	16	47	1	1		
≥ 2	145	548	1.29	1.35	0.72 - 2.51	0.35
Ever lived abroad in a sunny country						
No	140	455	1	1		
Yes	27	127	1.45	1.37*	0.86 - 2.18	0.18
Time spent outside						
Early adulthood (20-39 years)						
High	2	26	1	1		
Medium	24	90	1.35	1.29**	0.78 - 2.13	
Low	132	360	4.67	4.69**	1.11 - 19.80	0.08§
Middle age (40-60 years)						
High	10	38	1	1		
Medium	45	124	0.87	0.87**	0.57 - 1.32	
Low	100	310	1.2	1.14**	0.55 - 2.40	0.72§
Late adulthood (60+ years)						
High	1	13	1	1		
Medium	2	17	2.43	4.30**	0.45 - 41.46	
Low	4	14	3.71	7.45**	0.92 - 60.08	0.14§
Life course						
High	6	30	1	1		
Medium	10	38	1.13	1.09**	0.51 - 2.32	
Low	65	220	1.48	1.54**	0.62 - 3.82	0.65§
Sunbathing in life course						
High	34	118	1	1		
Medium	49	160	0.58	0.62**	0.36 - 1.05	
Low	30	173	0.61	0.62**	0.34 - 1.15	0.18§

*Adjusted for age of diagnosis, ethnicity and family history of prostate cancer **Adjusted for age of diagnosis, ethnicity, skin type and hair colour § p trend

Table 3.6: Odds Ratios and 95% Confidence intervals for UVR exposure comparing combined controls and high PSA case group - Results from The Birmingham Prostatic Neoplasms Association Study

	Combined Controls N	High PSA Cases N	Non Adjusted OR	Adjusted OR*	Adjusted 95%CI	p value or p trend§
Usual reaction to sunlight						
Always/easily burns	109	135	1	1		
Rarely/never burns	238	193	0.65	0.65*	0.47 - 0.90	0.01
No of times sunburnt as a child						
1	29	26	1	1		
≥ 2	299	290	1.08	1.18*	0.67 - 2.07	0.57
Ever lived abroad in a sunny country						
No	276	235	1	1		
Yes	57	78	1.61	1.61*	1.10 - 2.36	0.02
Time spent outside						
Early adulthood (20-39 years)						
High	4	12	1	1		
Medium	54	44	0.95	0.94**	0.60 - 1.47	
Low	236	201	3.5	3.71**	1.20 - 11.46	0.07§
Middle age (40-60 years)						
High	20	17	1	1		
Medium	79	61	0.79	0.79**	0.53 - 1.18	
Low	185	180	0.87	0.88**	0.44 - 1.77	0.5§
Late adulthood (60+ years)						
High	6	4	1	1		
Medium	5	5	-	1.68**	0.31 - 9.20	
Low	9	9	0.69	0.89**	0.16 - 4.92	0.76§
Life course						
High	12	15	1	1		
Medium	23	16	0.72	0.65**	0.31 - 1.36	
Low	120	117	1.29	1.55**	0.68 - 3.53	0.26§
Sunbathing in life course						
High	57	69	1	1		
Medium	89	77	0.75	0.82**	0.53 - 1.27	
Low	88	102	1.05	1.21**	0.74 - 1.99	0.29§

*Adjusted for age of diagnosis, ethnicity and family history of prostate cancer **Adjusted for age of diagnosis, ethnicity, skin type and hair colour § p trend

Table 3.7 Odds Ratios and 95% Confidence intervals for UVR after stratification for severity of disease - Results from The Birmingham Prostatic Neoplasms Association Study

		Localized n (%)	Advanced n (%)	OR	Adjusted OR	Adjusted 95%CI	p trend
Usual reaction to sunlight							
	Always/easilyburns	47 (36.4)	50 (30.7)				
	Rarely/never burns	82 (63.6)	113 (69.3)	1.3	1.36*	0.82 - 2.28	0.24
Number of times severely sunburnt as a child	1	14 (11.8)	7 (4.5)				
	≥ 2	105 (88.25)	150 (95.5)	2.85	2.51*	0.94 - 6.69	0.07
Ever lived abroad in a sunny country	No	102 (87.2)	117 (78)				
	Yes	15 (12.8)	33 (22)	1.92	1.50*	0.74 - 3.05	0.26
Time spent outside							
Early adulthood (20-39 years)	High	5 (5)	9 (7.6)				
	Medium	19 (19)	26 (22)	1.25	1.34§	0.65 - 2.73	
	Low	76 (76)	83 (70.4)	1.65	1.35§	0.43 - 4.20	0.67
Middleage adulthood (40 - 59)	High	9 (9.3)	12 (10.3)				
	Medium	23 (23.7)	39 (33.6)	1.7	1.80§	0.94 - 3.46	
	Low	65 (67)	65 (56.1)	1.33	1.11§	0.42 - 2.93	0.21
Life course	High	6 (9.7)	9 (11.8)				
	Medium	6 (9.7)	15 (19.7)	2.4	2.68§	0.97 - 7.43	
	Low	50 (80.6)	52 (68.5)	1.44	1.39§	0.43 - 4.52	0.16
Sunbathing in life course	High	21 (22.3)	28 (26.2)				
	Medium	41 (43.6)	41 (38.3)	0.84	0.70§	0.36 - 1.39	
	Low	32 (34.1)	38 (35.5)	1.12	1.03§	0.46 - 2.33	0.48

§ Adjusted for age at diagnosis, ethnicity, family history of prostate cancer, skin type and hair colour

* Adjusted for age at diagnosis, ethnicity and family history of prostate cancer Localized = Gleason < 7 Advanced = Gleason ≥ 10

Appendix 5: Additional Analysis for Chapter 4

Table 4.5: Odds Ratios and 95% Confidence intervals for physical activity comparing hospital controls and combined case group (aged 40+ years) - Results from The Birmingham Prostatic Neoplasms Association Study

	Hospital Controls	Combined Cases	Non Adjusted	Adjusted	Adjusted	p value or
	N	N	OR	OR*	95%CI	p trend
Walking hours per week						
Low (Never – 4)	100	387	1	1		
Medium (5-9)	41	140	0.88	0.88	0.54 – 1.43	
High (≥10)	19	52	0.71	0.96	0.46 – 2.01	0.67§
Cycling hours per week						
Never	86	356	1	1		
Ever	52	158	0.73	0.76	0.48 – 1.21	0.25
Gardening hours per week						
Low (Never – 4)	134	501	1	1		
Medium (5-9)	18	57	0.85	0.99	0.50 – 1.97	
High (≥10)	3	9	0.80	0.49	0.12 – 1.98	0.61§
Housework hours per week						
Low (Never – 4)	129	505	1	1		
Medium (5-9)	9	27	0.77	0.71	0.27 – 1.88	
High (≥10)	5	8	0.41	0.51	0.15 – 1.73	0.45§
Social Sport hours per week						
Never	83	307	1	1		
Ever	60	190	0.56	0.73	0.47 – 1.13	0.16
Competitive Sport hours per week						
Never	105	301	1	1		
Ever	37	202	1.90	1.81	1.15 – 2.86	0.01

* Adjusted for age at diagnosis, ethnicity, family history of prostate cancer, education, ever smoker and BMI § P trend
Combined cases = prostate cancer cases + high PSA cases

Table 4.6: Odds Ratios and 95% Confidence intervals for physical activity comparing population controls and combined case group (aged 40+ years) - Results from The Birmingham Prostatic Neoplasms Association Study

	Population Controls	Combined Cases	Non Adjusted	Adjusted	Adjusted	p value or
	N	N	OR	OR*	95%CI	p trend§
Walking hours per week						
Low (Never – 4)	125	387	1	1		
Medium (5-9)	39	140	1.16	1.35	0.85 – 2.14	
High (≥10)	8	52	2.10	2.43	1.09 – 5.44	0.06§
Cycling hours per week						
Never	117	356	1	1		
Ever	49	158	1.06	1.02	0.67 – 1.55	0.92
Gardening hours per week						
Low (Never – 4)	158	501	1	1		
Medium (5-9)	14	57	1.28	1.57	0.79 – 3.08	0.20
High (≥10)	0	9				
Housework hours per week						
Low (Never – 4)	162	505	1	1		
Medium (5-9)	10	27	0.87	0.94	0.37 – 2.38	0.90
High (≥10)	0	8				
Social Sport hours per week						
Never	108	307	1	1		
Ever	54	190	1.24	1.23	0.81 – 1.85	0.34
Competitive Sport hours per week						
Never	99	301	1	1		
Ever	67	202	0.99	1.03	0.70 – 1.53	0.87

* Adjusted for age at diagnosis, ethnicity, family history of prostate cancer, education, ever smoker and BMI § P trend
Combined cases = prostate cancer cases + high PSA cases

Table 4.7: Odds Ratios and 95% Confidence intervals for physical activity comparing high PSA cases and combined control group (aged 40+ years) - Results from The Birmingham Prostatic Neoplasms Association Study

	Combined Controls	High PSA Cases	Non Adjusted	Adjusted	Adjusted	p value or
	N	N	OR	OR*	95%CI	p trend
Walking hours per week						
Low (Never – 4)	225	214	1	1		
Medium (5-9)	80	71	0.93	1.10	0.72 – 1.67	
High (≥10)	27	29	1.13	1.75	0.91 – 3.36	0.24§
Cycling hours per week						
Never	203	188	1	1		
Ever	101	93	0.99	0.97	0.66 – 1.43	0.87
Gardening hours per week						
Low (Never – 4)	292	274	1	1		
Medium (5-9)	32	29	0.97	1.31	0.72 – 2.41	
High (≥10)	3	6	2.13	1.90	0.42 – 8.51	0.49§
Housework hours per week						
Low (Never – 4)	291	273	1	1		
Medium (5-9)	19	16	0.90	0.99	0.43 – 2.29	
High (≥10)	5	7	1.49	2.03	0.61 – 6.79	0.52§
Social Sport hours per week						
Never	191	156	1	1		
Ever	114	115	1.24	1.15	0.97 – 1.69	0.46
Competitive Sport hours per week						
Never	204	168	1	1		
Ever	104	102	1.19	1.15	0.78 – 1.67	0.48

*Adjusted for age at diagnosis, ethnicity, family history of prostate cancer, education, ever smoker and BMI § P trend
Combined controls = population controls + hospital controls

Table 4.8: Odds Ratios and 95% Confidence intervals for physical activity comparing combined cases and combined control group (aged 12 - 29 years) - Results from The Birmingham Prostatic Neoplasms Association Study

	Combined Controls	Combined Cases	Non Adjusted	Adjusted	Adjusted	p value or
	N	N	OR	OR*	95%CI	p trend
Walking hours per week						
Low (Never – 4)	195	312	1	1		
Medium (5-9)	100	164	1.03	1.13	0.80 - 1.61	
High (≥10)	36	75	1.3	1.71	1.02 - 2.85	0.12§
Cycling hours per week						
Never	65	112	1	1		
Ever	257	424	0.96	0.87	0.59 - 1.30	0.5
Gardening hours per week						
Low (Never – 4)	321	511	1	1		
Medium (5-9)	4	18	2.83	2.22	0.73 - 6.82	
High (≥10)	2	9	2.83	2.71	0.55 - 13.43	0.18§
Housework hours per week						
Low (Never – 4)	317	530	1	1		
Medium (5-9)	4	4	0.45	0.47	0.10 - 2.20	0.33
High (≥10)						
Social Sport hours per week	142	266	1	1		
Never	159	221	0.74	0.74	0.53 - 1.03	0.07
Ever						
Competitive Sport hours per week	120	190	1	1		
Never	186	305	1.04	1.12	0.81 - 1.56	0.49
Ever	104	102	1.19	1.15	0.78 – 1.67	0.48

* Adjusted for age at diagnosis, ethnicity, family history of prostate cancer, education, ever smoker and BMI § P trend
Combined cases = prostate cancer cases + high PSA cases
Combined controls = hospital controls + population controls

Table 4.9: Odds Ratios and 95% Confidence intervals for physical activity comparing combined cases and combined control group (aged 20 - 39 years) - Results from The Birmingham Prostatic Neoplasms Association Study

	Combined Controls	Combined Cases	Non Adjusted	Adjusted	Adjusted	p value or
	N	N	OR	OR*	95%CI	p trend
Walking hours per week						
Low (Never – 4)	182	315	1	1		
Medium (5-9)	105	172	0.95	1.11	0.78 - 1.57	
High (≥10)	39	68	1.01	1.53	0.91 - 2.56	0.27§
Cycling hours per week						
Never	173	297	1	1		
Ever	134	220	0.96	0.86	0.63 - 1.18	0.36
Gardening hours per week						
Low (Never – 4)	307	491	1	1		
Medium (5-9)	17	48	1.88	1.62	0.35 - 7.45	0.11
High (≥10)						
Housework hours per week	301	494	1	1		
Low (Never – 4)	12	24	1.42	2.61	0.54 - 12.76	0.44
Medium (5-9)						
High (≥10)	148	271	1	1		
Social Sport hours per week	154	213	0.76	0.68	0.49 - 0.95	0.02
Never						
Ever	141	215	1	1		
Competitive Sport hours per week	167	279	1.1	1.2	0.87 - 1.65	0.26
Never	186	305	1.04	1.12	0.81 - 1.56	0.49
Ever	104	102	1.19	1.15	0.78 – 1.67	0.48

* Adjusted for age at diagnosis, ethnicity, family history of prostate cancer, education, ever smoker and BMI § P trend
Combined cases = prostate cancer cases + high PSA cases
Combined controls = hospital controls + population controls

Appendix 6: Additional Analysis for Chapter 5

Table 5.6: Odds Ratios and 95% Confidence intervals for obesity comparing hospital controls and combined case group - Results from The Birmingham Prostatic Neoplasms Association Study

	Hospital Controls n (%)	Combined cases n (%)	Crude OR	Adjusted OR*	Adjusted 95%CI	p value/p trend
Waist Circumference						
<34.0	41 (24.7)	363 (53.5)	1	1		
34.1 - 37.0	50 (30.1)	128 (18.9)	0.29	0.34	0.21 - 0.55	
≥37.1	75 (45.2)	188 (27.6)	0.28	0.32	0.21 - 0.50	0.96§
BMI						
18.5 - 24.99	49 (28.0)	163 (23.6)	1	1		
25.0 - 29.99	78(44.6)	228 (33.0)	0.88	0.9	0.59 - 1.36	
≥30.0	48 (27.4)	299 (43.4)	1.87	1.49	0.94 - 2.35	0.84§
Body size age 20						
18.5 - 24.99	124 (74.7)	416 (72.3)	1	1		
25.0 - 29.99	42 (25.3)	150 (26.1)	1.06	1.05	0.70 - 1.56	0.81
≥30.0	0	9 (1.6)				
Body size age 30						
18.5 - 24.99	78 (46.7)	272 (47.6)	1	1		
25.0 - 29.99	87 (52.1)	289 (50.5)	0.95	0.94	0.66 - 1.33	0.78
≥30.0	2 (1.2)	11 (1.9)	1.58	1.56	0.33 - 7.31	0.73
Body size age 40						
18.5 - 24.99	38 (22.8)	155 (26.8)	1	1		
25.0 - 29.99	116 (69.5)	382 (66.3)	0.81	0.79	0.53 - 1.19	0.31
≥30.0	13 (7.7)	39 (66.9)	0.74	0.71	0.34 - 1.47	0.27
Body size current						
18.5 - 24.99	22 (13.1)	75 (13.0)	1	1		
25.0 - 29.99	105 (62.5)	363 (62.8)	1.01	1	0.59 - 1.69	0.99
≥30.0	41 (24.7)	140 (24.2)	1	0.99	0.55 - 1.78	0.97

* Adjusted for age at diagnosis, ethnicity & family history of prostate cancer § P trend
Combined cases = prostate cancer cases + high PSA cases

Table 5.7: Odds Ratios and 95% Confidence intervals for obesity comparing population controls and combined case group - Results from The Birmingham Prostatic Neoplasms Association Study

	Population Controls n (%)	Combined cases n (%)	Crude OR	Adjusted OR*	Adjusted 95%CI	p value/p trend
Waist Circumference						
<34.0	41 (24.7)	363 (53.5)	1	1		
34.1 - 37.0	50 (30.1)	128 (18.9)	0.29	0.34	0.21 - 0.55	
≥37.1	75 (45.2)	188 (27.6)	0.28	0.32	0.21 - 0.50	
BMI						
18.5 - 24.99	49 (28.0)	163 (23.6)	1	1		
25.0 - 29.99	78(44.6)	228 (33.0)	0.88	0.9	0.59 - 1.36	
≥30.0	48 (27.4)	299 (43.4)	1.87	1.49	0.94 - 2.35	0.05§
Body size age 20						
18.5 - 24.99	116 (67.1)	416 (72.3)	1	1		
25.0 - 29.99	56 (32.4)	150 (26.1)	0.75	0.75	0.59 - 1.69	0.99
≥30.0	1 (0.5)	9 (1.6)	2.51	2.65	0.35 - 19.95	0.34
Body size age 30						
18.5 - 24.99	78 (45.1)	272 (47.6)	1	1		
25.0 - 29.99	91 (52.6)	289 (50.5)	0.91	0.91	0.64 - 1.29	0.6
≥30.0	4 (2.3)	11 (1.9)	0.79	0.86	0.27 - 2.75	0.8
Body size age 40						
18.5 - 24.99	39 (22.5)	155 (26.8)	1	1		
25.0 - 29.99	126 (72.8)	382 (66.3)	0.76	0.79	0.53 - 1.19	0.26
≥30.0	8 (4.7)	39 (66.9)	1.23	1.37	0.59 - 3.17	0.46
Body size current						
18.5 - 24.99	22 (12.9)	75 (13.0)	1	1		
25.0 - 29.99	104 (61.2)	363 (62.8)	1.02	1.13	0.66 - 1.92	0.66
≥30.0	44 (25.9)	140 (24.2)	0.93	1.01	0.56 - 1.81	0.98

* Adjusted for age at diagnosis, ethnicity & family history of prostate cancer § P trend

Combined cases = prostate cancer cases + high PSA cases

Table 5.8: Odds Ratios and 95% Confidence intervals for obesity comparing high PSA cases and combined control group - Results from The Birmingham Prostatic Neoplasms Association Study

	Combined Controls n (%)	High PSA cases n (%)	Crude OR	Adjusted OR*	Adjusted 95%CI	p value/p trend
Waist Circumference						
<34.0	147 (40.8)	205 (55.0)	1	1		
34.1 - 37.0	85 (23.6)	71 (19.0)	0.6	0.68	0.46 - 1.02	
≥37.1	128 (35.6)	97 (26.0)	0.54	0.63	0.44 - 0.91	0.03§
BMI						
18.5 - 24.99	93 (24.9)	86 (22.7)	1	1		
25.0 - 29.99	141 (37.7)	131 (34.6)	1	0.99	0.98-1.45	
≥30.0	140 (37.4)	162 (42.7)	1.25	1.02	0.68 - 1.52	0.99§
Body size age 20						
18.5 - 24.99	240 (70.8)	220 (72.1)	1	1		
25.0 - 29.99	98 (28.9)	82 (26.9)	0.91	0.86	0.61 - 1.22	0.41
≥30.0	1 (0.3)	3 (1.0)	3.27	3.07	0.29 - 32.01	0.35
Body size age 30						
18.5 - 24.99	156 (45.9)	147 (48.4)	1	1		
25.0 - 29.99	178 (52.4)	150 (49.3)	0.89	0.88	0.64 - 1.20	0.41
≥30.0	6 (1.7)	7 (2.3)	1.24	1.15	0.37 - 3.34	0.81
Body size age 40						
18.5 - 24.99	77 (22.6)	92 (30.1)	1	1		
25.0 - 29.99	242 (71.2)	188 (61.4)	0.65	0.63	0.44 - 0.90	0.01
≥30.0	21 (6.2)	26 (8.5)	1.04	0.92	0.47 - 1.80	0.81
Body size current						
18.5 - 24.99	44 (13.0)	46 (15.0)	1	1		
25.0 - 29.99	209 (61.8)	197 (64.4)	0.9	0.86	0.54 - 1.36	0.52
≥30.0	85 (25.2)	63 (20.6)	0.71	0.66	0.39 - 1.13	0.13

* Adjusted for age at diagnosis, ethnicity & family history of prostate cancer § P trend
Combined controls = hospital controls + population controls

Appendix 7: Additional analyses for Chapter 7

Table 7.2 Additional analysis for DG8S737 alleles

ALLELES	REPEAT NUMBERS	CASES (n)	CONTROLS (n)	CRUDE ODDS RATIO (95%CI)	P	ODDS RATIO* (95%CI)	P
Allele -14 absent		371	361	1		1	
Allele -14 present	19	89	99	0.89 (0.63 – 1.25)	0.5	0.90 (0.64 – 1.27)	0.56
Allele -12 absent		453	452	1		1	
Allele -12 present	20	7	8	0.76 (0.24 – 2.35)	0.63	0.75 (0.24 – 2.36)	0.62
Allele -10 absent		417	430	1		1	
Allele -10 present	21	43	30	1.50 (0.89 – 2.53)	0.13	1.50 (0.88 – 2.55)	0.13
Allele -8 absent		417	394	1		1	
Allele -8 present	22	43	66	0.61 (0.39 – 0.94)	0.03	0.62 (0.40 – 0.96)	0.03
Allele -6 absent		390	374	1		1	
Allele -6 present	23	70	86	0.91 (0.63 – 1.31)	0.62	0.91 (0.63 – 1.32)	0.63
Allele -4 absent		444	449	1		1	
Allele -4 present	24	16	11	1.69 (0.74 – 3.87)	0.21	1.47 (0.63 – 3.43)	0.37
Allele -2 absent		416	408	1		1	
Allele -2 present	25	44	52	0.78 (0.50 -1.22)	0.28	0.79 (0.50 – 1.24)	0.31
Allele 0 absent		400	392	1		1	
Allele 0 present	26	60	68	0.82 (0.55 – 1.22)	0.33	0.84 (0.56 – 1.25)	0.39
Allele 2 absent		422	406	1		1	
Allele 2 present	27	38	54	0.76 (0.48 – 1.20)	0.24	0.75 (0.47 – 1.20)	0.24
Allele 4 absent		435	431	1		1	
Allele 4 present	28	25	29	0.79 (0.43 – 1.42)	0.43	0.78 (0.43 – 1.41)	0.41
Allele 6 absent		434	436	1		1	
Allele 6 present	29	26	24	1.15 (0.63 – 2.11)	0.66	1.06 (0.57 – 1.97)	0.85
Allele 8 absent		457	453	1		1	
Allele 8 present	30	3	7	0.68 (0.17 – 2.70)	0.58	0.63 (0.16 – 2.56)	0.52

* Adjusted for age, alcohol intake from wine, body mass index (BMI), energy intake, family history of prostate cancer and level of education

Appendix 8: Author contribution to BiPAS

Contributions to BiPAS

The author of this thesis contributed the study in the following ways:

- Co-ordinated the relevant approvals including research and development for all hospital sites and general practices
- Developed the database for data entry
- Implemented the protocol into clinics
- Trained clinicians, nurses and reception staff in recruitment of cases and controls
- Recruitment of patients
- Responsible for all entry and subsequent management of data
- Responsible for DNA extractions for all blood samples
- Responsible for data analysis and write up of publication arising from chapter 6
- Responsible for collaboration with PRACTICAL and all related data queries and writing of relevant sections in resulting publication

Appendix 9: Publications arising from this thesis (in print)