Genetic Predisposition to Prostate Cancer

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Abstract:

Introduction: Prostate cancer (PrCa) is the commonest non-cutaneous cancer in men in the UK. Epidemiological evidence as well as twin studies point towards a genetic component contributing to aetiology.

Sources of data: Key recently published literature.

Areas of agreement: A family history of PrCa doubles the risk of disease development in first degree relatives. Linkage and genetic sequencing studies identified rare moderate-high risk gene loci which predispose to PrCa development when altered by mutation. Genome wide association studies have identified common single nucleotide polypmorphisms (SNPs) which confer a cumulative risk of PrCa development with increasing number of risk alleles. There are emerging data that castrate resistant disease is associated with mutations in DNA repair genes.

Areas of controversy: Linkage studies investigating possible high risk loci leading to PrCa development identified possible loci on several chromosomes, but most have not been consistently replicated by subsequent studies. Germline SNPs related to PSA levels and to normal tissue radiosensitivity have also been identified though not all have been validated in subsequent studies.

Growing points: Utilising germline SNP profiles as well as identifying high risk genetic variants could target screening to high risk groups, avoiding the drawbacks of PSA screening.

Areas timely for developing research: Incorporating genetics into PrCa screening is being investigated currently using both common SNP profiles as well as higher risk rare variants. Knowledge of germline genetic defects will allow the development of targeted screening programs, preventive strategies and the personalised treatment of PrCa.

Introduction

Prostate cancer (PrCa) is the commonest non-cutaneous cancer in men in the UK with a lifetime risk of 13.24%, making it the cancer with the highest life time risk in UK males[1]. Although the mortality rate of PrCa has fallen since the 1990's (by approximately 20%), the incidence has risen significantly in the last 25 years; this is mostly attributed to the advent of PSA testing. In 2012, 43,400 new cases of PrCa were diagnosed in the UK and 10,800 men died of the disease. Worldwide, there is a distinct geographical variation in the incidence of PrCa with the highest rates observed in the Caribbean, Australia and New Zealand and the lowest in South Central Asia [1] (Fig. 1).

The aetiology of PrCa is not well understood, although epidemiological studies demonstrating a convergence of incidence rates in some populations migrating between areas with a low incidence to those with high incidence suggest environmental and lifestyle risk factors play a role;[2] this trend has been reported for a number of Asian-American populations in the USA, for example, in Korean and Vietnamese men for whom the incidence of PrCa rose linearly between 1990 and 2008.[3] This trend in incidence has not been observed in all populations migrating from Eastern countries to the West; in a study of migrants from the former Soviet Union to Germany, lower PrCa mortality and incidence were found in the migrants compared with the German rates with no increase in incidence in the longitudinal analysis[4]. This suggests a genetic effect on PrCa risk. Indeed, it has long been known that having a positive family history and/or a certain ethnic background such as Afro-Caribbean, is a risk factor for PrCa development. Evidence from twin studies where monozygotic twins were compared with dizygotic twins[5], as well as studies of familial PrCa highlight this. First degree relatives of PrCa patients have twice the risk of developing the disease compared to the general population[6]. In men diagnosed under the age of 60 years, the risk to their first degree relatives is more than fourfold that of those without a family history[7]. The variation in incidence according to ethnicity also suggests a genetic component to PrCa aetiology; rates are higher in African American men compared with Asian American men [8]. This review will focus on hereditary (germline) genetic factors contributing to PrCa development, rather than somatic genetics.

Germline genetics

With the strong epidemiological evidence pointing to a hereditary component to the development of PrCa, much research into causative genes has been explored. Linkage studies investigating possible high risk loci leading to PrCa development identified possible loci on several chromosomes, but most have not been consistently replicated by subsequent studies[9] with the exception of *HOXB13* (see later). Linkage studies investigate the co-segregation of genetic markers with a disease. The lack of significant findings from these studies suggests that the hereditary aetiology of PrCa has a significant polygenic inheritance.

With the advances in genomic technology and high throughput DNA sequencing techniques, and by utilising databases of millions of common (mean allele frequency >5%) single nucleotide polymorphisms (SNPs) such as the HapMap [10] and 1000 Genomes project [11], genome wide association studies (GWAS) have been developed to investigate the (common) genetic variants predisposing to cancer. GWAS allows investigators to take an unbiased approach when scanning the genomes of thousands of cases and controls to identify SNPs that associate with cancer[12]. GWASs have enabled the discovery of SNPs in or near genes previously not known to be involved in cancer development. From projects, such as the HapMap project, it is known that certain SNPs will tend to

occur together although they are located separately, and not always within the same gene[10]. This phenomenon known as linkage disequilibrium (LD) allows a GWAS to utilise several hundred thousand 'tag SNPs' to generate data on millions of SNPs. One of the first published GWASs was carried out in PrCa cases [13] and since then several GWASs have been carried out yielding about 100 PrCa risk SNPs, accounting for 33% of the familial relative risk in European ancestry populations (See list of SNPs in supplementary table online) [14]. Several GWASs have been carried out in non-European populations such as Korean, Japanese, Arab and West African men to reveal both shared risk SNPs as well as some SNPs that may be unique to these populations (all GWASs are listed in the National Human Genome Research Institute and European Bioinformatics Institute (NHGRI-EBI) Catalog of published GWASs: http://www.ebi.ac.uk/gwas).

PrCa risk SNPs identified in most GWAS analyses confer a low to moderate risk of disease development with odds ratios (OR) ranging from 0.74-1.62. [9] Therefore, single risk SNPs do not pose a clinically significant effect on their own, but the risk is cumulative (multiplicative or log additive) and increases with increasing numbers of risk alleles present in an individual. GWASs utilising catalogues of commonly occurring SNPs are not powered to detect rarer (MAF <1%) occurring variants which may have a higher relative risk of PrCa development. GWASs specifically investigating rarer variants may reveal these higher risk single nucleotide variants (SNV), but these studies will require very large populations to detect risk SNVs. With the formation of international consortia such as PRACTICAL (PRostate Cancer Association group To Investigate Cancer Associated Alterations in the genome), these types of GWAS will become more feasible. Some high risk SNVs are already known such as those residing in the *HOXB13* gene and were discovered by sequencing genes in regions of chromosome 17 identified by linkage studies of familial PrCa cases. The *HOXB1*3 G84E mutation was found to confer an OR of 5.1. [15]

Another rare variant conferring an increased relative risk of PrCa is mutation of the *BRCA2* gene. This was reported by the Breast Cancer Linkage Consortium (BCLC) which found that the *BRCA2* mutation conferred approximately a five-fold relative risk in PrCa development, which increases to a risk of more than sevenfold in families with young onset cases (<65 years)[16]. This association has been confirmed by several research groups worldwide and there is evidence that the *BRCA2* mutation is associated with more aggressive disease as well as earlier age of onset[17]. Germline variants in other DNA repair genes have also been implicated in PrCa development and will be discussed later.

Germline SNPs associated with prostate cancer

As well as SNPs associated with the development of PrCa, SNPs associated with outcome of treatment and radiotherapy toxicity have been identified. These germline genetic variants may prove to be useful clinically as the technology to identify them becomes more accessible within healthcare systems.

Over 100 SNPs associated with the development of PrCa have been identified thus far. The most recently identified variants that contributed to these data were found through a large meta-analysis by Al-Olama et al of >10 million SNPs from GWASs in populations of European, African, Japanese and Latino ancestry. By combining data from trans-ethnic GWASs, 23 new risk SNPs were identified, 7 of which were detected from the multi-ancestry analyses. As with most GWASs in oncology, the initial and largest studies have focused on European populations with limited availability of data from ethnic minority populations. By combining data across populations, study sample and study power is

increased. This approach also provides an independent replication sample set which can overcome concerns of subpopulation stratification effects in single population GWASs, and it enables the identification of rare variants by fine-mapping the association signals that persist across genetically diverse populations [18].

Of the latest 23 SNPs, one was found to be associated with early onset disease in men of European ancestry with an OR of 1.18 (rs636291 at 1p36; risk allele frequency= 0.16; $P=2.1x10^{-8}$) [14]. As with previous GWASs in PrCa, identified risk variants did not distinguish between aggressive and non-aggressive disease.

Although initial GWASs have focussed on European populations, recent studies in non-European cohorts have revealed population specific differences in the frequency of certain risk loci. This may partially explain the geographical and population differences in PrCa rates. For example, in the study of a Chinese population carried out via the PRACTICAL consortium using the iCOGS SNP array, 4 loci on 8q24 were found to contribute considerably to PrCa risk in a large proportion of men. Although, the SNPs didn't reach GWAS level of significance, the frequency of these loci are much higher than that reported in European men, highlighting population specific differences in PrCa risk. [19] Interestingly, differences in somatic genetic changes have also been noted between European and Chinese men, for example a much lower frequency in Chinese men was observed of the 21q22.2-22.3 deletion affecting the *ERG-TMPRSS2* fusion gene and 10q23 deletion, both of which are common in European PrCas; whether these differences are related to environmental or germline genetic factors is not known. [20]

Many of the PrCa risk SNPs identified in this study and previous GWASs are located in intronic non-coding regions of DNA. Therefore, the mechanism contributing to PrCa development is not clearly defined and functional characterisation of these genetic variants is needed to understand this further. Several mechanisms have been proposed to explain how these SNPs may contribute to cancer risk, for example, the identified SNP may in fact be in LD with the causative SNP and act as a proxy for a variant in a coding exon which is not detected by GWAS. Alternatively, a SNP may cause a structural change in the promoter binding site of a gene and in turn affect its expression[21]. For example, the PrCa risk SNP, rs10993994, which is located 2 bp upstream of the transcription start site of the *MSMB* gene, is thought to affect several predicted transcription factor binding sites. [22] *MSMB* encodes PSP94 (prostatic secretory protein of 94 amino acids) which is secreted by prostate tissue and is found at lower levels in prostate tumour tissue compared to healthy prostates. [23] It may be more specific as a diagnostic tool than PSA.[24] The region surrounding this SNP has been shown to be critical for *MSMB* promoter activation[25] and functional studies have demonstrated that mutations at rs10993994 directly affect expression of PSP94[26] and this may play a role in PrCa development through interactions with other genes such as *NCOA4* which lies adjacent to *MSMB*.[25]

A SNP may also cause a change in the expression of long non-coding RNAs (lncRNA). These are evolutionally conserved and biologically functional non-coding RNA transcripts. The mechanism by which they interact with phenotype-causing loci is unclear although studies suggest that they can regulate the expression of genes lying in close proximity or target distant transcriptional activators or repressors[27]. A complete list of PrCa associated SNPs are included in the review by Eeles et al. [9]

Clinical applications of GWAS data

Screening using PSA related SNPs

The most obvious use of GWAS data discussed so far would be in the context of screening, early diagnosis and ideally prevention of PrCa development. Germline DNA is an attractive entity for use in PrCa screening, as it acts as a constant risk factor which is easily accessible and requires a single measurement rather than serial measurements. Currently, PSA measurement with or without digital rectal examination (DRE) is used as a screening method in men who present with symptoms or concerns related to PrCa development. Screening questions often include enquiries related to a family history of PrCa. Studies assessing the use of these methods and particularly serum PSA measurement in large scale screening programs have suggested that the impact of overdiagnosis and over-treatment of indolent disease outweighs the benefit of detection of PrCa. [28] As a result, both the UK National Screening Committee (UK NSC) [29] and the U.S. Preventive Services Task Force (USPSTF) have made recommendations against PSA-based population screening. The UK NSC recommendation is currently under review but likely to remain unchanged despite recent evidence suggesting that screening may improve overall survival and metastasis free survival. [30, 31] PSA levels are prone to fluctuation and are affected by benign conditions such as prostatic hypertrophy as well as infection (both systemic and localised) and inflammation. Conversely, PrCa may be diagnosed in the presence of a 'normal' PSA level and therefore there is a risk of false negatives in the use of PSA screening.

Indeed, 40%-45% of the variation in PSA levels is accounted for by hereditary factors and GWAS have identified SNPs that associate with PSA level exclusively rather than PSA level and PrCa risk. Gudmundsson et al carried out a GWAS investigating SNPs correlating with PSA levels in men without PrCa [32]. Six SNPs were identified, 3 of which were not previously known. Although the 3 loci previously described had been shown to associate with PrCa as well as PSA levels, two of the newly identified SNPs (at 10q26 and 12q24) were found to associate exclusively with PSA levels, while the third (at 5p13) exerted only a moderate risk on PrCa development. The investigators propose using this knowledge of genetic variant association with PSA level to apply a 'genetic correction' to commonly used PSA thresholds. This will in turn produce a personalised PSA cutoff value to decide if a man should undergo a prostate biopsy. In their study population of Icelandic and UK men, this approach improved the accuracy of prediction of biopsy outcome. This improvement was greater when the 'genetic correction' of PSA level was combined with the effect of PrCa risk SNPs also present. [32] As knowledge of genetic variant associations develops, personalised PSA thresholds may become a feasible tool that could be incorporated into PrCa screening. It must be noted though that GWAS data and subsequent risk SNPs identified can only be applied to populations similar to that used in the original studies unless validation studies in other ethnic groups are carried out. Differences in SNP profiles between different ethnic groups exist and therefore, GWASs in other populations are needed; some have already been carried out such as in the Korean study by Kim et al which identified PSA related SNPs different to those observed in the Icelandic/UK GWAS. [33]

A UK study assessing the use of PSA related SNPs in improving the discrimination achieved by a single PSA threshold in men with raised PSA levels [34] found that genetically correcting PSA levels in men with a level between 3-10 ng/mL did not improve the differentiation between high and low risk PrCa. This suggests that current knowledge of SNPs affecting PrCa risk and prostate related variables may be most useful when used in conjunction with other biomarkers such as PrCa risk SNPs (as done

in the study by Gudmundsson et al) or clinical factors such as in the STHLM3 model (described below) and cannot be solely relied upon clinically.

Screening using prostate cancer risk SNPs

Screening models incorporating PrCa associated SNPs have been suggested. Using the currently known PrCa risk SNPs, men who are in the top 1% of the risk distribution are thought to have a relative risk of 4.7 fold compared with the average risk of the general reference population. Risk modelling suggests that the cumulative effect of risk SNPs could be used in large scale programs to target screening to high risk groups. [14]

A retrospective study using a cohort from the screened arm of the American Prostate, Lung, Colorectal and Ovary (PLCO) study showed that profiling germline PrCa SNPs (33 SNPs producing a prostate genetic score (PGS)) can identify men who have a higher risk of developing disease, with men in the top quartile of PGS-33 score having the highest risk detection rate of PrCa.[35]

In a recent prospective Swedish study, a screening model (STHLM3) combining plasma protein biomarkers (PSA, free PSA, intact PSA, hK2, MSMB and MIC1), 232 risk SNPs and a set of defined clinical variables (age, family history, previous prostate biopsy and prostate examination) were compared with PSA measurement alone (using a threshold of ≥3ng/ml). [36] The STHLM3 model performed significantly better than PSA measurement for the detection of Gleason 7 or higher PrCa. The investigators concluded that using the STHLM3 model would reduce the number of prostate biopsies by one third, the number of benign biopsies by 44% and the number of Gleason 6 PrCas biopsied by 17%. Gleason 6 PrCas are usually categorised as low risk and many don't require treatment. If the results using this screening model can be replicated and validated in other populations, this would partially address the overdiagnosis of indolent cancers observed in PSA screening studies as well as the risk of prostate biopsies to men without cancer, but further refinement of this model is needed as there was still a significant number of low grade cases diagnosed; over half of the tumours were Gleason 6 cancers. Sensitivity for the detection of high risk PrCas was significantly improved with the STHLM3 model; the AUC with PSA alone was 0.56 compared with 0.74 with the study model. Notably, 21% of the high risk PrCas diagnosed by the STHLM3 model had a PSA level in the range of 1-3 ng/ml and would not have been detected by PSA screening alone. It is unclear whether all the components of the STHLM3 algorithm would be needed in a large scale screening program, although all the variables used were significantly associated with high risk PrCa and contributed to a cumulative improvement in the AUC in the multivariate analysis. This was the first large prospective and population based PrCa screening study assessing a targeted approach to screening. As modifications of this strategy continue to be investigated, for example with the use of other biomarkers such as the four-kallikrein panel (included in STHLM3) and the incorporation of MRI and targeted biopsies (currently under investigation), it is likely that a practical and more feasible model for population screening will arise in the future. Further prospective studies are needed to assess how PrCa risk SNPs can be used to target screening to men at increased risk of developing cancer. As further PrCa risk SNPs are identified in future studies, effectively utilising these clinically, will require independent evaluation and validation of their association with PrCa development.

Germline SNPs as predictive and prognostic biomarkers

In the work up of newly diagnosed PrCa, management options are decided depending on factors such as T staging, Gleason score on pathology and presenting PSA level. Patients are assigned a risk category of low, intermediate or high. Although treatment is often straightforward for patients with high risk disease (e.g. Gleason score >7 or T4 stage), for low and intermediate risk cases, management can include active surveillance (AS). Many patients seek to delay interventional treatment due to the risk of long term side effects. In patients who are managed with AS, approximately 33% will progress to definitive treatment by 5 years.[37] Being able to prognosticate and predict outcome based on a profile of germline SNPs would enable a personalised approach to treatment and allow identification of men for whom interventional treatment should be applied.

Predicting aggressive disease

Kearns et al studied a cohort of White American men (n=950) with Gleason 6 PrCa who underwent a radical prostatectomy (RP) and assessed their germline DNA for a set of 23 SNPs to investigate a correlation with upgrading of Gleason score.[38] Three SNPs, rs11568818 on chromosome 11, rs2427345 on chromosome 20 and rs7141529 on chromosome 14, were found to be significant predictors of upgrading Gleason score. These SNPs can be biologically linked to PrCa development; rs11568818 on chromosome 11 lies within an exon of *MMP7* (matrix metalloproteinase 7) which may have a role in PrCa invasiveness. rs2427345 on chromosome 10 lies in an intron associated with *GATA5* and *CABLES2*, which are involved in cell cycle progression, and rs7141529 on chromosome 14 lies in an intron upstream of *RAD51B*, which is a component of the DNA mismatch repair pathway.[39]

The SNPs, rs11568818 (OR=1.46, P=0.0009), and rs2427345 (OR=1.32, P=0.02) remained significant predictors of upgrading Gleason score in a univariate logistic regression model. As well as these SNPs, age and serum PSA level were associated with upgrading disease, which suggests that genetic variant information could be combined with other clinical factors to assess risk category and allow more robust risk stratification. The 3 SNPs were also evaluated in an independent AS cohort analysing time to event. Although this cohort was much smaller, the HR (ranged from 3.7-5.3) for upgrading Gleason score was statistically significant for all 3 SNPs.

An earlier GWAS including only PrCa cases identified two loci that associated with Gleason score: rs35148638 at 5q14.3 and rs78943174 at 3q26.31.[40] A subsequent case-control analysis showed that the 5q14.3 SNP is specific for aggressive PrCa ($P=8.85 \times 10^{-5}$) and doesn't associate with non-aggressive disease compared to controls (P=0.57). Although both SNPs are intronic, they lie within genes that may have a biological role in PrCa development. The 5q14.3 SNP lies within *RASA1* which acts a RAS suppressor and is involved in vascular development as well as cellular proliferation and differentiation. The 3q26.31 SNP lies within the *NAALADL2* gene which is a member of the glutamate carboxypeptidase II family that also includes prostate-specific membrane antigen (PSMA/ *NAALADL1*). *NAALADL2* is overexpressed in prostate tumours and a higher level of expression in tumours has been associated with higher Gleason scores and poorer prognosis after radical prostatectomy. Further studies are needed to assess the use of SNP panels in disease prognostication and PrCa management.

Predicting radiation toxicity

Radiotherapy is the mainstay of curative treatment in a large proportion of PrCa cases. The improvement in techniques of radiation dose delivery and minimisation of the volume of normal

tissues irradiated has led to improved outcomes in acute and late toxicity rates. Despite this, some patients will be significantly troubled by radiation side effects and late effects may only be seen from 2 years after the end of treatment. GWASs in the area of radiogenomics have identified SNPs that appear to predict a predisposition to radiotherapy side effects.[41-45] Predictive markers of late radiotherapy toxicity such as urinary and erectile dysfunction would enable treatment to be personalised to reduce toxicity or permit dose escalation based on one's genetic profile of normal tissue sensitivity.[46] As cure rates and life expectancy continue to increase with improved treatments and earlier diagnosis, maintaining quality of life for patients treated curatively is an important priority.

Early genetic studies targeting candidate genes that may be involved in radiotherapy toxicity showed conflicting results. [47]With the advancement of the field of radiogenomics and utilising the GWAS approach, patterns of genetic variant association with specific toxicities are becoming apparent. Table 1 summarises identified SNPs related to radiotherapy toxicity in the PrCa setting identified by GWAS. Other genetic variants have been identified in candidate gene studies related to radiotherapy toxicities in breast and oesophageal cancer treatment[46].

Explorative successful GWASs require large patient numbers to establish significant association findings, as well as validation in subsequent replication cohorts. This is becoming more feasible with the formation of large consortiums such as the Radiogenomics Consortium and the EU funded REQUITE project (Validating predictive models and biomarkers of radiotherapy toxicity to reduce side effects and improve quality of life in cancer survivors). Once robust predictive genetic profiles are established in the field of radiogenomics, a true personalised approach to radiotherapy planning can be taken. As with the PrCa screening models previously discussed, the ideal way of utilising radiosensitivity SNPs is likely to involve incorporating them into normal tissue complication probability models (NTCP) along with other patient specific factors such as age, gender and race, to optimise radiotherapy delivery individually and minimise acute and long term toxicity.[48] (Fig. 2)

Rare genetic variants

The PrCa associated SNPs discussed so far confer a low to moderate risk of disease but do contribute cumulatively to a man's risk of PrCa development. The GWAS approach is based on the common disease common variant hypothesis and most are powered to detect SNPs with allele frequencies of ≥5%. To detect rare variants (MAF <1-5%) which may confer a higher risk of disease, larger populations of cases and controls are needed and even then very rare SNVs may be missed.

HOXB13

The *HOXB13* G84E germline mutation was identified in four PrCa families after sequencing 200 genes on 17q21 and 17q22 (these regions were previously identified by linkage studies) [15]. This mutation confers an OR of 5.1 for the development of PrCa and is more common in men with early onset disease (3.1%) compared with late onset (0.6%). A subsequent study showed that 5% of PrCa families carried this variant and were mostly of European descent[49], with the highest proportion (22.4%) observed in PrCa families from Finland and Sweden (8.2%). Two missense mutations of *HOXB13* were also detected, one in an African American family and the other in an African-Caribbean family. *HOXB* genes encode transcription factors of the homeobox family, but the mechanism by which mutation leads to prostate carcinogenesis is unknown. Mouse studies have

shown that the HOXB13 protein is involved in prostate development[50] and has been linked to the growth of PrCa cell lines in an androgen-independent manner[51].

BRCA1 and 2

Mutations of the *BRCA2* tumour suppressor gene have also been shown to increase the risk of PrCa development. A fivefold increased risk was reported by the BCLC [16]. *BRCA1* mutations also appear to increase the risk of PrCa development although this is less pronounced than with *BRCA2* mutations; the BCLC reported a 1.8-fold increased risk up to the age of 65 years with *BRCA1* mutation. This association was confirmed by Leongamornlert et al who found the frequency of *BRCA1* mutation in a cohort of 900 PrCa cases to be 0.45%, conferring a relative risk of 3.75 fold and an 8.6% cumulative risk by the age of 65 years. [52]

BRCA1 and *2* are involved in the homologous recombination (HR) DNA repair pathway. In the field of hereditary breast and ovarian cancer, *BRCA* mutation associated disease is known to be responsive to PARP inhibitors (PARPi), in keeping with the concept of synthetic lethality, as well as platinum based chemotherapy. These response patterns have led to the investigation of PARPi in the PrCa setting. In the recently published phase II TOPARP study [53], PrCa patients treated with the PARPi Olaparib were assessed for both germline and somatic mutations in DNA repair genes. Of the 49 patient cohort, 16 were found to have homozygous deletions, deleterious mutations or both in DNA repair genes. Of these, 6 had a germline mutation (3 in *BRCA2* and 3 in *ATM*). In the 16 patients with DNA repair gene mutations, 14 (88%) had a response to treatment including in all 3 germline *BRCA2* mutation carriers. Although the numbers of patients in this study are small, the clinical implications of these results are significant, both in the somatic and germline genetic settings. With the advancing progress of personalised medicine and molecularly targeted agents in oncology in the last 3 decades, identifying high risk genetic variants in the hereditary PrCa setting would extend this personalised approach further to allow targeted screening and surveillance of higher risk individuals thereby leading to earlier diagnosis and earlier access to curative treatment. Olaparib has recently been approved by the FDA and EMA for the treatment of platinum sensitive *BRCA*-mutated (germline and/or somatic) high grade serous epithelial ovarian cancer. If the responses to olaparib seen in TOPARP-A are replicated in the expansion cohort currently being enrolled into TOPARP-B (patients selected based on predictive DNA repair gene mutations in tumour tissue), it is highly likely that olaparib will be become an option for PrCa treatment in the near future.

The IMPACT study is addressing the area of PrCa screening (using biopsy in those with a PSA >3 ng/ml) in *BRCA1* and *BRCA2* carriers as well as men with Lynch Syndrome. Initial results from the first screening round in this study which involved *BRCA1* and *BRCA2* carriers and controls have shown a higher positive predictive value for PSA triggered biopsy in *BRCA2* carriers (PPV 48%) compared with controls (PPV 33%).[54] PrCa detected in *BRCA2* carriers was classified as intermediate or high risk in two thirds of cases. Similarly, in the *BRCA1* carriers, 61% were found to have intermediate or high risk disease. [54]

The findings of higher risk disease in *BRCA* mutation carriers may lead to worse outcomes as reported in a retrospective study by Castro et al [17], further emphasizing the argument for screening such high risk individuals. In this study of 67 *BRCA* mutation carriers and 1235 non-carriers, both metastasis free survival and cause specific survival were significantly lower in the *BRCA* mutation carriers. (Fig. 3)

Other DNA repair genes in prostate cancer

Previous to the TOPARP study revealing PrCa patients with germline mutations in *ATM* as well as *BRCA2*, there have been reports of PrCa associations with other DNA repair gene mutations. In a sample of 191 PrCa patients with ≥3 cases of PrCa in their family, 7.3% were found to harbour a germline loss of function (LoF) mutation in a tumour suppressor gene[55]. These genes included *BRCA 1* and *2*, *ATM,* mismatch repair (MMR) genes (*MLH1, MLH3, MSH2, MSH6*) and *RAD51C* among others (Fig. 4), and all belonged to one of four DNA damage response pathways. Similar to the correlation of *BRCA2* mutation with advanced disease, this retrospective study also showed that LoF mutation carriers have an increased risk of having advanced disease (defined by nodal or distant metastases or T4 disease) with an odds ratio of 15.09.[55]

Recently published data by Pritchard et al has reported an even higher frequency of germline mutations in a larger unselected group of PrCa.[56] In a cohort of 692 men with metastatic castration resistant PrCa, 11.8% (82 men) were found to carry a germline mutation in a DNA repair gene. Again, the highest rates were found in the *BRCA2* and *ATM* genes. The finding of higher than expected rates of germline mutations in men with advanced PrCa may lead to the use of genetic screening in the oncology setting after diagnosis with metastatic PrCa, similar to the recent initiation of *BRCA1/2* mutation screening for all women diagnosed with high grade serous ovarian cancer regardless of age or family history. [57]

Increasing our understanding of the germline genetic variants predisposing to PrCa will also complement the understanding of somatic mutations and prostate carcinogenesis. This is already happening in the ovarian cancer arena, where 50% of epithelial ovarian cancers (EOCs) display defects of the HR DNA repair pathway (termed HRD) which appear to confer sensitivity to PARPi as well as platinum chemotherapy, but only about 15% are accounted for by germline *BRCA* mutations. The remainder are thought to be caused by a range of genetic and epigenetic alterations of HR genes which has led to the investigation of candidate biomarkers of HRD to predict response to PARPi in non-*BRCA* mutated EOC. [58]

Similarly, as the evidence for the increased risk of PrCa in patients with germline mutations accumulates, clinical trials are also investigating somatic mutations that may predict response to targeted agents. After the presentation of the results of the TOPARP-A study, the TOPARP-B study is now enrolling PrCa patients whose tumour DNA harbours a mutation in a DNA repair gene. Only patients with a deleterious mutation will receive Olaparib. Similarly, a study of a PI3K inhibitor and enzalutamide in PrCa is selecting patients whose tumours are *PTEN* deficient (NCT02215096, www.clinicaltrials.gov).

Future of prostate cancer genetics

The rapid advances in genomic technology, along with the falling costs of genetic sequencing and the collaboration between large uro-oncology networks has led to an accumulation of data that we have yet to fully understand. For example, many of the PrCa risk SNPs conferring a low or moderate risk lie in non-coding regions of DNA and further studies are needed to identify how these SNPs interact with coding regions or whether in fact some SNPs are actually in linkage with a causative SNP elsewhere in the genome. Functional studies of causative SNPs are needed to understand how they promote prostate carcinogenesis. Studies of gene-environment interactions, under the umbrella of

the Collaborative Oncological Gene-Environment Study (COGS), are also underway to assess how germline SNPs interact with lifestyle factors to impact on the risk of PrCa development.

Even without complete functional information related to how SNPs cause PrCa, utilising known risk SNPs to target PrCa screening is being explored. Whether these SNP profiles can be used independent of other factors such as family history, age and PSA level is unclear but with future risk modelling studies, the ideal way of utilising one's genetic profile will become more apparent. By targeting PrCa screening to high risk groups, both in terms of common SNPs and rare genetic variants such as *BRCA 1* and *BRCA 2*, the adverse effects of population screening such as biopsy complications and over treatment of low risk disease may be avoided. If the risk of aggressive disease can also be predicted from one's genetic profile, then treatment of early stage disease that is not clearly aggressive may also be justified.

Although the focus of the current discussion has surrounded the germline genetics of PrCa development, the role of somatic genetics is evolving rapidly with the advent of personalised treatments targeted to tumour specific molecular defects. The most recent demonstration of this was seen in the TOPARP study where somatic mutations of DNA repair genes were targeted clinically with the PARP inhibitor, Olaparib.

With the efforts in developing personalised approaches to cancer management at the forefront of oncology research priorities, utilising germline genetic profiles to predict risk of disease development as well as other factors such as disease aggressiveness and treatment toxicity, combined with molecular target identification from somatic tumour profiles, will allow the development of a truly tailored approach to the screening for and treatment of PrCa.

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Figure 1: Top 20 countries worldwide with the highest age standardised rate (ASR) of prostate cancer per 100,000 population.

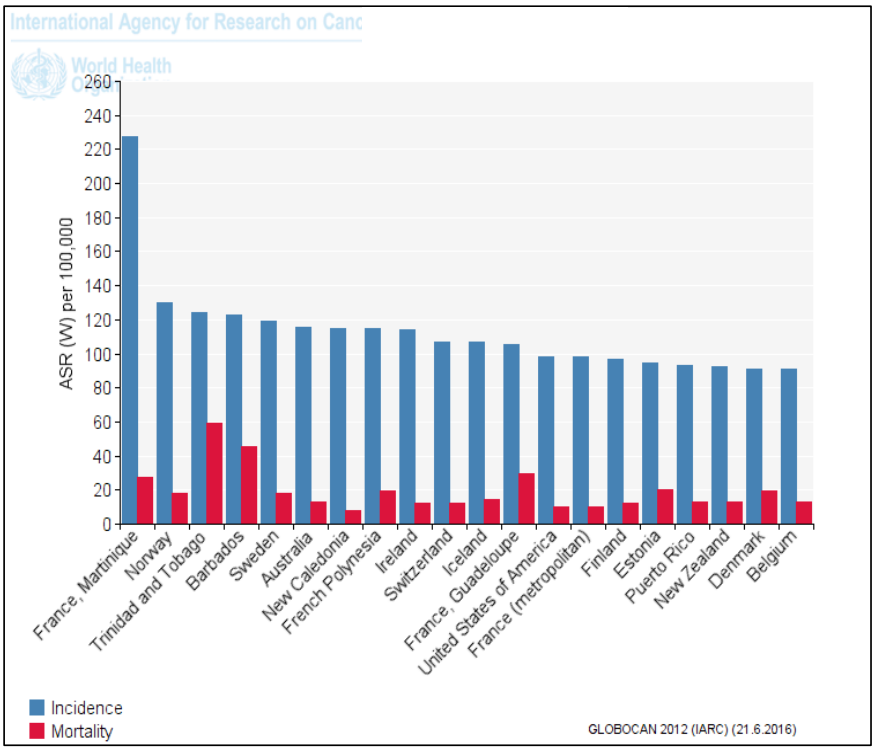
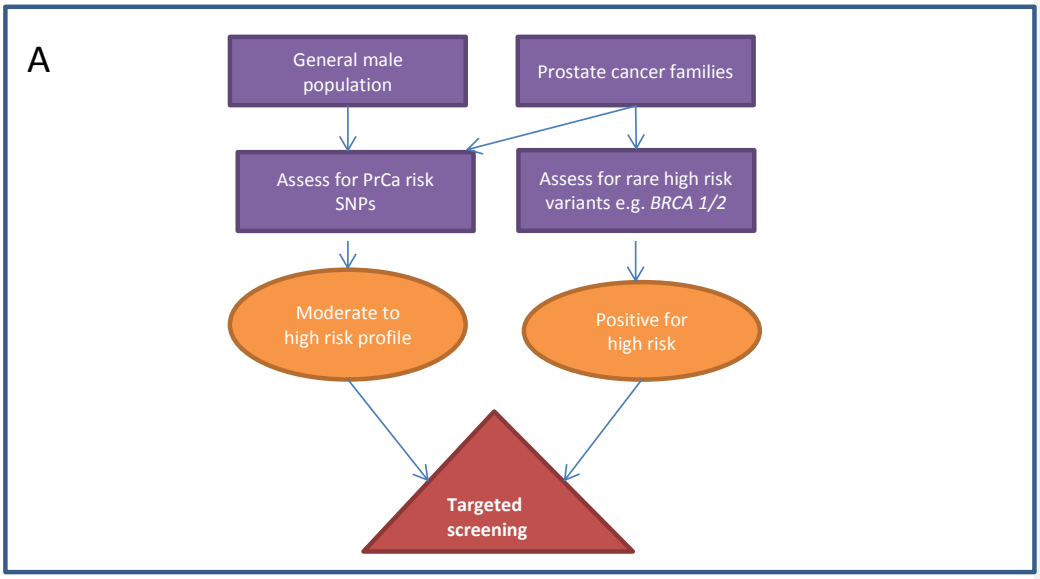


Figure 2: Knowledge of an individual's genetic profile can: A. Identify men at high risk of prostate cancer development and be incorporated into a targeted screening program. B. Allow the personalisation of treatment for example by modifying radiation dose or offering targeted agents such as PARP inhibitors.



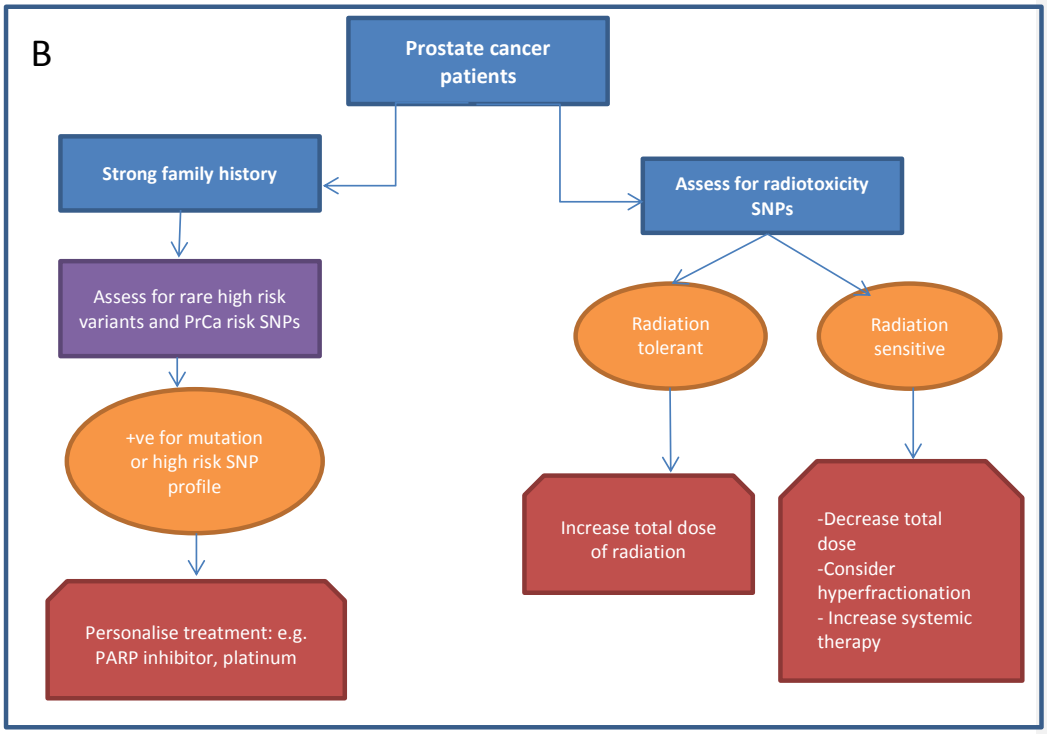


Figure 3: Kaplan-Meier survival curves for *BRCA 1/2* mutation carriers (orange) and controls (black); A. Metastasis free survival B. Cause specific survival.

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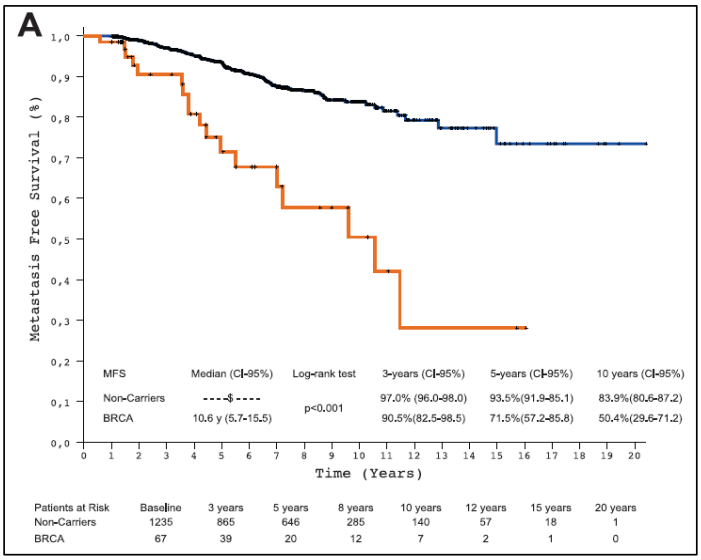

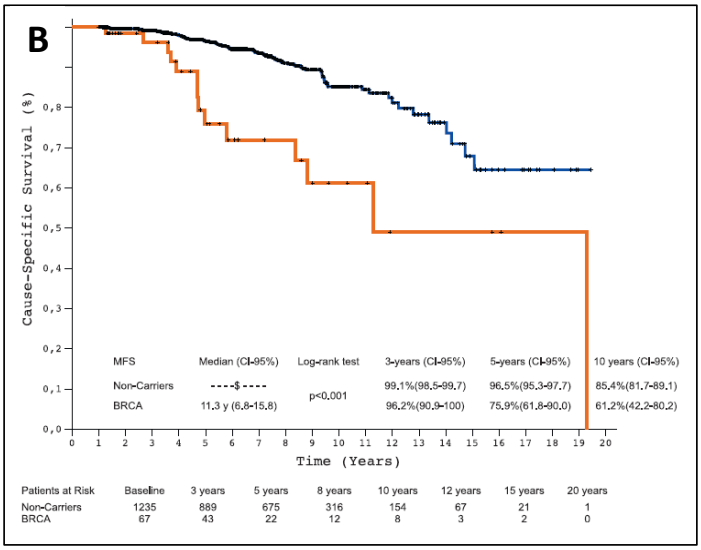

Figure 4: Proportion of loss of function mutations by gene in 14 of 191 cases of familial prostate cancer

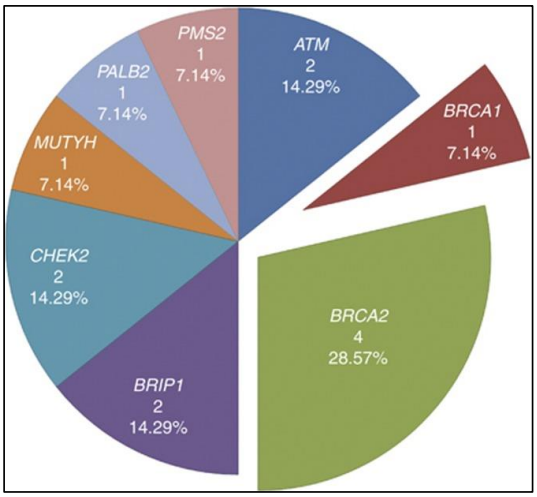

Table 1: Common prostate cancer risk SNPs identified through GWAS and other studies

Locus	SNP	Effect allele	Major Allele	Per allele OR*	Nearby genes	Reference
3p12	rs2660753	C	T	1.13 (1.08-1.19)		45
6q25	rs9364554	C	T	1.10 (1.06-1.14)	*SLC22A3*	45
7q21	rs6465657	T	C	1.10 (1.07-1.13)	*LMTK2*	45
10q11	rs10993994	C	T	1.24 (1.20-1.28)	*MSMB*	45
11q13	rs7931342	G	G	1.20 (1.16-1.23)		45
19q13	rs2735839	G	G	1.23 (1.18-1.30)	*KLK2/3*	45
Xp11	rs5945619	T	C	1.28 (1.21-1.35)	*NUDT11*	45
2p21	rs1465618	C	T	1.07 (1.04-1.11)	*THADA*	45
2q31	rs12621278	A	A	1.33 (1.25-1.43)	*ITGA6*	45
4q22	rs17021918	C	C	1.14 (1.10-1.18)	*PDLIM5*	45
4q22	rs12500426	C	A	1.10 (1.06-1.13)	*PDLIM5*	45
4q24	rs7679673	C	C	1.15 (1.11-1.18)	*TET2*	45
8p21	rs2928679	G	A	1.04 (1.01-1.07)	*SLC25A37*	45
8p21	rs1512268	C	T	1.13 (1.10-1.17)	*NKX3.1*	45
11p15	rs7127900	G	A	1.23 (1.18-1.28)		45
22q13	rs5759167	G	G	1.19 (1.15-1.22)		45
8q24	rs10086908	T	T	1.15 (1.06-1.23)		46
8q24	rs12543663	A	C	1.08 (1.00-1.16)		46
8q24	rs620861	C	C	1.11 (1.04-1.19)		46
19q13	rs11672691	G	A	1.08 (1.05-1.12)	*PCAT19*	47, 48
2p11	rs10187424	T	T	1.09 (1.06-1.12)		49
2q37	rs7584330	A	G	1.06 (1.02-1.09)	*MLPH*	49, 50
3q23	rs6763931	G	A	1.04 (1.01-1.07)	*ZBTB38*	49
3q26	rs10936632	A	A	1.11 (1.08-1.14)		49

5p12	rs2121875	A	C	1.05 (1.02-1.08)	*FGF10*	49
5p15	rs2242652	C	C	1.15 (1.11-1.19)	*TERT*	49, 51
5p15	rs2853676	C	T	1.09 (1.05-1.12)	*TERT*	51
5p15	rs2736107	C	T	1.12 (1.08-1.15)	*TERT*	51
5p15	rs13190087	A	C	1.20 (1.12-1.29)	*TERT*	51
6p21	rs130067	T	G	1.05 (1.02-1.09)	*CCHCR1*	49
12q13	rs10875943	T	C	1.07 (1.04-1.10)		49
Xq12	rs5919432	T	C	1.06 (1.02-1.12)	*AR*	49
1q21	rs1218582	A	G	1.06 (1.03-1.09)		52
1q32	rs4245739	A	A	1.10 (1.05-1.14)		52
2p25	rs11902236	C	T	1.07 (1.03-1.10)		52
2q37	rs3771570	C	T	1.12 (1.08-1.17)	*FARP2*	52
3q13	rs7611694	A	A	1.10 (1.08-1.14)		52
4q13	rs1894292	G	G	1.10 (1.06-1.12)		52
5q35	rs6869841	C	T	1.07 (1.04-1.11)		52
6p21	rs3096702	G	A	1.07 (1.04-1.10)	*NOTCH4*	52
6q21	rs2273669	A	G	1.07 (1.03-1.11)		52
6q25	rs1933488	A	A	1.12 (1.09-1.15)		52
7p15	rs12155172	G	A	1.11 (1.07-1.15)	*SP8*	52
8p21	rs11135910	C	T	1.11 (1.07-1.16)	*EBF2*	52
10q24	rs3850699	A	A	1.10 (1.06-1.12)	*TRIM8*	52
11q22	rs11568818	T	T	1.10 (1.06-1.14)	*MMP7*	52
12q24	rs1270884	G	A	1.07 (1.04-1.10)	*TBX5*	52
14q22	rs8008270	C	C	1.12 (1.08-1.16)	*FERMT2*	52
14q24	rs7141529	T	C	1.09 (1.06-1.12)	*RAD51B*	52
17p13	rs684232	T	C	1.10 (1.07-1.14)		52
17q21	rs11650494	G	A	1.15 (1.09-1.22)		52
18q23	rs7241993	C	C	1.09 (1.05-1.12)		52

20q13	rs2427345	C	C	1.06 (1.03-1.10)		52
20q13	rs6062509	T	T	1.12 (1.09-1.16)	*ZGPAT*	52
Xp22	rs2405942	A	A	1.14 (1.09-1.20)		52
1q21	rs17599629	A	G	1.10 (1.07-1.13)	*GOLPH3L*	13
2p25	rs9287719	T	C	1.07 (1.04-1.09)	*NOL10*	13
4q13	rs10009409	C	T	1.09 (1.06-1.12)		13
6p21	rs3129859	G	G	1.08 (1.06-1.11)	*HLA-DRA*	13
6p22	rs7767188	G	A	1.08 (1.06-1.11)	*TRIM31*	13
6p24	rs4713266	C	C	1.08 (1.04-1.09)	*NEDD9*	13
7p12	rs56232506	G	A	1.07 (1.05-1.09)	*TNS3*	13
9p21	rs17694493	C	G	1.10 (1.06-1.13)	*CDKN2B-AS1*	13
10q11	rs76934034	T	T	1.14 (1.10-1.18)	*Mar-08*	13
11q23	rs11214775	G	G	1.08 (1.05-1.11)	*HTR3B*	13
12q13	rs80130819	A	A	1.12 (1.08-1.18)		13
14q24	rs8014671	G	G	1.08 (1.05-1.10)		13
Xp11	rs2807031	T	C	1.07 (1.04-1.09)	*XAGE3*	13
Xq13	rs6625711	T	A	1.07 (1.05-1.08)		13
Xq13	rs4844289	A	G	1.05 (1.04-1.07)		13
1q32	rs1775148	T	C	1.06 (1.03-1.08)	*SLC41A1*	13
6q14	rs9443189	A	G	1.07 (1.04-1.11)	*MYO6*	13
14q23	rs7153648	G	C	1.09 (1.04-1.13)	*SIX1*	13
16q22	rs12051443	G	A	1.06 (1.03-1.08)	*PHLPP2*	13
20q13	rs12480328	T	T	1.14 (1.08-1.18)	*ADNP*	13
21q22	rs1041449	A	G	1.06 (1.04-1.09)	*TMPRSS2*	13
22q11	rs2238776	G	G	1.09 (1.06-1.12)	*TBX1*	13
1p35	rs636291	G	A	1.18(1.12-1.24)	*PEX14*	13
2p15	rs721048	G	A	1.12 (1.07-1.16)		13
3q21	rs10934853	C	A	1.12 (1.08-1.16)	*EEFSEC*	13

7p15	rs10486567	G	G	1.18 (1.12-1.22)	*JAZF1*	55
8q24	rs1447295	C	A	1.42 (1.35-1.49)		56
8q24	rs6983267	G	G	1.22 (1.19-1.27)		57
8q24	rs16901979	C	A	1.55 (1.43-1.68)		12
9q33	rs1571801	G	T	1.27 (1.10-1.48)	*DAB2IP*	58
10q26	rs4962416	T	C	1.04 (1.00-1.09)	*CTBP2*	55
12q13	rs902774	G	A	1.17 (1.11-1.24)	*KRT8*	50
17q12	rs4430796	A	A	1.22 (1.19-1.27)	*HNF1B*	59
17q12	rs11649743	G	G	1.14 (1.10-1.19)	*HNF1B*	60
17q24	rs1859962	T	G	1.19 (1.14-1.23)		59, 61
19q13	rs8102476	C	C	1.12 (1.08-1.15)		54
22q13	rs9623117	T	C	1.11 (1.04-1.19)	*TNRC6B*	62
2p24	rs13385191	G	G	1.15 (1.10-1.21)	*C2orf43*	63
3p11	rs2055109	T	C	1.20 (1.13-1.29)		64
5p15	rs12653946	C	T	1.26 (1.20-1.33)	*IRX4*	63
6p21	rs1983891	C	T	1.15 (1.09-1.21)	*FOXP4*	63
6q22	rs339331	T	T	1.22 (1.15-1.28)	*RFX6*	63
9q31	rs817826	T	C	1.41 (1.29-1.54)	*RAD23B*	65
10q26	rs2252004	C	C	1.16 (1.10-1.22)		64
17q21	rs7210100	G	A	1.51 (1.35-1.69)	*ZNF652*	66

Table 2: SNPs associated with radiation toxicity

Comment [RE1]: Have we included the ED one?

Locus- Nearest Gene(s)	SNP	Toxicity endpoint	OR (95% CI)	Proposed mechanistic relationship	Ref
2q24.1 - *TANC1*	rs264663	Overall toxicity, late toxicity	6.6 (2.2-19.6)	*TANC1* involved in repair of muscle damage	33
9p21.2 - *IFNK* and *MOB3B*	rs17779457 (one of 8 SNPs in a haplotype block)	Urinary symptoms e.g. incomplete emptying, intermittency, frequency	No OR published. Beta coefficient 2.4	*IFNK* a member of type 1 IFN family with a role in inflammatory response to radiation induced tissue damage. *MOB3B* essential for mitotic checkpoint regulation.	31
11q14.3 - *SLC36A4*	rs7120482 rs17630638	Rectal bleeding	3.1 (1.7-5.6) 2.9 (1.6-5.2	SLC36A4 encodes amino acid transporter needed for cellular proliferation.	67
10q26.3 - *GLRX3* 19q13.43 - *NLRP11*	rs11017104 rs7245988 (two of 12 SNPs discovered in this GWAS)	Erectile dysfunction	1.5 (0.7-3.0) 2.0 (0.9-4.4)	12 SNPs identified that lie near genes involved in biological activities of erectile function.	68