Prostate cancer genomics by high-throughput technologies: genome-wide association study and sequencing analysis

Hidewaki Nakagawa

Laboratory for Genome Sequencing Analysis, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan

Correspondence should be addressed to H Nakagawa
Email
hidewaki@ims.u-tokyo.ac.jp

Abstract

Prostate cancer (PC) is the most common malignancy in males. It is evident that genetic factors at both germline and somatic levels play critical roles in prostate carcinogenesis. Recently, genome-wide association studies (GWAS) by high-throughput genotyping technology have identified more than 70 germline variants of various genes or chromosome loci that are significantly associated with PC susceptibility. They include multiple *8q24* loci, prostate-specific genes, and metabolism-related genes. Somatic alterations in PC genomes have been explored by high-throughput sequencing technologies such as whole-genome sequencing and RNA sequencing, which have identified a variety of androgen-responsive events and fusion transcripts represented by E26 transformation-specific (ETS) gene fusions. Recent innovations in high-throughput genomic technologies have enabled us to analyze PC genomics more comprehensively, more precisely, and on a larger scale in multiple ethnic groups to increase our understanding of PC genomics and biology in germline and somatic studies, which can ultimately lead to personalized medicine for PC diagnosis, prevention, and therapy. However, these data indicate that the PC genome is more complex and heterogeneous than we expected from GWAS and sequencing analyses.

Key Words

- ▶ prostate
- ▶ molecular genetics

Endocrine-Related Cancer
(2013) 20, R171–R181

Introduction

Prostate cancer (PC) is the most common malignancy in men and the second leading cause of cancer-related deaths in Western countries. Incidence and mortality rates vary widely across geographic regions and ethnic groups (Gronberg 2003). In particular, Asian populations have a substantially lower incidence rate than Caucasians or African Americans, indicating the contribution of different genetic backgrounds to PC susceptibility (Gronberg 2003, Schaid 2004, Nakagawa *et al.* 2012). Although the precise mechanisms of prostate carcinogenesis have not been fully elucidated, it is evident that

genetic factors play important roles in PC etiology. A positive family history of PC has been recognized as one of the most important risk factors for PC, as well as African ethnicity and older age (Schaid 2004); twin studies have suggested that the contribution of genetic factors to the development of PC is larger than in other types of common human tumors (Lichtenstein *et al.* 2001). Over the past 20 years, genetic research by linkage analysis on several hereditary PC families had been conducted to clarify PC genomics and to identify genes responsible for PC susceptibility (Schaid 2004). This strategy identified

some candidate genes predisposing to hereditary PC, but they were not replicated in other studies, and this approach was not successful for understanding PC genomics. However, recent genome-wide association studies (GWAS) using high-throughput genotyping technologies on several thousand samples of several ethnic groups have successfully identified more than 70 single-nucleotide polymorphisms (SNPs) of various genes or chromosome loci that are known to be significantly associated with PC susceptibility (Amundadottir *et al.* 2006, Gudmundsson *et al.* 2007, Haiman *et al.* 2007, Eeles *et al.* 2008, 2013, Nakagawa *et al.* 2012).

On the other hand, somatic events in the development and progression of PC have been recently explored more comprehensively through genomic technologies. *NKX3.1* (*NKX3-1*) loss and *PTEN* loss or point mutations were frequently observed in advanced PCs. The androgen/androgen receptor (AR) signaling pathway plays a central role in PC development and progression, and PC growth is usually androgen-dependent. Hence, most of the patients with relapsed or advanced disease respond well to androgen-ablation therapy (castration). Nonetheless, they eventually acquire castration resistance (CR) and progress to a more aggressive phenotype (Scher & Sawyers 2005). In this phase, AR amplification (10–20%) or AR point mutations (<10%) are frequently observed (Taplin *et al.* 1995, Bubendorf *et al.* 1999), indicating that somatic AR alterations play a critical role in CRPC progression (Scher & Sawyers 2005). Furthermore, a new link was established in 2005 between the androgen signaling pathway and PC through the discovery of a genetic rearrangement that drives the fusion of two genes: the androgen-regulated gene *TMPRSS2* and the E26 transformation-specific (ETS) transcription factor *ERG* (Tomlins *et al.* 2005).

Cancer is essentially a disease of the genome that develops and evolves with the accumulation of a variety of somatic mutations with the background of germline variants, and these genomic alterations have now been targeted for cancer treatment and diagnosis. Recent explosive advances of high-throughput genotyping and high-throughput sequencing technologies (next-generation sequencing (NGS)) with bioinformatics approaches enable us to comprehensively analyze a number of cancer genomes. GWAS, whole-genome sequencing (WGS), whole exome sequencing (WES), and RNA sequencing (RNA-Seq) have now been conducted for many types of cancer genomes worldwide, including the International Cancer Genome Consortium (ICGC; Hudson *et al.* 2010) and the Prostate Cancer Association Group to

Investigate Cancer Associated Alterations in the Genome (PRACTICAL Consortium; Kote-Jarai *et al.* 2008, Eeles *et al.* 2013), to explore cancer genomic alterations and diversity. The findings from GWAS using high-throughput genotyping contribute to a better understanding of prostate carcinogenesis and potential application for PC risk prediction and prevention, whereas the findings from somatic mutation analysis, such as WGS and RNA-Seq, may contribute to the discovery of novel therapeutic targets and biomarkers for PC and molecular subclassifications of PC (Fig. 1). This genomic research could ultimately lead to personalized medicine for PC diagnosis, treatment, and prevention. This review describes how recent high-throughput technologies have been applied to PC genomics research, in germline and somatic studies, and what has been achieved to date.

GWAS by high-throughput genotyping to identify germline PC susceptibility loci

GWAS is typically based on a case-control design in which around a hundred thousand SNPs across the human genome are genotyped, and it can scan for germline variants that are associated with disease risk. Such associations are consistent with the ‘common disease–common variant’ hypothesis that assumes that genetic influences on susceptibility to common diseases such as PC are attributable to some number of common variants present in more than 5% of the population (Manolio 2010). GWAS for PC have been remarkably successful in identifying more than 70 common genetic variants or loci, which is quite high compared with the corresponding numbers for other types of cancers and other common diseases. A Manhattan plot of a PC GWAS (Fig. 1) typically indicates many peaks of $-\log(P \text{ value})$ across the genome, including an extremely high peak (low P value for the association) in chromosome 8q24. Subsequent replication and follow-up studies have identified additional associated variants that show genome-wide significance with P values $< 5 \times 10^{-8}$ after adjustment for multiple testing. Recent meta-analyses combining several GWAS cohorts in several ethnic groups (more than 50 000 samples) identified additional PC-susceptibility genes or variants (Eeles *et al.* 2009, 2013, Kote-Jarai *et al.* 2011); larger meta-analysis are now being conducted worldwide and are expected to identify additional PC-susceptibility loci and reach more than 100 PC-susceptibility loci in the near future. As PC incidence and heritability remarkably differ for each race, it is important to perform meta-GWAS on several ethnic

Figure 1

Recent innovations in high-throughput genomic technologies enable us to analyze prostate cancer genomics more comprehensively, more precisely, and on a larger scale. High-throughput genotyping technologies can genotype 60–100 K SNPs and are applicable for GWAS on 10 000 samples. Next-generation sequencing (NGS) can analyze whole genomes (3 Gb) and

the whole transcriptome on dozens of samples to identify point mutations, structural changes, and fusion genes comprehensively. Rare germline variants discovered by NGS have also been analyzed by high-throughput genotyping technologies for GWAS targeting of rare variants.

or racial groups such as Asian and African populations (Takata *et al.* 2010, Haiman *et al.* 2011). However, many of the identified PC-susceptibility variants are located in the intergenic regions, as observed for other disease GWAS, and there are variants for which the biological significance has been proved to be associated with PC. The function or biological significance of only a few variants or genes has been elucidated so far. The greatest challenge of the GWAS loci for prostate genomics is to understand the functional consequences of these many PC-associated loci and to attempt to further understand PC biology and prostate carcinogenesis (Freedman *et al.* 2011). Identification of additional PC-susceptibility loci in larger multi-ethnic GWAS cohorts and biological insights from such functional analysis on GWAS loci can be translated into clinical benefits, including reliable risk-prediction biomarkers and effective strategies for screening and prevention of PC.

Chromosome 8q24 region

Many GWAS in multiple ethnic groups reported strong and consistent association of multiple variants at chromosome 8q24 with PC susceptibility (Gudmundsson *et al.* 2007, Haiman *et al.* 2007, Yeager *et al.* 2007, Al Olama *et al.* 2009). This region contains various independent PC-susceptibility loci within an ~1 Mb segment, and some of them were found to be significantly associated with other types of cancers such as colon cancer and breast cancer (Easton *et al.* 2007, Tomlinson *et al.* 2007). However, no gene has been annotated in this ~1 Mb region, and its biological significance in cancer remains unclear. There are at least five separate loci (Region1–3 or Block1–5) associated with PC susceptibility in this region (Ghoussaini *et al.* 2008). The MYC proto-oncogene is located at ~200 kb downstream, and recent studies have indicated that one of the loci at 8q24 (rs6983267

represents Region1/Block5) could be associated with the WNT signaling pathway (Tuupanen *et al.* 2009) in colorectal cancer and with MYC expression in several cancers (Pomerantz *et al.* 2009). The association of its most centromeric region (Region2: Chr8: 128.14–128.28 Mb) with PC is stronger in Japanese and African populations, and this region was transcribed as an ~10 kb intron-less long non-coding RNA (ncRNA). The expression of this ncRNA was upregulated in PC cells and its precursor lesions, and it is likely to be involved with transcriptional AR activity (Chung *et al.* 2011). Another study reported that a variant on Region2 was correlated with serum testosterone levels (Chu *et al.* 2010), and because Region2 is associated only with PC, not with breast cancer and others, it is likely to be specifically involved with PC through the androgen/AR signaling pathway.

Prostate-specific genes

Several GWAS of PC identified a variant (rs2735839) of *KLK3* at chromosome 19q13, encoding prostate-specific antigen (PSA) as an indicator of PC susceptibility (Eeles *et al.* 2008). However, several groups observed an association between rs2735839 and serum PSA levels (Gudmundsson *et al.* 2010), and this genetic variant may be merely associated with the likelihood of diagnosis by virtue of its link with serum PSA levels. A SNP at chromosome 10q11 (rs10993994) was validated to be associated with several GWAS in some populations (Eeles *et al.* 2008, Thomas *et al.* 2008). It is located at the 5' region of the *MSMB* gene, encoding β -microseminoprotein. Fine mapping and functional analysis demonstrated that this SNP could influence the transcriptional activity of the promoter of *MSMB* (Chang *et al.* 2009). *MSMB* is synthesized by epithelial cells in the prostate gland and secreted into the seminal plasma, and its expression progressively decreased during development of PC from early to late stages, suggesting its potential effect as a tumor suppressor (Beke *et al.* 2007). Some GWAS showed the consistent association of rs1512268 at chromosome 8p21 with PC susceptibility, which represents a 57 kb genetic region in which the *NKX3.1* gene is solely annotated (Eeles *et al.* 2009, Takata *et al.* 2010). *NKX3.1* is exclusively expressed in the prostate tissue, and variants in the 5'-UTR of *NKX3.1* regulate the proximal promoter activity of the *NKX3.1* gene (Akamatsu *et al.* 2010). *NKX3.1* is an androgen-regulated homeobox gene, which plays a key role in the regulation of growth and differentiation of prostate epithelium in a normal prostate and marks a stem cell population that functions

during prostate regeneration (Wang *et al.* 2009). Iroquois homeobox 4 (*IRX4*) at chromosome 5p15 was also identified as a PC-susceptibility gene by GWAS (Nguyen *et al.* 2012), and it is specifically expressed in the adult prostate and heart, suggesting its involvement with the development of the prostate and heart. The associated variants are located at the 5' region of the *IRX4* gene, and regulate the transcriptional activity of *IRX4* through direct interaction with the vitamin D pathway (Nguyen *et al.* 2012). GWAS have demonstrated that these prostate-specific genes are strongly associated with prostate carcinogenesis as well as prostate development.

Metabolism-related genes

It is interesting that GWAS for PC identified several loci or SNPs that were validated to be inversely associated with type 2 diabetes (T2D). Epidemiological studies suggest that men with T2D are less likely to develop PC than non-diabetic men (Bonovas *et al.* 2004). GWAS identified variants of the hepatocyte nuclear factor 1 homeobox B gene (*HNF1B*), which were also related to T2D predisposition (Sun *et al.* 2008). These variants were shown to be associated with decreased risk of PC. *HNF1B* encodes a transcription factor, which plays a key role in the development and function of the pancreas and kidney by regulating the expression of numerous genes in these tissues (Pontoglio 2000). *HNF1B* was previously shown to be mutated in individuals with maturity-onset diabetes of the young type 5 (MODY5; Lindner *et al.* 1999). Interestingly, this variant at *HNF1B* was also found to be associated with the risk of endometrial cancer in women, which is also thought to be dependent on some metabolic and hormone factors, as well as PC in men (Spurdle *et al.* 2011). GWAS showed that variants of juxtaposed with another zinc finger 1 (*JAZF1*) were also significantly associated with T2D and associated with decreased risk of PC (Thomas *et al.* 2008). *JAZF1* encodes a nuclear protein with three zinc fingers and functions as a transcriptional repressor and it may be involved in lipid metabolism in the liver and adipocytes (Li *et al.* 2011). *JAZF1* variants were also shown to be associated with human height, supporting a role for this gene in the regulation of growth and metabolism (Soranzo *et al.* 2009). Thus, it appears possible that genetic variations in *JAZF1* and *HNF1B* may influence PC risk by changing the levels of hormones or growth-related factors previously suggested to be related to T2D. A GWAS identified that rs339331 at 6q22 was significantly associated with PC susceptibility (Takata *et al.* 2010), and this locus includes

the G protein-coupled receptor, family C, group 6, member A gene (*GPRC6A*). *Gprc6a*-null mice exhibited a metabolic syndrome characterized by impaired bone mineralization, increased fat mass, fatty liver, glucose intolerance, testicular feminization, and abnormal steroidogenesis (Pi *et al.* 2008), indicating that it can affect PC susceptibility by altering *GPRC6A*-mediated sex hormone production and metabolic pathways. Diet and lifestyle are now proposed to be critical risk factors for PC development, and indeed, in Asia, there is rapidly increasing incidence of PC partially due to the prevalence of a Western lifestyle, which includes high consumption of a high-fat diet. These interesting observations illustrate a biological phenomenon connecting metabolism and prostate carcinogenesis.

Inflammation-related gene

Japanese GWAS on PC identified a PC-susceptibility locus at *19q13.4* (rs103294), and this SNP is located in the *FOXP4* gene (Takata *et al.* 2010). The FOXP family of forkhead transcription factors are essential for normal T-cell lineage development. A new member of the FOXP family, FOXP4, has also been reported to be dispensable for T-cell development, and it may be involved in the immune response to pathogen infection (Wiehagen *et al.* 2012). GWAS on Chinese PCs identified a PC-susceptibility locus at *19q13.4* (rs103294), and this SNP was tightly linked with a 6.7 kb deletion that removed the first six exons of leukocyte immunoglobulin-like receptor, subfamily A, member 3 (*LILRA3*) and induced the loss of its expression in T cells (Xu *et al.* 2012). The LIR family members, including *LILRA3*, bind to major histocompatibility complex antigens and regulate the immune and inflammatory response. Although the role of *LILRA3* in prostate carcinogenesis is largely unknown, these findings suggest a potential role in chronic inflammation or the immune response in prostate carcinogenesis.

Variants associated with aggressive PCs

It remains unclear whether these genetic variants are associated with PC aggressiveness. PC is primarily a disease associated with old age, and many indolent PC cases in elderly people are subject to a watch-and-wait strategy without any PC treatment (Albertsen 2010). The rate of latent PC in autopsies may be as high as 80% by the age of 80 years (Konety *et al.* 2005), and PC is a very 'common' disease in older men. Hence, distinguishing between indolent PC and aggressive PC, which requires treatment,

including medical castration, surgery, and radiation therapy, and predicting the risk of aggressive PC are very important current goals in PC research and clinics. Recent meta-analysis of GWAS showed that rs4054823 at *17p12* is associated with aggressive disease of PC, but there is no annotated gene around this SNP and no explanation for its biological association with PC aggressiveness (Xu *et al.* 2010). Another meta-analysis identified rs11672691 at *19q13* as being significantly associated with aggressive PCs, but this association was also observed in indolent PCs, and the responsible genes and biological significance of this locus was not clear (Al Olama *et al.* 2013). To date, most of the PC-susceptibility loci or SNPs have not been significantly associated with aggressive PC disease specifically.

PC risk estimation by multiple SNPs

GWAS have identified more than 50 variants or loci significantly associated with PC risk, but each of them has limited use in the assessment of PC risk in an individual because each of these genetic markers confers a modest effect (OR: 1.1–2.0). It is required for clinical use of these risk variants to combine individual variants for PC risk assessment (Manolio 2010). Zheng *et al.* (2008) combined five variants for PC risk assessment plus a family history of PC and confirmed a cumulative and significant association with PC. However, several studies using five or more SNPs have since indicated that the area under the curve (AUC) in the ROC curve was around 0.6, and this SNP panel may have limited clinical utility (Nam *et al.* 2009, Zheng *et al.* 2009). Even when the sample number was increased, the AUC was predicted to be <0.7 (Chatterjee *et al.* 2013). The AUC of the PSA test was found to be 0.65–0.7; to apply the risk estimation model for PC screening, the AUC should be larger than that of PSA. Otherwise, the risk estimation model should focus on individuals with gray-zone or normal-range PSA levels, in which the efficiency of PSA testing to detect PC is questionable (Akamatsu *et al.* 2012). And also it is important to construct a SNP prediction model for each ethnic group because the frequency and OR of each SNP is different in each ethnic group.

Rare variants associated with PC susceptibility

GWAS have thus far focused on common SNPs, but most of these disease-associated SNPs have a very small effect. Even when multiple variants are combined to predict PC risk, they can explain only 10–20% of the genetic risk of

the given disease, indicating missing heritability (Cirulli & Goldstein 2010). Furthermore, GWAS signals have rarely been tracked to causal variants. Although it is still not easy to identify rare variants that are assumed to have a larger effect on susceptibility to common diseases (Cirulli & Goldstein 2010), a few rare variants have been identified that confer a substantial disease risk. Several linkage studies on hereditary PCs and early-onset PCs identified the chromosome 7q21–22 region as one of the most critical regions responsible for hereditary PCs. There are 202 genes in this critical region and NGS analysis identified rare variants of the *HOXB13* gene, which is highly expressed in the prostate and is likely to be involved with the development of the prostate or urological system. While a rare variant of *HOXB13* G84E was observed in 0.1–0.2% of the control population, the patients of hereditary PC or early-onset PC had 1–3% with an OR of 2.7–5.1 (Ewing *et al.* 2012). WGS in an Icelandic population that identified a rare variant in the PC critical region at chromosome 8q24, whose frequency was 0.5% in the control population, which is independent of the reported SNPs or loci at chromosome 8q24 (Gudmundsson *et al.* 2012). Now next-generation GWAS are focusing on rare variants. WGS or WES can identify rare variants (frequency ~0.1 or 0.5%) in thousands of the affected population, and these variants on custom SNP chips can be screened for GWAS on ~10 000 case controls. Owing to their low frequency, these analyses are required to scan a large number of samples to produce sufficient statistical power. Hence, it is critical to collect such a large sample size in the bio-banks or consortium worldwide.

Somatic mutation analysis by NGS technology

There is increasing evidence that the somatic mutation burden in an individual's cancer drives tumor formation, influences disease progression, and affects sensitivity to therapy (Fig. 2). Common somatic mutations in PC include loss of *NKX3.1* and *PTEN*, and *AR* amplification or point mutations. *NKX3.1* at chromosome 8p21 is frequently subject to loss of heterozygosity (LOH) in human PCs, and it has been recognized to function as a tumor suppressor of prostate (Bova *et al.* 1993), although somatic point mutations of *NKX3.1* were not frequently detected in PCs (Voeller *et al.* 1997). *PTEN* at chromosome 10q23 is also frequently subject to LOH or homozygous deletion in many types of human cancers, and it is inactivated by point mutations in 20–40% of PCs (Li *et al.* 1997). *Pten*^{+/-} mice have prostatic hyperplasia and dysplasia; prostatic intraepithelial neoplasia (PIN)

develops in *Pten*^{+/-}*Nkx3.1*^{+/-} and *Pten*^{+/-}*Nkx3.1*^{-/-} mice (Kim *et al.* 2002), which indicate their tumor suppressor feature in prostate. In 2005, fusion transcripts of ETS family transcription factors with androgen-responsive promoters were discovered, which were driven by a genomic rearrangement (Tomlins *et al.* 2005). The *TMPRSS2-ERG* gene fusion and other ETS fusion genes have been causally linked to cancer progression because it promotes invasion, and overexpression of the fusion product in mice shows greatly enhanced PC development (Carver *et al.* 2009). Now recent rapid advances in sequencing technology (NGS) have enabled us to perform more comprehensive analysis such as WGS for human genome and cancer genome for \$5000 in a few days, and its cost is expected to become less than \$1000 in a few years. WGS can comprehensively detect almost all single-nucleotide variants, copy number variants, structure changes, and foreign genomes such as integrated virus (Fig. 1). Some projects in ICGC and other groups are analyzing PC genomes by WGS or WES integrated with RNA-Seq and DNA methylation analysis (<http://www.icgc.org/>) now. It is also required to examine somatic mutations of PC in different ethnic groups (African and Asian PCs) as well as germline variants because PC genomics and its biological behavior show some ethnic differences.

WGS and WES of PC

Initial WGS analysis on seven PCs with a Gleason grade 7 or higher (Berger *et al.* 2011) revealed that a number of genomic rearrangements were present in PC genomes and their breakpoints were enriched near open chromatin, AR, and ERG DNA binding site, leading to several types of ETS gene fusions. Other genomic rearrangements were observed to affect *PTEN* and *PTEN*-interacting proteins, indicating the effect of the *PTEN* pathway on prostate carcinogenesis. On average, PCs harbored 13–40 non-silent coding somatic mutations and ~1.0 mutations per Mb. This number is relatively low compared with the mutation numbers of other types of solid tumors. WES on 23 PC xenografts in mice (Kumar *et al.* 2011) was reported, and this study identified a number of somatic mutations, because they did not analyze the normal control genome and their data were likely to be contaminated by mouse genome sequences. WES on 112 PC samples (Barbieri *et al.* 2012) identified recurrent mutations in multiple genes, including *SPOP*, *MED12*, and *FOXA1*. *SPOP* mutations were identified in 6–13% of PCs, and it is likely to be involved in cell invasion. It is interesting that *SPOP* mutations occurred mutually exclusively in PC with *ERG*

Figure 2

Prostate cancer (PC) development and progression with the accumulation of various somatic mutations on the background of germline variants.

The androgen/AR signaling pathway plays a consistent and critical role in all steps of PC development and progression.

rearrangements, and it was also detected in PC precursor, high-grade PIN, suggesting that *SPOP* mutations comprise an early event in prostate carcinogenesis as well as *ERG* rearrangements. Another WES analysis was performed focusing on 50 CRPCs (Grasso *et al.* 2012). This study identified recurrent mutations of chromatin regulators, including *MLL2*, *CHD1*, and *FOXA1*. These mutated genes are likely to be involved with androgen/AR signaling and confer deregulated AR signaling and a CR phenotype. WGS on 11 early-onset PCs was also conducted, which showed age-related differences in structure rearrangement formations. Compared with elderly-onset PC, these early-onset PCs demonstrated enrichment for androgen-driven structural rearrangements involving ETS family genes (Weischenfeldt *et al.* 2013), suggesting that early-onset PC development is predominantly driven by the androgen and AR signaling pathway.

RNA-Seq and fusion genes in PC

RNA-Seq (whole transcriptome sequencing) can provide not only a gene expression profile but also information about splicing changes, non-annotated ncRNA expression, and RNA editing. Most importantly, for PC genomics, RNA-Seq analysis can detect many types of recurrent fusion genes. ETS family gene fusions, including *TPRSS2-ERG*, were discovered in 2005, and most PCs are characterized by the presence of recurrent gene fusions

primarily involving androgen-regulated upstream genes, *TPRSS2*, *KLK2*, and *SLC45A3*, fused to one of the genes of the ETS family of oncogenic transcription factors (*ERG*, *ETV1*, *ETV4*, or *ETV5*) (Maher *et al.* 2009). These fusion events are usually driven by genomic rearrangements in PC genomes. Recent RNA-Seq analysis detected one read-through chimera, *SLC45A3-ELK4*, between the fourth exon of *SLC45A3* with exon 2 of *ELK4*, a member of the ETS transcription factor family, and this fusion event was not driven by genomic rearrangements, but *cis*-splicing between the two adjacent genes (Maher *et al.* 2009, Zhang *et al.* 2012). In addition to the ETS family gene fusions, RNA-Seq analysis on 25 PC samples discovered fusion transcripts involving non-ETS genes such as *CDKN1A* (*p21*), *CD9*, and *IKBKB* (*IKK β*), genes known to exhibit key biological roles in cellular homeostasis, as well as the oncogene *PIGU* and the tumor suppressor gene *RSRC2* (Pflueger *et al.* 2011). Another RNA-Seq analysis on 14 Chinese PCs uncovered a recurrent fusion gene between *USP9Y* and *TTY15* on chromosome Y, and a fusion event that arose from an interchromosomal translocation involving *CTAGE5* and *KHDRBS3* (Ren *et al.* 2012). A recent study by RNA-Seq discovered *RAF* kinase gene fusions: *SLC45A3-BRAF*, *ESRP1-RAF1*, and *RAF1-ESRP1* in advanced PCs. Although they were detected in only 1–2% of PCs, *RAF* kinase fusions represent the first ‘actionable’ fusions in PCs that do not involve an ETS family member (Palanisamy *et al.* 2010). RNA-Seq

analysis can detect non-annotated transcripts, different from the microarrays, and systematic RNA-Seq analysis on 102 PC samples discovered more than 100 novel PC-specific ncRNAs and demonstrated that a novel lincRNA *PCAT1* is a transcriptional repressor implicated in a subset of PC patients (Prensner *et al.* 2011).

Neuroendocrine PC genome

Neuroendocrine PC (NEPC) is a rare subtype of PC affecting only 0.5–2% of patients with PC, but NEPCs more frequently arise after castration therapy for prostate adenocarcinoma, although it can arise *de novo* (Palmgren *et al.* 2007). Focal neuroendocrine differentiation is detected in some 10% of localized PCs and increases with disease progression, indicating that these clones are evolved to a NEPC phenotype after castration (Beltran *et al.* 2011). New AR inhibitors and blockers of androgen production are now available, and ‘complete’ castration will be established and be a standard therapy for recurrent and metastatic CRPCs (Haddad & Garcia 2012). Hence, the number of patients with the NEPC phenotype will increase (Fig. 2), and more research and clinical focus will be put into the study of NEPCs. *TMPRSS2-ERG* has been reported in ~50% of NEPCs, suggesting that NEPC is clonally derived and evolved from PC (Lotan *et al.* 2011). RNA-Seq analysis of seven NEPCs revealed overexpression and amplification of *AURKA* and *MYCN* in 40% of NEPCs and 5% of PCs (Beltran *et al.* 2011).

Conclusions

Recent innovation of high-throughput genomic technology has enabled us to analyze PC genomics more comprehensively, more precisely, and on a larger scale. Based on this valuable information, our understanding of PC genomics and biology is increasing. Germline research can help to establish the risk prediction and prevention of PC, while somatic research can provide information on novel molecular targets and biomarkers and a rationale for molecular sub-classification. The final goal is personalized medicine for PC diagnosis, treatment, and prevention (Fig. 1). However, through GWAS and sequencing analysis, we have recognized more complexity and heterogeneity in the human PC genome than expected. Therefore, to gain further new insights into PC genomics, we have to analyze a larger sample size in each ethnic or racial group. It is still too early and primitive to translate these high-throughput data into the clinic because the majority of these genomic data lack biological

significance; it is difficult to interpret these huge data sets in all biological and clinical aspects. Hence, in addition to discovery by large-scale analysis, we have to employ functional and informatic approaches to better capitalize on the genomic data and to shed light on the biological significance of the genomic alterations or variants that have been identified by high-throughput genomic technologies.

Declaration of interest

The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

This work has been financially supported by the BioBank Japan Project of the Ministry of Education, Culture, Sports, Sciences and Technology of the Japanese government, by Research grant #22390306 (H Nakagawa) from the Japan Society for the Promotion of Science, and by the Takeda Science Foundation.

References

- Akamatsu S, Takata R, Ashikawa K, Hosono N, Kamatani N, Fujioka T, Ogawa O, Kubo M, Nakamura Y & Nakagawa H 2010 A functional variant in NKX3.1 associated with prostate cancer susceptibility down-regulates NKX3.1 expression. *Human Molecular Genetics* **19** 4265–4272. (doi:10.1093/hmg/ddq350)
- Akamatsu S, Takahashi A, Takata R, Kubo M, Inoue T, Morizono T, Tsunoda T, Kamatani N, Haiman CA, Wan P *et al.* 2012 Reproducibility, performance, and clinical utility of a genetic risk prediction model for prostate cancer in Japanese. *PLoS ONE* **7** e46454. (doi:10.1371/journal.pone.0046454)
- Albertsen PC 2010 Treatment of localized prostate cancer: when is active surveillance appropriate? *Nature Reviews. Clinical Oncology* **7** 394–400. (doi:10.1038/nrclinonc.2010.63)
- Al Olama AA, Kote-Jarai Z, Giles GG, Guy M, Morrison J, Severi G, Leongamornlert DA, Tymrakiewicz M, Jhavar S, Saunders E *et al.* 2009 Multiple loci on 8q24 associated with prostate cancer susceptibility. *Nature Genetics* **41** 1058–1060. (doi:10.1038/ng.452)
- Al Olama A, Kote-Jarai Z, Schumacher FR, Wiklund F, Berndt SI, Benlloch S, Giles GG, Severi G, Neal DE, Hamdy FC *et al.* 2013 A meta-analysis of genome-wide association studies to identify prostate cancer susceptibility loci associated with aggressive and non-aggressive disease. *Human Molecular Genetics* **22** 408–415. (doi:10.1093/hmg/ddt425)
- Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, Sigurdsson A, Benediksdottir KR, Cazier JB, Sainz J *et al.* 2006 A common variant associated with prostate cancer in European and African populations. *Nature Genetics* **38** 652–658. (doi:10.1038/ng1808)
- Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, White TA, Stojanov P, Van Allen E, Stransky N *et al.* 2012 Exome sequencing identifies recurrent SPOP, FOXA1 and MED12 mutations in prostate cancer. *Nature Genetics* **44** 685–689. (doi:10.1038/ng.2279)
- Beke L, Nuytten M, Van Eynde A, Beullens M & Bollen M 2007 The gene encoding the prostatic tumor suppressor PSP94 is a target for repression by the Polycomb group protein EZH2. *Oncogene* **26** 4590–4595. (doi:10.1038/sj.onc.1210248)

- Beltran H, Rickman DS, Park K, Chae SS, Sboner A, MacDonald TY, Wang Y, Sheikh KL, Terry S, Tagawa ST *et al.* 2011 Molecular characterization of neuroendocrine prostate cancer and identification of new drug targets. *Cancer Discovery* **1** 487–495. (doi:10.1158/2159-8290.CD-11-0130)
- Berger MF, Lawrence MS, Demicheli F, Drier Y, Cibulskis K, Sivachenko AY, Sboner A, Esgueva R, Pflueger D, Sougnez C *et al.* 2011 The genomic complexity of primary human prostate cancer. *Nature* **470** 214–220. (doi:10.1038/nature09744)
- Bonovas S, Filioussi K & Tsantes A 2004 Diabetes mellitus and risk of prostate cancer: a meta-analysis. *Diabetologia* **47** 1071–1078. (doi:10.1007/s00125-004-1415-6)
- Bova GS, Carter BS, Bussemakers MJ, Emi M, Fujiwara Y, Kyprianou N, Jacobs SC, Robinson JC, Epstein JI, Walsh PC *et al.* 1993 Homozygous deletion and frequent allelic loss of chromosome 8p22 loci in human prostate cancer. *Cancer Research* **53** 3869–3873.
- Bubendorf L, Kononen J, Koivisto P, Schraml P, Moch H, Gasser TC, Willi N, Mihatsch MJ, Sauter G & Kallioniemi OP 1999 Survey of gene amplifications during prostate cancer progression by high-throughput fluorescence *in situ* hybridization on tissue microarrays. *Cancer Research* **59** 803–806.
- Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, Alimonti A, Nardella C, Varmeh S, Scardino PT *et al.* 2009 Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. *Nature Genetics* **41** 619–624. (doi:10.1038/ng.370)
- Chang BL, Cramer SD, Wiklund F, Isaacs SD, Stevens VL, Sun J, Smith S, Pruett K, Romero LM, Wiley KE *et al.* 2009 Fine mapping association study and functional analysis implicate a SNP in MSMB at 10q11 as a causal variant for prostate cancer risk. *Human Molecular Genetics* **18** 1368–1375. (doi:10.1093/hmg/ddp035)
- Chatterjee N, Wheeler B, Sampson J, Hartge P, Chanock SJ & Park JH 2013 Projecting the performance of risk prediction based on polygenic analyses of genome-wide association studies. *Nature Genetics* **45** 400–405. (doi:10.1038/ng.2579)
- Chu LW, Meyer TE, Li Q, Menashe I, Yu K, Rosenberg PS, Huang WY, Quraishi SM, Kaaks R, Weiss JM *et al.* 2010 Association between genetic variants in the 8q24 cancer risk regions and circulating levels of androgens and sex hormone-binding globulin. *Cancer Epidemiology, Biomarkers & Prevention* **19** 1848–1854. (doi:10.1158/1055-9965.EPI-10-0101)
- Chung S, Nakagawa H, Uemura M, Piao L, Ashikawa K, Hosono N, Takata R, Akamatsu S, Kawaguchi T, Morizono T *et al.* 2011 Association of a novel long non-coding RNA in 8q24 with prostate cancer susceptibility. *Cancer Science* **102** 245–252. (doi:10.1111/j.1349-7006.2010.01737.x)
- Cirulli ET & Goldstein DB 2010 Uncovering the roles of rare variants in common disease through whole-genome sequencing. *Nature Reviews. Genetics* **11** 415–425. (doi:10.1038/nrg2779)
- Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, Struwing JP, Morrison J, Field H, Luben R *et al.* 2007 Genome-wide association study identifies novel breast cancer susceptibility loci. *Nature* **447** 1087–1093. (doi:10.1038/nature05887)
- Eeles RA, Kote-Jarai Z, Giles GG, Al Olama A, Guy M, Jugurnauth SK, Mulholland S, Leongamornlert DA, Edwards SM, Morrison J *et al.* 2008 Multiple newly identified loci associated with prostate cancer susceptibility. *Nature Genetics* **40** 316–321. (doi:10.1038/ng.90)
- Eeles RA, Kote-Jarai Z, Al Olama AA, Giles GG, Guy M, Severi G, Muir K, Hopper JL, Henderson BE, Haiman CA *et al.* 2009 Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. *Nature Genetics* **41** 1116–1121. (doi:10.1038/ng.450)
- Eeles RA, Al Olama A, Benlloch S, Saunders EJ, Leongamornlert DA, Tymrakiewicz M, Ghousaini M, Luccarini C, Dennis J, Jugurnauth-Little S *et al.* 2013 Identification of 23 new prostate cancer susceptibility loci using the iCOGS custom genotyping array. *Nature Genetics* **45** 385–391. (doi:10.1038/ng.2560)
- Ewing CM, Ray AM, Lange EM, Zuhlke KA, Robbins CM, Tembe WD, Wiley KE, Isaacs SD, Johng D, Wang Y *et al.* 2012 Germline mutations in HOXB13 and prostate-cancer risk. *New England Journal of Medicine* **366** 141–149. (doi:10.1056/NEJMoa1110000)
- Freedman ML, Monteiro AN, Gayther SA, Coetzee GA, Risch A, Plass C, Casey G, De Biasi M, Carlson C, Duggan D *et al.* 2011 Principles for the post-GWAS functional characterization of cancer risk loci. *Nature Genetics* **43** 513–518. (doi:10.1038/ng.840)
- Ghoussaini M, Song H, Koessler T, Al Olama AA, Kote-Jarai Z, Driver KE, Pooley KA, Ramus SJ, Kjaer SK, Hogdall E *et al.* 2008 Multiple loci with different cancer specificities within the 8q24 gene desert. *Journal of the National Cancer Institute* **100** 962–966. (doi:10.1093/jnci/djn190)
- Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC *et al.* 2012 The mutational landscape of lethal castration-resistant prostate cancer. *Nature* **487** 239–243. (doi:10.1038/nature11125)
- Gronberg H 2003 Prostate cancer epidemiology. *Lancet* **361** 859–864. (doi:10.1016/S0140-6736(03)12713-4)
- Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, Rafnar T, Bergthorsson JT, Agnarsson BA, Baker A *et al.* 2007 Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. *Nature Genetics* **39** 631–637. (doi:10.1038/ng1999)
- Gudmundsson J, Besenbacher S, Sulem P, Gudbjartsson DF, Olafsson I, Arinbjarnarson S, Agnarsson BA, Benediktsson KR, Isaksson HJ, Kostic JP *et al.* 2010 Genetic correction of PSA values using sequence variants associated with PSA levels. *Science Translational Medicine* **2** 62ra92. (doi:10.1126/scitranslmed.3001513)
- Gudmundsson J, Sulem P, Gudbjartsson DF, Masson G, Agnarsson BA, Benediktsson KR, Sigurdsson A, Magnusson OT, Gudjonsson SA, Magnusdottir DN *et al.* 2012 A study based on whole-genome sequencing yields a rare variant at 8q24 associated with prostate cancer. *Nature Genetics* **44** 1326–1329. (doi:10.1038/ng.2437)
- Haddad H & Garcia JA 2012 Novel agents for the management of castration-resistant prostate cancer. *Current Opinion in Urology* **22** 175–182. (doi:10.1097/MOU.0b013e3283523ba0)
- Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, Neubauer J, Tandon A, Schirmer C, McDonald GJ *et al.* 2007 Multiple regions within 8q24 independently affect risk for prostate cancer. *Nature Genetics* **39** 638–644. (doi:10.1038/ng2015)
- Haiman CA, Chen GK, Blot WJ, Strom SS, Berndt SI, Kittles RA, Rybicki BA, Isaacs WB, Ingles SA, Stanford JL *et al.* 2011 Genome-wide association study of prostate cancer in men of African ancestry identified a susceptibility locus at 17q21. *Nature Genetics* **43** 570–573. (doi:10.1038/ng.839)
- Hudson TJ, Anderson W, Artez A, Barker AD, Bell C, Bernabé RR, Bhan MK, Calvo F, Eerola I, Gerhard DS *et al.* 2010 International network of cancer genome projects. *Nature* **464** 993–998. (doi:10.1038/nature08987)
- Kim MJ, Cardiff RD, Desai N, Banach-Petrosky WA, Parsons R, Shen MM & Abate-Shen C 2002 Cooperativity of Nkx3.1 and Pten loss of function in a mouse model of prostate carcinogenesis. *PNAS* **99** 2884–2889. (doi:10.1073/pnas.042688999)
- Konety BR, Bird VY, Deorah S & Dahmouch L 2005 Comparison of the incidence of latent prostate cancer detected by autopsy before and after the prostate specific antigen era. *Journal of Urology* **174** 1785–1788. (doi:10.1097/01.ju.0000177470.84735.55)
- Kote-Jarai Z, Easton DF, Stanford JL, Ostrander EA, Schleutker J, Ingles SA, Schaid D, Thibodeau S, Dörk T, Neal D *et al.* 2008 Multiple novel prostate cancer predisposition loci confirmed by an international study: the PRACTICAL Consortium. *Cancer Epidemiology, Biomarkers & Prevention* **17** 2052–2061. (doi:10.1158/1055-9965.EPI-08-0317)
- Kote-Jarai Z, Al Olama A, Giles GG, Severi G, Schleutker J, Weischer M, Campa D, Riboli E, Key T, Gronberg H *et al.* 2011 Seven prostate cancer susceptibility loci identified by a multi-stage genome-wide association study. *Nature Genetics* **43** 785–789. (doi:10.1038/ng.882)
- Kumar A, White TA, MacKenzie AP, Clegg N, Lee C, Dumpit RF, Coleman I, Ng SB, Salipante SJ, Rieder MJ *et al.* 2011 Exome sequencing identifies a

- spectrum of mutation frequencies in advanced and lethal prostate cancers. *PNAS* **108** 17087–17092. (doi:10.1073/pnas.1108745108)
- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliareis C, Rodgers L, McCombie R *et al.* 1997 PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* **275** 1943–1947. (doi:10.1126/science.275.5308.1943)
- Li L, Yang Y, Yang G, Lu C, Yang M, Liu H & Zong H 2011 The role of JAZF1 on lipid metabolism and related genes *in vitro*. *Metabolism* **60** 523–530. (doi:10.1016/j.metabol.2010.04.021)
- Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytté A & Hemminki K 2001 Environmental and heritable factors in the causation of cancer – analyses of cohorts of twins from Sweden, Denmark, and Finland. *New England Journal of Medicine* **343** 78–85. (doi:10.1056/NEJM200007133430201)
- Lindner TH, Njolstad PR, Horikawa Y, Bostad L, Bell GI & Sovik O 1999 A novel syndrome of diabetes mellitus, renal dysfunction and genital malformation associated with a partial deletion of the pseudo-POU domain of hepatocyte nuclear factor-1 β . *Human Molecular Genetics* **8** 2001–2008. (doi:10.1093/hmg/8.11.2001)
- Lotan TL, Gupta NS, Wang W, Toubaji A, Haffner MC, Chaux A, Hicks JL, Meeker AK, Bieberich CJ, De Marzo AM *et al.* 2011 ERG gene rearrangements are common in prostatic small cell carcinomas. *Modern Pathology* **24** 820–828. (doi:10.1038/modpathol.2011.7)
- Maher CA, Kumar-Sinha C, Cao X, Kalyana-Sundaram S, Han B, Jing X, Sam L, Barrette T, Palanisamy N & Chinnaiyan AM 2009 Transcriptome sequencing to detect gene fusions in cancer. *Nature* **458** 97–101. (doi:10.1038/nature07638)
- Manolio TA 2010 Genomewide association studies and assessment of the risk of disease. *New England Journal of Medicine* **363** 166–176. (doi:10.1056/NEJMra0905980)
- Nakagawa H, Akamatsu S, Takata R, Takahashi A, Kubo M & Nakamura Y 2012 Prostate cancer genomics, biology, and risk assessment through genome-wide association studies. *Cancer Science* **103** 607–613. (doi:10.1111/j.1349-7006.2011.02193.x)
- Nam RK, Zhang WW, Trachtenberg J, Seth A, Klotz LH, Stanimirovic A, Punnen S, Venkateswaran V, Toi A, Loblaw DA *et al.* 2009 Utility of incorporating genetic variants for the early detection of prostate cancer. *Clinical Cancer Research* **15** 1787–1793. (doi:10.1158/1078-0432.CCR-08-1593)
- Nguyen HH, Takata R, Akamatsu S, Shigemizu D, Tsunoda T, Furihata M, Takahashi A, Kubo M, Kamatani N, Ogawa O *et al.* 2012 IRX4 at 5p15 suppresses prostate cancer growth through the interaction with vitamin D receptor, conferring prostate cancer susceptibility. *Human Molecular Genetics* **21** 2076–2085. (doi:10.1093/hmg/ddso25)
- Palanisamy N, Ateeq B, Kalyana-Sundaram S, Pflueger D, Ramnarayanan K, Shankar S, Han B, Cao Q, Cao X, Suleman K *et al.* 2010 Rearrangements of the RAF kinase pathway in prostate cancer, gastric cancer and melanoma. *Nature Medicine* **16** 793–798. (doi:10.1038/nm.2166)
- Palmgren JS, Karavadia SS & Wakefield MR 2007 Unusual and under-appreciated: small cell carcinoma of the prostate. *Seminars in Oncology* **34** 22–29. (doi:10.1053/j.seminoncol.2006.10.026)
- Pflueger D, Terry S, Sboner A, Habegger L, Esgueva R, Lin PC, Svensson MA, Kitabayashi N, Moss BJ, MacDonald TY *et al.* 2011 Discovery of non-ETS gene fusions in human prostate cancer using next-generation RNA sequencing. *Genome Research* **21** 56–67. (doi:10.1101/gr.110684.110)
- Pi M, Chen L, Huang MZ, Zhu W, Ringhofer B, Luo J, Christenson L, Li B, Zhang J, Jackson PD *et al.* 2008 GPRC6A null mice exhibit osteopenia, feminization and metabolic syndrome. *PLoS ONE* **3** e3858. (doi:10.1371/journal.pone.0003858)
- Pomerantz MM, Ahmadiyeh N, Jia L, Herman P, Verzi MP, Doddapaneni H, Beckwith CA, Chan JA, Hills A, Davis ML *et al.* 2009 The 8q24 cancer risk variant rs6983267 shows long-range interaction with MYC in colorectal cancer. *Nature Genetics* **41** 882–884. (doi:10.1038/ng.403)
- Pontoglio M 2000 Hepatocyte nuclear factor 1, a transcription factor at the crossroads of glucose homeostasis. *Journal of the American Society of Nephrology* **11** S140–S143.
- Prensner JR, Iyer MK, Balbin OA, Dhanasekaran SM, Cao Q, Brenner JC, Laxman B, Asangani IA, Grasso CS, Kominsky HD *et al.* 2011 Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression. *Nature Biotechnology* **29** 742–749. (doi:10.1038/nbt.1914)
- Ren S, Peng Z, Mao JH, Yu Y, Yin C, Gao X, Cui Z, Zhang J, Yi K, Xu W *et al.* 2012 RNA-seq analysis of prostate cancer in the Chinese population identifies recurrent gene fusions, cancer-associated long noncoding RNAs and aberrant alternative splicings. *Cell Research* **22** 806–821. (doi:10.1038/cr.2012.30)
- Schaid DJ 2004 The complex genetic epidemiology of prostate cancer. *Human Molecular Genetics* **13** R103–R121. (doi:10.1093/hmg/ddh072)
- Scher HI & Sawyers CL 2005 Biology of progressive, castration-resistant prostate cancer: directed therapies targeting the androgen-receptor signaling axis. *Journal of Clinical Oncology* **23** 8253–8261. (doi:10.1200/JCO.2005.03.4777)
- Soranzo N, Rivadeneira F, Chinappan-Horsley U, Malkina I, Richards JB, Hammond N, Stolk L, Nica A, Inouye M, Hofman A *et al.* 2009 Meta-analysis of genome-wide scans for human adult stature identifies novel loci and associations with measures of skeletal frame size. *PLoS Genetics* **5** e1000445. (doi:10.1371/journal.pgen.1000445)
- Spurdle AB, Thompson DJ, Ahmed S, Ferguson K, Healey CS, O'Mara T, Walker LC, Montgomery SB, Dermizakis ET, Fahey P *et al.* 2011 Genome-wide association study identifies a common variant associated with risk of endometrial cancer. *Nature Genetics* **43** 451–454. (doi:10.1038/ng.812)
- Sun J, Zheng SL, Wiklund F, Isaacs SD, Purcell LD, Gao Z, Hsu FC, Kim ST, Liu W, Zhu Y *et al.* 2008 Evidence for two independent prostate cancer risk-associated loci in the HNF1B gene at 17q12. *Nature Genetics* **40** 1153–1155. (doi:10.1038/ng.214)
- Takata R, Akamatsu S, Kubo M, Takahashi A, Hosono N, Kawaguchi T, Tsunoda T, Inazawa J, Kamatani N, Ogawa O *et al.* 2010 Genome-wide association study identifies five new susceptibility loci for prostate cancer in the Japanese population. *Nature Genetics* **42** 751–754. (doi:10.1038/ng.635)
- Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, Keer HN & Balk SP 1995 Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. *New England Journal of Medicine* **332** 1393–1398. (doi:10.1056/NEJM199505253322101)
- Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N, Yu K, Chatterjee N, Welch R, Hutchinson A *et al.* 2008 Multiple loci identified in a genome-wide association study of prostate cancer. *Nature Genetics* **40** 310–315. (doi:10.1038/ng.91)
- Tomkins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, Varambally S, Cao X, Tchinda J, Kuefer R *et al.* 2005 Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. *Science* **310** 644–648. (doi:10.1126/science.1117679)
- Tomlinson I, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, Spain S, Penegar S, Chandler I, Gorman M, Wood W *et al.* 2007 A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. *Nature Genetics* **39** 984–988. (doi:10.1038/ng2085)
- Tuupainen S, Turunen M, Lehtonen R, Hallikas O, Vanharanta S, Kivioja T, Björklund M, Wei G, Yan J, Niittymäki I *et al.* 2009 The common colorectal cancer predisposition SNP rs6983267 at chromosome 8q24 confers potential to enhanced Wnt signaling. *Nature Genetics* **41** 885–890. (doi:10.1038/ng.406)
- Voeller HJ, Augustus M, Madike V, Bova GS, Carter KC & Gelmann EP 1997 Coding region of NKX3.1, a prostate-specific homeobox gene on 8p21, is not mutated in human prostate cancers. *Cancer Research* **57** 4455–4459.
- Wang X, Kruijthof-de Julio M, Economides KD, Walker D, Yu H, Halili MV, Hu Y-P, Price SM, Abate-Shen C & Shen MM 2009 A luminal epithelial stem cell that is a cell of origin for prostate cancer. *Nature* **461** 495–500. (doi:10.1038/nature08361)

- Weischenfeldt J, Simon R, Feuerbach L, Schlangen K, Weichenhan D, Minner S, Wuttig D, Warnatz HJ, Stehr H, Rausch T *et al.* 2013 Integrative genomic analyses reveal an androgen-driven somatic alteration landscape in early-onset prostate cancer. *Cancer Cell* **23** 159–170. (doi:10.1016/j.ccr.2013.01.002)
- Wiehagen KR, Corbo-Rodgers E, Li S, Staub ES, Hunter CA, Morrissey EE & Maltzman JS 2012 Foxp4 is dispensable for T cell development, but required for robust recall responses. *PLoS ONE* **7** e42273. (doi:10.1371/journal.pone.0042273)
- Xu J, Zheng SL, Isaacs SD, Wiley KE, Wiklund F, Sun J, Kader AK, Li G, Purcell LD, Kim ST *et al.* 2010 Inherited genetic variant predisposes to aggressive but not indolent prostate cancer. *PNAS* **107** 2136–2140. (doi:10.1073/pnas.0914061107)
- Xu J, Mo Z, Ye D, Wang M, Liu F, Jin G, Xu C, Wang X, Shao Q, Chen Z *et al.* 2012 Genome-wide association study in Chinese men identifies two new prostate cancer risk loci at 9q31.2 and 19q13.4. *Nature Genetics* **44** 1231–1235. (doi:10.1038/ng.2424)
- Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, Minichiello MJ, Fearnhead P, Yu K, Chatterjee N *et al.* 2007 Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. *Nature Genetics* **39** 645–649. (doi:10.1038/ng2022)
- Zhang Y, Gong M, Yuan H, Park HG, Frierson HF & Li H 2012 Chimeric transcript generated by cis-splicing of adjacent genes regulates prostate cancer cell proliferation. *Cancer Discovery* **2** 598–607. (doi:10.1158/2159-8290.CD-12-0042)
- Zheng SL, Sun J, Wiklund F, Smith S, Stattin P, Li G, Adami H-O, Hsu F-C, Zhu Y, Bälter K *et al.* 2008 Cumulative association of five genetic variants with prostate cancer. *New England Journal of Medicine* **358** 910–919. (doi:10.1056/NEJMoa075819)
- Zheng SL, Sun J, Wiklund F, Gao Z, Stattin P, Purcell LD, Adami HO, Hsu FC, Zhu Y, Adolfsson J *et al.* 2009 Genetic variants and family history predict prostate cancer similar to prostate-specific antigen. *Clinical Cancer Research* **15** 1105–1111. (doi:10.1158/1078-0432.CCR-08-1743)

Received in final form 22 April 2013

Accepted 23 April 2013

Made available online as an Accepted Preprint
26 April 2013