Acta Oncologica, 2011; 50(Suppl 1): 18–23

informa
healthcare

REVIEW ARTICLE

Early detection of prostate cancer with emphasis on genetic markers

MARKUS ALY[1,2], FREDRIK WIKLUND[1] & HENRIK GRÖNBERG[1]

[1]*Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden and* [2]*Division of Urology, Department of Clinical Sciences, Danderyd Hospital, Karolinska Institutet, Stockholm, Sweden*

Abstract

Background. The recent advances in genomic research have made it possible to identify several new genomic-based biomarkers for prostate cancer. In this review we evaluate these new markers and speculate about future scenarios. *Results.* Today 35 single nucleotide polymorphisms (SNPs) have been identified and independently validated to associate with prostate cancer. These SNPs are common in the population (>5%) but the effect of these SNPs in these regions on prostate cancer risk is modest with odds ratios typically ranging between 1.1 and 1.3. It is estimated that these markers explain 25% of the familial risk of prostate cancer. However, it is anticipated that additional 50–75 prostate cancer SNPs will be identified in the near future. The SNPs associated with prostate cancer so far are not associated with disease stage or outcome. There are several efforts to identify germline genetic markers that can be used as prognostic markers. There are also tumor-based methods that are promising in identifying new genetic markers that can be easily measured in plasma or urine. *Conclusion.* There are several new "genetic" markers that in the near future might be used in clinical routine. These markers are easy to measure and stable over time. However the challenge is not only to identify new biomarkers but the real test is to validate new biomarkers in several large well-characterized patient populations. This validation must be done together will all other known biomarkers at the same time as it not likely that one single marker is enough, but a panel of different markers. Today 2010 there are over 19 000 publications in the area of biomarkers and prostate cancer, but only one biomarker, PSA, is used in the clinic today!

Since the late 1980s Prostate Specific Antigen (PSA) has been used as a clinical biomarker initially used to follow treatment outcome but later as a tool to identify patients at a high risk of being diagnosed with prostate cancer.

Two recent studies have presented different outcomes as to whether PSA can serve as a tool for screening or not. The European study, ERPC, showed a 20% reduction in prostate cancer mortality compared to the non-intervention arm. The PLCO study did not show any reduction in mortality, probably due to contamination of the control group and relatively few events in the intervention group. Although it seems as if screening would affect the prostate cancer related deaths it has not led to general screening in most western countries mostly due to the fact that screening inevitably leads to overdiagnosis and overtreatment which also was shown in the ERCP study where 1 400 men had to be screened and 48 men had to be treated to avoid one death related to prostate cancer [1,2]. In an updated analysis of the Göteborg part of the ERPC, a 44% reduction in prostate cancer mortality was reported. This results reduces the numbers needed to screen to 293 and number needed to treat to only 12 [3].

Still this calls for novel biomarkers with a better specificity in order to better predict which men would benefit from further diagnostic interventions and eventually treatment. Biological markers associated with aggressiveness would be very valuable in order to better be able to individualize treatment.

In this review we are focusing on genetic markers that can be measured in blood, either from DNA or plasma/serum. Genetic markers measured in urine or tumors are covered in other articles. We have the following definitions of a genetic marker:

Correspondence: Henrik Grönberg, Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, PO Box 281, SE-171 77 Stockholm, Sweden. Tel: +46 8 52482347. Fax: +46 8 314975. E-mail: Henrik.Gronberg@ki.se

(Received 22 September 2010; accepted 4 October 2010)

ISSN 0284-186X print/ISSN 1651-226X online
DOI: 10.3109/0284186X.2010.529824

Germline (inherited) genetic markers

Point mutations/copy number variation in the human genome. A single nucleotide polymorphism (SNP) is an inherited mutation that is present in more than 1% of the population. These markers can be analyzed from any normal tissue in the body but in most cases DNA from white blood cells are used.

Germline genetic markers and prostate cancer risk

A family history of prostate cancer is one of the strongest risk factors and twin studies suggest that as much as 42% of the disease risk is explained by heritable factors [4]. Attempts to decipher the heritable component of prostate cancer based on candidate gene association studies and genome-wide linkage studies in multiple case families have suggested numerous prostate cancer susceptibility genes and loci. However, an inability to replicate reported linkage and association findings suggest that prostate cancer is genetically complex with multiple common low-penetrance genes involved in prostate cancer predisposition [5].

Recently, genome-wide association studies (GWAS) have emerged as a powerful method to identify genomic low-risk susceptibility regions for complex diseases including cancer [6]. Through genotyping platforms that explore hundreds of thousands of single nucleotide polymorphisms (SNPs) simultaneously it is possible to screen the complete genome for common genetic variation associated with the disease of interest. In 2006 the first prostate cancer susceptibility region was identified at chromosome 8q24. Subsequent GWAS and region-focused studies have revealed five distinct linkage disequilibrium blocks harbouring prostate cancer susceptibility alleles at 8q24 [7–13]. The 8q24 region has also been shown to harbour susceptibility alleles for breast cancer [14], colorectal cancer [15], bladder cancer [16], and ovarian cancer [10]. The 1.2-Mb sequence at 8q24 containing all observed risk alleles does not code for any known genes and the biologic mechanisms underlying these associations are unknown. The oncogene *c-MYC* is the closest distal gene to this region and it has been suggested that the observed associations reflect long-range control of myc expression. To date, 38 distinct genetic loci harbouring prostate cancer risk alleles have been identified and consistently replicated (Table I). In general, the effect of variants in these regions on prostate cancer risk is modest with odds ratios typically ranging between 1.1 and 1.3. It has been estimated 17 that hitherto identified variants together explain approximately 22% of the familial risk of prostate cancer and it is anticipated that many more prostate cancer susceptibility variants will be identified in the future. All these studies have been conducted on European/North American populations making the translation to other ethnic groups uncertain. However, a recent Japanese study showed that x/x of known prostate cancer susceptibility loci was confirmed in Japanese men [18]. In addition five new loci was reported and it is unknown if these loci are specific to the Japanese population or not.

Germline genetic markers and disease aggressiveness

To date there is no reliable way of predicting whether prostate cancer will be an aggressive, fast-growing disease or a non-aggressive, slow-growing type of cancer. In general, a combination of tumor staging (using the tumor-node-metastasis staging system [19]), tumor grading (using the Gleason scoring system [20]) and diagnostic PSA serum levels are used to classify patients into different prognostic risk groups to guide clinicians in treatment decisions. In genetic association studies, prostate cancer patients are commonly classified as having a more aggressive form of the disease if they fulfill any of the following criteria: 1) disease spread outside of the prostate gland, or presence of cancer in the lymph nodes or other metastatic sites; 2) presence of poorly differentiated cancer as indicated by a high Gleason score (i.e. 4+3=7 or higher); or 3) a serum PSA level associated with a high likelihood of extensive disease (i.e. >20 ng/ml).

Several studies have explored the capacity of established prostate cancer risk variants to distinguish between less aggressive and more aggressive disease [7–9,21–43]. Overall, results are inconclusive, with some studies reporting stronger associations for some of these variants among more aggressive prostate cancer patients, while others did not. In a large replication study from the PRACTICAL (Prostate cancer association group to Investigate cancer associated alterations in the genome) consortium, which evaluated genetic variants at chromosome 3p12, 6q25, 7q21, 10q11, 11q13, 19q13 and Xp11 among 7 370 prostate cancer cases and 5 742 controls, no association with tumor grade was observed for any of the explored variants [42]. Fitzgerald and coworkers assessed the same seven variants and an additional six variants at chromosome 7p15, 8q24, 10q26, and 17q12 in a population-based study comprising 1 308 cases and 1 267 controls for association with family history and clinical features of more aggressive disease [43]. No association was observed between any of the evaluated risk variants and a composite measure of disease aggressiveness; however, two variants, rs10993994 at 10q11

Table I. Validity SNPs and chromosomal loci associated with prostate cancer risk.

dbSNP No.	Chromosome	Gene*	Risk Allele†	Per Allele OR	Study
rs1465618	2p21	THADA	A	1.08	Eeles et al. 2009 [17]
rs721048	2p15	EHBP1	A	1.15	Gudmundsson et al. 2008 [24]
rs12621278	2q31.1	ITGA6	A	1.33	Eeles et al. 2009 [17]
rs4857841	3q21.3	EEFSEC	A	1.12	Gudmundsson et al. 2009 [55]
rs12500426	4q22.3	PDLIM5	A	1.08	Eeles et al. 2009 [17]
rs17021918	4q22.3	PDLIM5	C	1.11	Eeles et al. 2009 [17]
rs7679673	4q24	FLJ20032	C	1.10	Eeles et al. 2009 [17]
rs9364554	6q25.3	SLC22A3	T	1.17	Eeles et al. 2008 [25]
rs10486567	7p15.2	JAZF1	G	1.35	Thomas et al. 2008 [23]
rs6465657	7q21.3	LMTK2	C	1.12	Eeles et al. 2008 [25]
rs1512268	8p21.2	NKX3-1	T	1.18	Eeles et al. 2009 [17]
rs12543663	8q24.21		C	1.08	Al Olama et al. 2009 [12]
rs10086908	8q24.21		T	1.15	Al Olama et al. 2009 [12]
rs1016343	8q24.21		T	1.21	Al Olama et al. 2009 [12]
rs13252298	8q24.21		A	1.19	Al Olama et al. 2009 [12]
rs6983561	8q24.21		C	1,47	Al Olama et al. 2009 [12]
rs16901979	8q24.21		A	1,79	Gudmundsson et al. 2007 [7]
rs16902094	8q24.21		G	1.21	Gudmundsson et al. 2009 [55]
rs445114	8q24.21		T	1.14	Gudmundsson et al. 2009 [55]
rs620861	8q24.21		C	1.11	Al Olama et al. 2009 [12]
rs6983267	8q24.21		G	1.26	Al Olama et al. 2009 [12]
rs1447295	8q24.21		A	1.29	Amundadottir et al. 2006 [56]
rs10993994	10q11.23	MSMB	T	1.25	Eeles et al. 2008 [25]
rs4962416	10q26.13	CTBP2	C	1.20	Thomas et al. 2008 [23]
rs7127900	11p15.5		A	1.22	Eeles et al. 2009 [17]
rs12418451	11q13.2		A	1.15	Zheng et al. 2009 [31]
rs11228565	11q13.2		A	1.23	Gudmundsson et al. 2009 [55]
rs10896449	11q13.2		G	1.28	Thomas et al. 2008 [23]
rs11649743	17q12	HNF1B	G	1.28	Sun et al. 2008 [27]
rs4430796	17q12	HNF1B	A	1.22	Gudmundsson et al. 2007 [22]
rs1859962	17q24.3		G	1.20	Gudmundsson et al. 2007 [22]
rs8102476	19q13.2	PPP1R14A	C	1.12	Gudmundsson et al. 2009 [55]
rs2735839	19q13.33	KLK3	A	1.20	Eeles et al. 2008 [25]
rs9623117	22q13.1	TNRC6B	C	1.11	Sun et al. 2009 [28]
rs5759167	22q13.2	BIK	G	1.16	Eeles et al. 2009 [17]
rs5945619	Xp11.22	NUDT11	C	1.19	Eeles et al. 2008 [25]

*These genes are within the linkage-disequilibrium block defined by the associated variant. THADA denotes thyroid adenoma associated isoform 1, EHBP1 the EH domain binding protein 1, ITGA6 the integrin alpha chain 6, EEFSEC the elongation factor for selenoprotein translation, PDLIM5 the PDZ and LIM domain 5 isoform d, FLJ20032 the hypothetical protein LOC54790, SLC22A3 the solute carrier family 22 member 3, JAZF1 the juxtaposed with another zinc finger gene 1, LMTK2 the lemur tyrosine kinase 2, SLC25A37 the mitochondrial solute carrier protein, NKX3-1 the NK3 transcription factor related locus 1, MSMB the beta-microseminoprotein isoform a precursor, CTBP2 the C-terminal binding protein 2 isoform 2, HNF1B hepatocyte nuclear factor 1 homeobox B, PPP1R14A the protein phosphatase 1, regulatory inhibitor, KLK3 the kallikrein 3 gene, TNRC6B the trinucleotide repeat containing 6B isoform 2, BIK the BCL2-interacting killer, NUDT11 the nudix-type motif 11.
†Risk alleles as defined from published data cited in the column.

(p=0.02) and rs5945619 at Xp11 (p=0.03) were nominally significantly associated with Gleason score.

Most of the published studies exploring established risk variants with respect to prostate cancer aggressiveness had several limitations including small sample size, heterogeneous definition of aggressive disease across multiple study populations, and reliance on clinical grading and staging of tumors. To address these limitations Xu and coworkers evaluated 20 established risk variants in 17 distinct genomic regions among 5 895 prostate cancer patients of European descent who underwent radical prostatectomy for treatment of prostate cancer. Based on the entire prostate gland each tumor was uniformly graded and staged using the same protocol. For 18 of the 20 variants explored no significant difference was observed in risk allele frequencies between patients with more aggressive and less aggressive disease. Two variants were significantly associated with disease aggressiveness; SNP rs2735839 downstream of the kallikrein 3 gene (*KLK3*, $p=8.4\times10^{-7}$), the gene coding for PSA, and SNP rs10993994 in the microseminoprotein beta gene (*MSMB*, p=0.046). Since these risk alleles have been shown to strongly associate with higher PSA levels among population controls [25,44,45], it is possible that the observed association with aggressive disease may partly reflect a PSA detection bias.

Somatic (tumor) genetic marker

Acquired genetic mutations/rearrangement in a tumor during the development of the tumor. These changes can be detected from DNA or RNA from the specific tumor. New techniques make it possible to detect these somatic mutations (DNA) or copies of gene expression (RNA) directly in blood plasma.

Since the 1970s it has been known that elevated levels of free circulating DNA can be detected in blood in patients with malignant disease [46]. It was also shown in the same paper that the levels correlated with metastatic status and that the levels decreased after therapy. Chun et al. described 2006 that plasma DNA level is predictive and highly accurate to assess the risk of positive biopsy outcome in prostate cancer. Increased levels of cDNA have also been shown in patients with metastatic disease, although they also found elevated levels in patients with benign prostatic hyperplasia [47]. Papadopoulou et al. showed that cell-free DNA could be used to distinguish between patients with prostate cancer and those not diagnosed with the disease [48]. Cherepanova et al. has also shown that patients with prostate cancers have elevated levels in blood in comparison to patients with benign diseases of the prostate [49]. The utility of detecting tumor-specific rearrangements in plasma is currently limited by the heterogeneity of solid tumors. In an attempt to map structural variation in breast cancer, none of 24 tumors harbored any identical rearrangements [50]. Therefore, for most cancer types, the primary tumor needs to be investigated first, which limits the possibility to use circulating DNA for early detection. Although it would be possible to detect individual-specific tumor rearrangements directly in plasma or serum it is not practically feasible due to costs since tumor DNA constitutes $<30\%$ of the total amount of circulating nucleic acids [51]. Unlike other adenocarcinomas, prostate tumors harbors a rearrangement (TMPRSS2-ERG gene fusion) that occur in 50% of cases [52]. These rearrangements may be detectable in blood and could be of diagnostic and perhaps also of prognostic value. Further studies are needed to assess this as a clinically useful marker.

miRNA

MikroRNAs are small, up to 22 nucleotides long, noncoding functional RNAs. It is estimated that 1 000 such small RNAs exists and they are all expressed in a tissue specific manner suggesting that they could be used as markers for various conditions. miRNAs are known to regulate gene expression. Porrka et al. identified 51 different miRNA being either up- or down from an expression profile of 319 genes encoding miRNA in prostatic cancer tissue as compared to normal prostatic tissue. Twenty two of these were downregulated in all prostate cancer samples and 15 were only downregulated in castration resistant specimens. It has also been demonstrated that miRNA profiles were consistent with prostate cancer disease process [53]. miRNA plays a biological role that may imply their correlation with diagnosis and therapeutic outcome and miRNA are possible to detect in plasma samples.

Identification of somatic mutations in prostate tumors

With the introduction of massive parallel sequencing it is now possible to screen for somatic mutation in the entire coding parts of the genome (all genes) or even the entire genome in 10–100 of tumors. This has been demonstrated to be successful in several other tumors as breast cancer, colon cancer and lung cancer in which several new key genes and pathways have been identified. If protein products from these altered genes are secreted in either the urine or the bloodstream new biomarkers might be identified.

Conclusion

There are several promising new "genetic" markers that in the near future might be used in clinical routine. These markers are easy to measure and stabile over time. We foresee the following areas as most promising:

1. Identifying high-risk populations using a combination of prostate cancer susceptibility alleles (SNPs).

Individually, each risk variant has a modest effect on disease risk and they will clearly not be useful for individualized risk prediction. However, risk profiles based on a combination of risk variants lead to an appreciable increased risk of disease [32] and there is potential for the predictive power to increase considerably as more risk variants are detected [54]. Combining the first 28 prostate cancer SNPs in the Swedish CAPS study, the top 8% of the population had three times of more increased lifetime risk of prostate cancer. These high-risk men can be selected for targeted screening or chemoprevention.

2. Using prostate cancer susceptibility alleles (SNPs) in current risk calculators for prostate cancer.

These risk variants might be used in combination with current risk calculation aiding clinicians when to do prostate biopsy or not.

3. The identification of new prognostic markers both germline genetic variants and other biomarkers based somatic mutations in the tumors are highly warranted.

However the challenge is not only to identify new biomarkers but the real test is to validate

new biomarkers in several large well-characterized patient populations. This validation must be done together will all other known biomarkers at the same time as it not likely that one single marker is enough, but a panel of different markers. Today 2010 there are over 19 000 publications in the area of biomarkers and prostate cancer, but only one biomarker, PSA, is used in the clinic today!

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Schroder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, et al. Screening and prostate-cancer mortality in a randomized European study. N Engl J Med 2009;360: 1320–8.

[2] Andriole GL, Crawford ED, Grubb RL, 3rd, Buys SS, Chia D, Church TR, et al. Mortality results from a randomized prostate-cancer screening trial. N Engl J Med 2009;360: 1310–9.

[3] Hugosson J, Carlsson S, Aus G, Bergdahl S, Khatami A, Lodding P, et al. Mortality results from the Goteborg randomised population-based prostate-cancer screening trial. Lancet Oncol 2010;11:725–32.

[4] Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, et al. Environmental and heritable factors in the causation of cancer – analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343:78–85.

[5] Schaid DJ. The complex genetic epidemiology of prostate cancer. Hum Mol Genet 2004;13(Spec No. 1):R103–21.

[6] Chung CC, Magalhaes WC, Gonzalez-Bosquet J, Chanock SJ. Genome-wide association studies in cancer – current and future directions. Carcinogenesis 2010;31:111–20.

[7] Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, et al. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. Nat Genet 2007;39:631–7.

[8] Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, et al. Multiple regions within 8q24 independently affect risk for prostate cancer. Nat Genet 2007;39:638–44.

[9] Zheng SL, Sun J, Cheng Y, Li G, Hsu FC, Zhu Y, et al. Association between two unlinked loci at 8q24 and prostate cancer risk among European Americans. J Natl Cancer Inst 2007;99:1525–33.

[10] Ghoussaini M, Song H, Koessler T, Al Olama AA, Kote-Jarai Z, Driver KE, et al. Multiple loci with different cancer specificities within the 8q24 gene desert. J Natl Cancer Inst 2008;100:962–6.

[11] Yeager M, Xiao N, Hayes RB, Bouffard P, Desany B, Burdett L, et al. Comprehensive resequence analysis of a 136 kb region of human chromosome 8q24 associated with prostate and colon cancers. Hum Genet 2008;124:161–70.

[12] Al Olama AA, Kote-Jarai Z, Giles GG, Guy M, Morrison J, Severi G, et al. Multiple loci on 8q24 associated with prostate cancer susceptibility. Nat Genet 2009;41:1058–60.

[13] Yeager M, Chatterjee N, Ciampa J, Jacobs KB, Gonzalez-Bosquet J, Hayes RB, et al. Identification of a new prostate cancer susceptibility locus on chromosome 8q24. Nat Genet 2009;41:1055–7.

[14] Easton DF, Pooley KA, Dunning AM, Pharoah PD, Thompson D, Ballinger DG, et al. Genome-wide association study identifies novel breast cancer susceptibility loci. Nature 2007;447:1087–93.

[15] Tomlinson I, Webb E, Carvajal-Carmona L, Broderick P, Kemp Z, Spain S, et al. A genome-wide association scan of tag SNPs identifies a susceptibility variant for colorectal cancer at 8q24.21. Nat Genet 2007;39:984–8.

[16] Kiemeney LA, Thorlacius S, Sulem P, Geller F, Aben KK, Stacey SN, et al. Sequence variant on 8q24 confers susceptibility to urinary bladder cancer. Nat Genet 2008;40: 1307–12.

[17] Eeles RA, Kote-Jarai Z, Al Olama AA, Giles GG, Guy M, Severi G, et al. Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. Nat Genet 2009;41:1116–21.

[18] Takata R Akamatsu S, Kubo M, Takahashi A, Hosono N, Kawaguchi T, et al. Genome-wide association study identifies five new susceptibility loci for prostate cancer in the Japanese population. Nat Genet 2010;42:751–4.

[19] Hoedemaeker RF, Vis AN, Van Der Kwast TH. Staging prostate cancer. Microsc Res Tech 2000;51:423–9.

[20] Epstein JI, Allsbrook WC, Jr., Amin MB, Egevad LL. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Surg Pathol 2005;29:1228–42.

[21] Yeager M, Orr N, Hayes RB, Jacobs KB, Kraft P, Wacholder S, et al. Genome-wide association study of prostate cancer identifies a second risk locus at 8q24. Nat Genet 2007; 39:645–9.

[22] Gudmundsson J, Sulem P, Steinthorsdottir V, Bergthorsson JT, Thorleifsson G, Manolescu A, et al. Two variants on chromosome 17 confer prostate cancer risk and the one in TCF2 protects against type 2 diabetes. Nat Genet 2007;39: 977–83.

[23] Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N, et al. Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 2008;40:310–5.

[24] Gudmundsson J, Sulem P, Rafnar T, Bergthorsson JT, Manolescu A, Gudbjartsson D, et al. Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. Nat Genet 2008;40:281–3.

[25] Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, Jugurnauth SK, et al. Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 2008;40:316–21.

[26] Duggan D, Zheng SL, Knowlton M, Benitez D, Dimitrov L, Wiklund F, et al. Two genome-wide association studies of aggressive prostate cancer implicate putative prostate tumor suppressor gene DAB2IP. J Natl Cancer Inst 2007;99: 1836–44.

[27] Sun J, Zheng SL, Wiklund F, Isaacs SD, Purcell LD, Gao Z, et al. Evidence for two independent prostate cancer risk-associated loci in the HNF1B gene at 17q12. Nat Genet 2008;40:1153–5.

[28] Sun J, Zheng SL, Wiklund F, Isaacs SD, Li G, Wiley KE, et al. Sequence variants at 22q13 are associated with prostate cancer risk. Cancer Res 2009;69:10–5.

[29] Chang BL, Cramer SD, Wiklund F, Isaacs SD, Stevens VL, Sun J, et al. Fine mapping association study and functional analysis implicate a SNP in MSMB at 10q11 as a causal variant for prostate cancer risk. Hum Mol Genet 2009;18: 1368–75.

[30] Hsu FC, Sun J, Wiklund F, Isaacs SD, Wiley KE, Purcell LD, et al. A novel prostate cancer susceptibility locus at 19q13. Cancer Res 2009;69:2720–3.

[31] Zheng SL, Stevens VL, Wiklund F, Isaacs SD, Sun J, Smith S, et al. Two independent prostate cancer risk-associated Loci at 11q13. Cancer Epidemiol Biomarkers Prev 2009; 18:1815–20.

[32] Zheng SL, Sun J, Wiklund F, Smith S, Stattin P, Li G, et al. Cumulative association of five genetic variants with prostate cancer. N Engl J Med 2008;358:910–9.

[33] Sun J, Chang BL, Isaacs SD, Wiley KE, Wiklund F, Stattin P, et al. Cumulative effect of five genetic variants on prostate cancer risk in multiple study populations. Prostate 2008; 68:1257–62.

[34] Xu J, Isaacs SD, Sun J, Li G, Wiley KE, Zhu Y, et al. Association of prostate cancer risk variants with clinicopathologic characteristics of the disease. Clin Cancer Res 2008;14: 5819–24.

[35] Wang L, McDonnell SK, Slusser JP, Hebbring SJ, Cunningham JM, Jacobsen SJ, et al. Two common chromosome 8q24 variants are associated with increased risk for prostate cancer. Cancer Res 2007;67:2944–50.

[36] Suuriniemi M, Agalliu I, Schaid DJ, Johanneson B, McDonnell SK, Iwasaki L, et al. Confirmation of a positive association between prostate cancer risk and a locus at chromosome 8q24. Cancer Epidemiol Biomarkers Prev 2007;16:809–14.

[37] Cheng I, Plummer SJ, Jorgenson E, Liu X, Rybicki BA, Casey G, et al. 8q24 and prostate cancer: Association with advanced disease and meta-analysis. Eur J Hum Genet 2008;16:496–505.

[38] Helfand BT, Loeb S, Cashy J, Meeks JJ, Thaxton CS, Han M, et al. Tumor characteristics of carriers and noncarriers of the deCODE 8q24 prostate cancer susceptibility alleles. J Urol 2008;179:2197–201; Discussion 202.

[39] Tan YC, Zeigler-Johnson C, Mittal RD, Mandhani A, Mital B, Rebbeck TR, et al. Common 8q24 sequence variations are associated with Asian Indian advanced prostate cancer risk. Cancer Epidemiol Biomarkers Prev 2008;17:2431–5.

[40] Terada N, Tsuchiya N, Ma Z, Shimizu Y, Kobayashi T, Nakamura E, et al. Association of genetic polymorphisms at 8q24 with the risk of prostate cancer in a Japanese population. Prostate 2008;68:1689–95.

[41] Robbins C, Torres JB, Hooker S, Bonilla C, Hernandez W, Candreva A, et al. Confirmation study of prostate cancer risk variants at 8q24 in African Americans identifies a novel risk locus. Genome Res 2007;17:1717–22.

[42] Kote-Jarai Z, Easton DF, Stanford JL, Ostrander EA, Schleutker J, Ingles SA, et al. Multiple novel prostate cancer predisposition loci confirmed by an international study: The PRACTICAL Consortium. Cancer Epidemiol Biomarkers Prev 2008;17:2052–61.

[43] Fitzgerald LM, Kwon EM, Koopmeiners JS, Salinas CA, Stanford JL, Ostrander EA. Analysis of recently identified prostate cancer susceptibility loci in a population-based study: Associations with family history and clinical features. Clin Cancer Res 2009;15:3231–7.

[44] Ahn J, Berndt SI, Wacholder S, Kraft P, Kibel AS, Yeager M, et al. Variation in KLK genes, prostate-specific antigen and risk of prostate cancer. Nat Genet 2008;40:1032–4; Author reply 35–6.

[45] Wiklund F, Zheng SL, Sun J, Adami HO, Lilja H, Hsu FC, et al. Association of reported prostate cancer risk alleles with PSA levels among men without a diagnosis of prostate cancer. Prostate 2009;69:419–27.

[46] Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. Free DNA in the serum of cancer patients and the effect of therapy. Cancer Res 1977;37:646–50.

[47] Jung K, Stephan C, Lewandowski M, Klotzek S, Jung M, Kristiansen G, et al. Increased cell-free DNA in plasma of patients with metastatic spread in prostate cancer. Cancer Lett 2004;205:173–80.

[48] Papadopoulou E, Davilas E, Sotiriou V, Koliopanos A, Aggelakis F, Dardoufas K, et al. Cell-free DNA and RNA in plasma as a new molecular marker for prostate cancer. Oncol Res 2004;14:439–45.

[49] Cherepanova AV, Tamkovich SN, Bryzgunova OE, Vlassov VV, Laktionov PP. Deoxyribonuclease activity and circulating DNA concentration in blood plasma of patients with prostate tumors. Ann N Y Acad Sci 2008;1137:218–21.

[50] Stephens PJ, McBride DJ, Lin ML, Varela, I, Pleasance ED, Simpson JT, et al. Complex landscapes of somatic rearrangement in human breast cancer genomes. Nature 2009; 462:1005–10.

[51] Diehl F, Li M, Dressman D, He Y, Shen D, Szabo S, et al. Detection and quantification of mutations in the plasma of patients with colorectal tumors. Proc Natl Acad Sci U S A 2005;102:16368–73.

[52] Perner S, Svensson MA, Hossain RR, Day JR, Groskopf J, Slaughter RC, et al. ERG rearrangement metastasis patterns in locally advanced prostate cancer. Urology 2010;75:762–7.

[53] Ozen M, Creighton CJ, Ozdemir M, Ittmann M. Widespread deregulation of microRNA expression in human prostate cancer. Oncogene 2008;27:1788–93.

[54] Pharoah PD, Antoniou AC, Easton DF, Ponder BA. Polygenes, risk prediction, and targeted prevention of breast cancer. N Engl J Med 2008;358:2796–803.

[55] Gudmundsson J, Sulem P, Gudbjartsson DF, Blondal T, Gylfason A, Agnarsson BA, et al. Genome-wide association and replication studies identify four variants associated with prostate cancer susceptibility. Nat Genet 2009;41: 1122–6.

[56] Amundadottir LT, Sulem P, Gudmundsson J, Helgason A, Baker A, Agnarsson BA, et al. A common variant associated with prostate cancer in European and African populations. Nat Genet 2006;38:652–8.