ORIGINAL ARTICLE

A common nonsense mutation in *EphB2* is associated with prostate cancer risk in African American men with a positive family history

R A Kittles, A B Baffoe-Bonnie, T Y Moses, C M Robbins, C Ahaghotu, P Huusko, C Pettaway, S Vijayakumar, J Bennett, G Hoke, T Mason, S Weinrich, J M Trent, F S Collins, S Mousses, J Bailey-Wilson, P Furbert-Harris, G Dunston, I J Powell, J D Carpten

J Med Genet 2006;**43**:507–511. doi: 10.1136/jmg.2005.035790

See end of article for authors' affiliations

Correspondence to:
Dr R A Kittles, Department
of Molecular Virology,
Immunology and Medical
Genetics, Arthur G James
Cancer Hospital and
Richard J Solove Research
Institute, The Ohio State
University, 494 Medical
Research Facility, 420
West 12th Avenue,
Columbus, OH 43210,
USA; pkittles.2@osu.edu

Received 12 June 2005
Revised version received
31 August 2005
Accepted for publication
4 September 2005
Published Online First
9 September 2005

Background: The *EphB2* gene was recently implicated as a prostate cancer (PC) tumour suppressor gene, with somatic inactivating mutations occurring in ~10% of sporadic tumours. We evaluated the contribution of *EphB2* to inherited PC susceptibility in African Americans (AA) by screening the gene for germline polymorphisms.

Methods: Direct sequencing of the coding region of *EphB2* was performed on 72 probands from the African American Hereditary Prostate Cancer Study (AAHPC). A case-control association analysis was then carried out using the AAHPC probands and an additional 183 cases of sporadic PC compared with 329 healthy AA male controls. In addition, we performed an ancestry adjusted association study where we adjusted for individual ancestry among all subjects, in order to rule out a spurious association due to population stratification.

Results: Ten coding sequence variants were identified, including the K1019X (3055A→T) nonsense mutation which was present in 15.3% of the AAHPC probands but only 1.7% of 231 European American (EA) control samples. We observed that the 3055A→T mutation significantly increased risk for prostate cancer over twofold (Fisher's two sided test, $p=0.003$). The T allele was significantly more common among AAHPC probands (15.3%) than among healthy AA male controls (5.2%) (odds ratio 3.31; 95% confidence interval 1.5 to 7.4; $p=0.008$). The ancestry adjusted analyses confirmed the association.

Conclusions: Our data show that the K1019X mutation in the *EphB2* gene differs in frequency between AA and EA, is associated with increased risk for PC in AA men with a positive family history, and may be an important genetic risk factor for prostate cancer in AA.

Prostate cancer is the most common male specific malignancy in the US and disproportionately affects AA (AA) men, who have higher incidence (~40% of all cancer cases) and mortality rates compared with other ethnic groups.^{1–2} The underlying reasons for these disparities are not well understood, although existing evidence implicates an important genetic component.^{3–8} Many studies of hereditary prostate cancer (HPC) have been reported; however, few, if any, genes that are reproducibly associated with increased risk for prostate cancer across different study populations have been identified, emphasising the heterogeneous nature of this disease.⁹ Despite AA men having the highest incidence and mortality rates of prostate cancer in the US, very few data are available on the genetics of familial prostate cancer in this ethnic group. Studying the genetic contributions for prostate cancer in this high risk population will have important implications for addressing the disparity of prostate cancer in AA.

Germline mutations have been found within three candidate genes for hereditary prostate cancer: *ELAC2* at 17p11,¹⁰ *RNASEL* at 1q25,^{11–12} and *MSR1* at 8p22.¹³ The frequency spectrum of rare nonsense and missense mutations within these candidate genes vary significantly across ethnicities. These mutations in addition to common polymorphisms within the three candidate genes may also contribute to sporadic disease in populations of European descent.^{6–12–14–15} To date, there has not been a clear candidate identified as contributing to HPC in AA.

Recently, the *EphB2* gene, which encodes the EPHB2 receptor tyrosine kinase, was found to be completely inactivated in the DU145 PC cell line using nonsense mediated RNA decay inhibition in combination with array CGH, to enrich for genes likely to harbour mutations.¹⁶ The exact function of the gene is unknown, but evidence suggests that *EphB2* may be a tumour suppressor gene, as wild type *EphB2* significantly reduced clonogenic growth of DU145 PC cells (which have biallelic inactivation of *EphB2*).¹ *EphB2* maps to 1p36, a region previously shown to be linked with HPC among ethnically diverse sets of families,^{17–18} including AA.¹⁹ The strong genomic and functional characteristics of *EphB2* along with its map position near a putative HPC locus make it a strong candidate PC susceptibility gene. Therefore, we set out to screen the *EphB2* gene by direct sequencing for the presence of germline mutations in 72 unrelated AA cases of HPC in order to determine if this gene is associated with PC predisposition in this high risk population. A common nonsense mutation, K1019X, was genotyped in an additional 183 AA cases of sporadic PC and 329 healthy age matched controls. We provide evidence of an association of the *EphB2* nonsense mutation with the risk of PC in AA.

Abbreviations: AA, African American; AAHPC, African American Hereditary Prostate Cancer Study; AIM, ancestry informative marker; EA, European American; HPC, hereditary prostate cancer; PC, prostate cancer; PS, population stratification; PSA, prostate specific antigen

METHODS

African American hereditary prostate cancer cases

The case group of HPC consisted of 72 AA from unrelated HPC multiplex families recruited as part of the African American Hereditary Prostate Cancer (AAHPC) Study Network.^{20–22} Ascertainment of multiplex PC families and the clinical description of the AA cases of HPC have been previously described.^{20–21} Owing to barriers in recruiting AA men into HPC studies, the AAHPC Study Network developed a nationwide effort to establish collaborative recruitment centres in regions of the US with large numbers of AA including Atlanta, Chicago, Detroit, Harlem, Houston, rural South Carolina, and Washington.²⁰ Inclusion criteria were: (a) four or more cases of PC in a family, preferably first degree relatives; (b) at least three cases available for sampling; and (c) an average age at diagnosis of <65 years for the family. These families all self identified as AA and were verified by the recruitment staff. We performed mutational analysis for *EphB2* using DNA samples from 72 probands from unrelated AA families with HPC. The average age at diagnosis for these probands was 64.9 years of age. Clinical characteristics of the AAHPC dataset have been previously described.²² All participants gave informed consent and recruitment was approved by the appropriate institutional review boards).

African American cases of sporadic prostate cancer and controls

Unrelated men self described as AA were enrolled for case-control studies of risk factors for PC. The subjects consisted of 512 AA men (183 cases of PC and 329 healthy male controls) recruited from the Howard University Hospital in Washington.^{4–23} Incident cases were identified through the Division of Urology at the university and confirmed by review of medical records. Healthy control subjects unrelated to the cases and matched for age (± 5 years) were ascertained from the Division of Urology and also from men participating in screening programmes for PC at the Howard University Hospital. Control subjects were excluded from the study if they had ever been diagnosed with benign prostatic hyperplasia, had ever had an elevated prostate specific antigen test (>4.0 ng/ml), or had ever had an abnormal digital rectal examination. The demographic characteristics of participants in the screening programme were similar to the patient population seen in the Division of Urology clinics. Recruitment of sporadic PC cases and healthy controls occurred concurrently, and each donated a blood sample for research purposes. The participation response rates for cases and controls were 92% and 90%, respectively. All PC cases were aged 40–85 years and had been diagnosed with the disease within the past 4 years. Clinical characteristics including Gleason grade, prostatic specific antigen (PSA), tumour node metastasis stage, age at diagnosis, and family history were obtained for all cases from medical records. Disease aggressiveness was defined as low ($<T2c$ and/or Gleason grade <7) or high ($>T2c$ and/or Gleason grade >7). The institutional review board of Howard University approved the study and written consent was obtained from all subjects. In addition, we used previously published data from 231 European American (EA) population control genomic DNA samples, commercially available from the Coriell Institute for Medical Research, in order to compare the *EphB2* K1019X allele and genotype frequencies.¹⁶

Mutation detection and genotyping

DNA specimens were amplified using standard PCR protocol and intronic primer pairs with M13 tails (sequences available on request). The PCR products were purified using a commercial kit and system (QiaQuick PCR purification kit

and BioRobot 8000 Automated nucleic acid purification and liquid handling system; both Qiagen). Cycle sequencing reactions (quarter or eighth volumes) were prepared in a 96 well plate using standard M13 forward or reverse primers with a reaction kit (Big Dye terminator chemistry; PE Applied Biosystems, Piscataway, NJ, USA). Following Sephadex purification, sequence products were separated on a DNA analyser (ABI 3700 or ABI 3730 Capillary DNA Analyzer; PE Applied Biosystems) using the manufacturer's protocols. Sequence chromatograms were aligned and analysed using Sequencher software (version 4.1; Gene Codes). Owing to the complexity of the mutations (lying within a A(9) mononucleotide stretch) we used DNA sequencing for all samples. We achieved a 100% success rate for the 72 samples. For all 584 samples analysed we achieved an overall success rate of $>95\%$. Reproducibility was $>99\%$ based upon comparison of data from duplicate experiments for a subset of samples.

Controlling for population stratification

To control for possible confounding by population stratification in this study, a panel of 34 ancestry informative markers (AIM) was also genotyped in the AA samples. These markers show large differences in frequency between the parental populations (West Africans and Europeans), and were used to control for the presence of population stratification (PS) due to admixture.^{24–25} Information regarding primer sequences, polymorphic sites and other relevant information on the AIM can be found at the dbSNP NCBI database site, under the submitter handle PSU-ANTH.

Statistical analysis

Odds ratios (OR) and p values were determined by logistic regression analyses from comparison of genotypes between subjects with PC and healthy controls using SAS software (version 6.91; AAS Institute, Inc, Cary, NC, USA). Further analyses were performed on the combined dataset consisting of all subjects with PC, and for the hereditary and sporadic cases separately. For all analyses, genetic effects were adjusted for age (at time of diagnosis for cases and at time of ascertainment for controls). Statistical control of PS was achieved by introducing individual ancestry (IA) as a covariate in the analyses. Individual ancestry was estimated by a Bayesian method implemented in the STRUCTURE program (version 2.0),²⁶ and the estimate was then used as a covariate in the regression analyses. Given the large number of potential false positive marker associations (type II error) throughout the genome it would be appropriate to adjust the traditional thresholds of significance for p values based on some correction for multiple testing; however, given our modest sample size and because we explored the effect of only one single nucleotide polymorphism PC risk, the only corrections on p values we made were due to ancestry.

RESULTS

The clinical characteristics of the 72 AA probands with HPC, the 183 sporadic cases of PC, and the 329 healthy AA male controls are presented in table 1. The mean age of 69 years for the sporadic cases of PC was higher than that for the controls (66.1 years) and the HPC probands (64.9 years). The Wilcoxon signed rank test showed that the mean PSA for both the HPC probands and the sporadic cases of PC compared with the controls was significantly different ($p<0.01$). For 52 HPC probands for whom disease aggressiveness categorisation was available, only 17% had a high index compared with 47% of those with sporadic disease.

Mutational analysis in 72 AA HPC probands resulted in the discovery of 10 unique coding sequence variants within the *EphB2* gene. All sequence variants are summarised in table 2. Six silent exonic variants were observed, with frequencies

Table 1 Clinical characteristics of the AA patients with PC and population based control subjects

Characteristic	Hereditary PC (n = 72)	Sporadic PC (n = 183)	Controls (n = 329)
Mean (SD) age, years	64.9 (20.8)	69.0 (8.9)	66.1 (12.6)
Serum PSA, ng/ml*			
Mean (SD)	52.3 (90.1)†	71.3 (195.1)†	2.8 (1.1)
Median (range)	7.6 (0.1–230)	8.6 (0.1–150)	1.0 (0.1–3.6)
Disease aggressiveness:‡			
Low, n (%)	43 (83)	54 (53)	–
High, n (%)	9 (17)	48 (47)	–
Unknown, n	20	81	–

*Serum PSA measured at time of diagnosis for cases and at most recent clinical visit for controls; †p<0.01 (Wilcoxon signed rank test), comparison with control population; ‡low aggressiveness: Gleason <7 and T <T2c, high aggressiveness: Gleason ≥7 or T ≥T2c.

ranging from 1.4% to 30% among the probands. Only four of the 10 variants actually resulted in amino acid changes and are considered mutations (table 2). Two of the amino acid substitutions, V650A and M883V, were novel in contrast to the previously observed A279S variant¹⁶ but all three were observed at a frequency of 2.8%. Both V650A and M883V appear to be located in the mutation rich kinase domain of the EphB2 protein. The kinase domain is essential for receptor signalling.¹⁶ Also observed among the coding mutations was the previously reported K1019X nonsense mutation (3055 A→T) in exon 15 of *EphB2*. The K1019X mutation was present in 15.3% (11 of 72) of the AA HPC probands, although it was previously shown to be present in 1.7% (4 of 231) of control DNA samples from the Coriell Institute for Medical Research (two sided Fisher's exact test, p = 0.000043). We observed that this nonsense mutation was three times more common in the AA controls (5.17%; 17 of 329) than in the EA controls.

An association analysis of the K1019X variant (3055 A→T) was performed combining all AA cases of HPC (n = 72) and sporadic cases of PC (n = 183) and comparing them with AA male controls (n = 329), controlling for age at diagnosis. We did not examine the other coding variants because they were less frequent (<3.0%) and thus would require an extremely large number of cases and controls in order to perform a reliable association study. Table 3 reveals that the presence of the T allele significantly increased risk for PC (OR = 2.44; 95% confidence interval (CI) 1.4 to 4.3; Fisher's two sided test, p = 0.003) in the combined dataset. Subset analyses revealed that the frequency of K1019X was significantly higher for the AA HPC (15.3%) compared with AA healthy male controls (5.2%) (OR = 3.31; 95% CI 1.48 to 7.41; Fisher's

two sided test, p = 0.008). We compared the 6.6% (12 of 183) frequency of the mutation among the 183 sporadic PC cases with that in the AA healthy male controls, and found no significant difference between the two groups (OR = 1.3; 95% CI 0.60 to 2.76; Fisher's two sided test, p = 0.55) (table 3). Stratifying the analysis by PC disease aggressiveness revealed no significant associations (data not shown).

We found that the K1019X variant was not in Hardy-Weinberg equilibrium within our AA sporadic case and control samples (p<0.05). The observed departure from equilibrium was not unexpected given that the AA population is the product of recent admixture²⁷ and there are significant differences in K1019X allele frequency between AA and EA. Thus, in order to rule out a spurious association of K1019X with PC in AA due to admixture stratification, we controlled for ancestral differences between the PC cases and controls by estimating individual ancestry for each subject using 34 AIM. We used the individual ancestry estimate for each subject as a covariate in the association analysis in order to take into account differences in ancestral proportions between cases and controls.^{24–25} Individual ancestry (West African) ranged from 10% to 93.5% in the cases with a mean (SD) individual ancestry estimate of 71.3 (1.9). The estimates for West African ancestry for the controls ranged from 6.5% to 95.3% (mean (SD) was 69.0 (0.8)). After adjusting for individual ancestry, the association of the *EphB2* K1019X mutation with PC was still significant in the AAHPC probands compared with the AA healthy male controls (p = 0.01).

DISCUSSION

The potential genetic basis for the high incidence and mortality rates of PC among AA is still under investigation. In this study, we identified 10 sequence variants in the *EphB2* gene, including a common nonsense mutation K1019X among 72 AA HPC patients. Further, we revealed an association of *EphB2* K1019X and PC risk in AA. The K1019X variant was observed in much higher frequency among AA patients with PC than among healthy AA male controls (p = 0.003). The association was mainly due to men with HPC (p = 0.008); in fact, the risk for PC was increased threefold among AA men who carried at least one copy of the K1019X allele and had a family history of PC. Given its high frequency in hereditary cases, K1019X is probably associated with familial PC in AA men. Of course, further replication is necessary before these results can be accepted as a true positive. The most reliable replication study should use patients with PC with strong family history, not sporadic cases. A larger case-control study could also evaluate the rare missense variants such as A279S, V650A, and M883V, which may also contribute to PC risk.

The results of this study, and the recent identification of biallelic inactivation of the *EphB2* gene using the nonsense mediated decay microarray technique,¹⁶ further implicates *EphB2* as a PC tumour suppressor gene. K1019X is an A→T transversion within a poly-A tract of the last exon of the *EphB2* gene. This polymorphism may exhibit an effect similar to the APC I1307K polymorphism, carried by 6.1% of Ashkenazi Jews, which is associated with colorectal cancer.^{28–29} Mononucleotide repeat sequences such as in the region of K1019X have been shown to be genetically unstable and prone to somatic mutation.^{28–30} Performing somatic mutational analyses surrounding the poly-A tract region is strongly warranted in order to confirm the role of *EphB2* in prostate tumorigenesis.

We noted previously that the prevalence of K1019X was significantly higher among AA controls than among EA controls (P<0.001), suggesting that it could be in admixture disequilibrium in the AA population. These findings were

Table 2 *EphB2* sequence variants discovered in 72 AA HPC probands

Nucleotide position	Amino acid change	dbSNP	Frequency in probands
510 C/T	None	–	5.6%
624 G/A	None	–	1.4%
657 G/A	None	rs1371869	1.4%
835 G/T	A279S	–	2.8%
930 C/T	None	–	5.6%
1377 G/A	None	rs2229872	30%
1949 T/C	V650A	–	2.8%
2640 G/A	None	–	1.4%
2647 A/G	M883V	–	2.8%
3055 A/T	K1019X	–	15.3%

*Nucleotide and amino acid positions are based upon coding sequence for Genbank accession file NM_017449.

Table 3 Association between prostate cancer and the *Ephb2* K1019X polymorphism

Population	n	No. (%) of subjects with			OR (95% CI)*	p
		K/K	K/X	X/X		
All cases	255	234 (91.7)	19 (7.5)	2 (0.7)	2.44 (1.4 to 4.3)	0.003
AA HPC	72	61 (84.7)	11 (15.3)	–	3.31 (1.5 to 7.4)	0.008
AA sporadic PC	183	173 (94.5)	8 (4.4)	2 (1.1)	1.28 (0.6 to 2.8)	0.55
AA controls	329	312 (94.8)	9 (3.2)	8 (1.9)	1.00 (reference)	–
Coriell controls	231	270 (98.5)	4 (1.5)	–		

*OR by logistic regression for K/X and X/X genotype comparisons with AA controls after adjusting for age at diagnosis.

extremely interesting and inspired us to further investigate the role of this mutation as a PC genetic risk factor; however, several questions remained. Ethnic differences in allele frequency and disease risk can create false positive results in case-control studies, especially when using recently admixed populations such as AA. Thus, in order to control for possible confounding we introduced individual ancestry as a covariate in our analyses. This approach has been used to limit spurious associations that are the result of differences in ancestral proportions (admixture). Our ancestry adjusted analyses provided additional support for a strong association of the K1019X and PC in AA.

The frequencies of sequence variants in a number of candidate genes for PC differ significantly between AA and EA. Among the examples are the CAG repeat tract within the androgen receptor gene,^{31–32} a TA repeat tract within the *SRD5A2* gene,³³ the *CYP3A4* promoter variant,^{5–23} and frequent variants within *MSR1*.⁶ The *EphB2* K1019X mutation represents a novel addition to this group of allelic variants. Population differences in allele frequencies for many of the candidate genes are real but whether the differences contribute to differences in disease susceptibility is difficult to assess with the current available data and traditional approaches. One potential confounding issue is PS, created by admixture.^{34–35} Here we typed unlinked genetic markers informative for ancestry in order to detect, quantify and correct for PS.^{26–36–39} Even though studies have consistently shown evidence of PS in AA,^{25–27–40–41} there continues to be debate on whether PS exists^{42–43} and whether its impact is significant.⁴⁴ A recent study has shown that even in highly inbred populations, significant PS exists, lending support for the notion that this phenomenon must exist in highly outbred populations such as AA.⁴⁵

Our examination of sequence variants in the *EphB2* gene and subsequent case-control study among AA men suggest that *EphB2* may play an important role in familial PC. This finding is potentially significant given the higher frequency of the K1019X nonsense mutation and the higher prevalence of PC among AA men compared with their US counterparts. The functional significance of the common K1019X nonsense mutation is not known; however, our results in addition to previous functional analyses of wild type *EphB2* using the DU145 PC cell line,¹⁶ suggests a pathogenic role for *EphB2* in PC and further study is warranted.

ACKNOWLEDGEMENTS

The authors would like to first thank the families and study participants for their continued involvement in this research. We would also like to thank the study coordinators of the AAHPC study network, including M Franklin, P Roberson, E Johnson, L Faison-Smith, C Meegan, M Johnson, L Kososki, C Jones, and R Mejia. In addition, we thank F Akereyeni and C Bonilla for technical assistance. This research was funded in part by the Intramural Research Program of the National Human Genome Research Institute, NIH, NIH Center for Minority Health and Health Disparities (1-HG-75418), the NCI (1U54CA91431-01) and the Department of Defense (DAMD17-00-1-0025 and DAMD 17-02-1-

0067). A B Baffoe-Bonnie also received support from USPHS grant CA-06927 and an appropriation from the Commonwealth of Pennsylvania.

Authors' affiliations

R A Kittles, Department of Molecular Virology, Immunology and Medical Genetics, Arthur G James Cancer Hospital and Richard J Solove Research Institute, Ohio State University, Columbus, OH, USA

A B Baffoe-Bonnie, Population Science Division, Human Genetics Program, Fox Chase Cancer Center, Philadelphia, PA, USA

T Y Moses, **C M Robbins**, **P Huusko**, **J M Trent**, **S Mousses**, **J D Carpten**, Genetic Basis of Human Disease Research Division, Translational Genomics Research Institute, Phoenix, AZ, USA

C Ahaghotu, **R A Kittles**, **P Furbert-Harris**, **G Dunston**, National Genome Center at Howard University, Washington, DC, USA

C Pettaway, Department of Urology, University of Texas MD Anderson Cancer Center, Houston, TX, USA

S Vijayakumar, Department of Radiation Oncology, UC Davis Cancer Center, University of California, Davis, Sacramento, CA, USA

J Bennett, Midtown Urology Surgical Center, Atlanta, GA, USA

G Hoke, Department of Urology, Columbia University Medical Center, Columbia University, New York, NY, USA

T Mason, Department of Urology, University of Illinois at Chicago, Chicago, IL, USA

S Weinrich, Medical College of Georgia, School of Nursing, Augusta, GA, USA

F S Collins, National Human Genome Research Institute, Bethesda, MD, USA

J Bailey-Wilson, Center for Inherited Disease Research, National Institutes of Health, Baltimore, MD, USA

I J Powell, Karmanos Cancer Institute, Wayne State University, Detroit, MI, USA

Competing interests: there are no competing interests

The NCBI dbSNP website is available at www.ncbi.nlm.nih.gov/SNP.

REFERENCES

- Ghafoor A**, Jemal A, Cokkinides V, Cardinez C, Murray T, Samuels A, Thun MJ. Cancer statistics for African Americans. *CA Cancer J Clin* 2002;**52**:326–41.
- Jemal A**, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ, American Cancer Society. Cancer statistics, 2004. *CA Cancer J Clin* 2004;**54**:8–29.
- Makridakis NM**, Ross RK, Pike MC, Crocitto LE, Kolonel LN, Pearce CL, Henderson BE, Reichardt JK. Association of mis-sense substitution in *SRD5A2* gene with prostate cancer in African-American and Hispanic men in Los Angeles, USA. *Lancet* 1999;**354**:975–8.
- Kittles RA**, Panguluri RK, Chen W, Massac A, Ahaghotu C, Jackson A, Ukoli F, Adams-Campbell L, Isaacs W, Dunston GM. Cyp17 promoter variant associated with prostate cancer aggressiveness in African Americans. *Cancer Epidemiol Biomarkers Prev* 2001;**10**:943–7.
- Zeigler-Johnson CM**, Walker AH, Mancke B, Spangler E, Jalloh M, McBride S, Deitz A, Malkowicz SB, Ofori-Adjei D, Gueye SM, Rebbeck TR. Ethnic differences in the frequency of prostate cancer susceptibility alleles at *SRD5A2* and *CYP3A4*. *Hum Hered* 2002;**54**:13–21.
- Miller DC**, Zheng SL, Dunn RL, Sarma AV, Montie JE, Lange EM, Meyers DA, Xu J, Cooney KA. Germ-line mutations of the macrophage scavenger receptor 1 gene: association with prostate cancer risk in African-American men. *Cancer Res* 2003;**63**:3486–9.
- Cunningham GR**, Ashton CM, Annegers JF, Soucek J, Klima M, Miles B. Familial aggregation of prostate cancer in African-Americans and white Americans. *Prostate* 2003;**56**:256–62.
- Oakley-Girvan I**, Feldman D, Eccleshall TR, Gallagher RP, Wu AH, Kolonel LN, Halpern J, Balise RR, West DW, Paffenbarger RS Jr,

- Whittemore AS. Risk of early-onset prostate cancer in relation to germ line polymorphisms of the vitamin D receptor. *Cancer Epidemiol Biomarkers Prev* 2004;**13**:1325–30.
- 9 Easton DF, Schaid DJ, Whittemore AS, Isaacs WJ, International Consortium for Prostate Cancer Genetics. Where are the prostate cancer genes? A summary of eight genome wide searches. *Prostate* 2003;**57**:261–69.
 - 10 Tavtigian SV, Simard J, Teng DH, Abtin V, Baumgard M, Beck A, Camp NJ, Carillo AR, Chen Y, Dayananth P, Desrochers M, Dumont M, Farnham JM, Frank D, Frye C, Ghaffari S, Gupta JS, Hu R, Iliev D, Janecki T, Kort EN, Laity KE, Leavitt A, Leblanc G, McArthur-Morrison J, Pederson A, Penn B, Peterson KT, Reid JE, Richards S, Schroeder M, Smith R, Snyder SC, Swedlund B, Swensen J, Thomas A, Tranchant M, Woodland AM, Labrie F, Skolnick MH, Neuhausen S, Rommens J, Cannon-Albright LA. A candidate prostate cancer susceptibility gene at chromosome 17p. *Nat Genet* 2001;**27**:172–80.
 - 11 Carpten J, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M, Moses T, Ewing C, Gillanders E, Hu P, Bujnovszky P, Makalowska I, Baffoe-Bonnie A, Faith D, Smith J, Stephan D, Wiley K, Brownstein M, Gildea D, Kelly B, Jenkins R, Hostetter G, Matikainen M, Schleutker J, Klinger K, Connors T, Xiang Y, Wang Z, De Marzo A, Papadopoulos N, Kallioniemi OP, Burk R, Meyers D, Gronberg H, Meltzer P, Silverman R, Bailey-Wilson J, Walsh P, Isaacs W, Trent J. Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. *Nat Genet* 2002;**30**:181–4.
 - 12 Casey G, Neville PJ, Plummer SJ, Xiang Y, Krumroy LM, Klein EA, Catalona WJ, Nupponen N, Carpten JD, Trent JM, Silverman RH, Witte JS. RNASEL Arg462Gln variant is implicated in up to 13% of prostate cancer cases. *Nat Genet* 2002;**32**:581–3.
 - 13 Xu J, Zheng SL, Komiya A, Mychaleckyj JC, Isaacs SD, Hu JJ, Sterling D, Lange EM, Hawkins GA, Turner A, Ewing CM, Faith DA, Johnson JR, Suzuki H, Bujnovszky P, Wiley KE, DeMarzo AM, Bova GS, Chang B, Hall MC, McCullough DL, Partin AW, Kassabian VS, Carpten JD, Bailey-Wilson JE, Trent JM, Ohar J, Bleecker ER, Walsh PC, Isaacs WB, Meyers DA. Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. *Nat Genet* 2002;**32**:321–5.
 - 14 Rebbeck TR, Walker AH, Zeigler-Johnson C, Weisburg S, Martin AM, Nathanson KL, Wein AJ, Malkowicz SB. Association of HPC2/ELAC2 genotypes and prostate cancer. *Am J Hum Genet* 2000;**67**:1014–19.
 - 15 Stanford JL, Sabacan LP, Noonan EA, Iwasaki L, Shu J, Feng Z, Ostrander EA. Association of HPC2/ELAC2 polymorphisms with risk of prostate cancer in a population-based study. *Cancer Epidemiol Biomarkers Prev* 2003;**12**:876–81.
 - 16 Huusko P, Ponciano-Jackson D, Wolf M, Kiefer JA, Azorsa DO, Tuzmen S, Weaver D, Robbins C, Moses T, Allinen M, Hautaniemi S, Chen Y, Elkahoun A, Basik M, Bova GS, Bubendorf L, Lugli A, Sauter G, Schleutker J, Ozzelik H, Elowe S, Pawson T, Trent JM, Carpten JD, Kallioniemi OP, Mousset S. Nonsense-mediated decay microarray analysis identifies mutations of EPHB2 in human prostate cancer. *Nat Genet* 2004;**36**:979–83.
 - 17 Gibbs M, Stanford JL, McIndoe RA, Jarvik GP, Kolb S, Goode EL, Chakrabarti L, Schuster EF, Buckley VA, Miller EL, Brandzel S, Li S, Hood L, Ostrander EA. Evidence for a rare prostate cancer-susceptibility locus at chromosome 1p36. *Am J Hum Genet* 1999;**64**:776–87.
 - 18 Matsui H, Suzuki K, Ohtake N, Nakata S, Takeuchi T, Yamanaka H, Inoue I. Genome-wide linkage analysis of familial prostate cancer in the Japanese population. *J Hum Genet* 2004;**49**:9–15.
 - 19 Brown WM, Lange EM, Chen H, Zheng SL, Chang B, Wiley KE, Isaacs SD, Walsh PC, Isaacs WB, Xu J, Cooney KA. Hereditary prostate cancer in African American families: linkage analysis using markers that map to five candidate susceptibility loci. *Br J Cancer* 2004;**90**:510–14.
 - 20 Royal C, Baffoe-Bonnie A, Kittles R, Powell I, Bennett J, Hoke G, Pettaway C, Weinrich S, Vijayakumar S, Ahaghotu C, Mason T, Johnson E, Obeikwe M, Simpson C, Mejia R, Boykin W, Roberson P, Frost J, Faison-Smith L, Meegan C, Foster N, Furbert-Harris P, Carpten J, Bailey-Wilson J, Trent J, Berg K, Dunston G, Collins F. Recruitment experience in the first phase of the African American Hereditary Prostate Cancer (AAHPC) study. *Ann Epidemiol* 2000;**10**(suppl):S68–77.
 - 21 Powell U, Carpten J, Dunston G, Kittles R, Bennett J, Hoke G, Pettaway C, Weinrich S, Vijayakumar S, Ahaghotu CA, Boykin W, Mason T, Royal C, Baffoe-Bonnie A, Bailey-Wilson J, Berg K, Trent J, Collins F. African-American hereditary prostate cancer study: a model for genetic research. *J Natl Med Assoc* 2001;**93**(suppl):25–85.
 - 22 Ahaghotu C, Baffoe-Bonnie A, Kittles R, Pettaway C, Powell I, Royal C, Wang H, Vijayakumar S, Bennett J, Hoke G, Mason T, Bailey-Wilson J, Boykin W, Berg K, Carpten J, Weinrich S, Trent J, Dunston G, Collins F. Clinical characteristics of African-American men with hereditary prostate cancer: the AAHPC Study. *Prostate Cancer Prostatic Dis* 2004;**7**:165–9.
 - 23 Panguluri RC, Long LO, Chen W, Wang S, Coulbaly A, Ukoli F, Jackson A, Weinrich S, Ahaghotu C, Isaacs W, Kittles RA. COX-2 gene promoter haplotypes and prostate cancer risk. *Carcinogenesis* 2004;**25**:961–6.
 - 24 Hoggart CJ, Parra EJ, Shriver MD, Bonilla C, Kittles RA, Clayton DG, McKeigue PM. Control of confounding of genetic associations in stratified populations. *Am J Hum Genet* 2003;**72**:1492–504.
 - 25 Kittles RA, Chen W, Panguluri RK, Ahaghotu C, Jackson A, Adebamowo CA, Griffin R, Williams T, Ukoli F, Adams-Campbell L, Kwagyan J, Isaacs W, Freeman V, Dunston GM. CYP3A4-V and prostate cancer in African Americans: causal or confounding association because of population stratification? *Hum Genet* 2002;**110**:553–60.
 - 26 Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. *Genetics* 2000;**155**:945–59.
 - 27 Freedman ML, Reich D, Penney KL, McDonald GJ, Mignault AA, Patterson N, Gabriel SB, Topol EJ, Smoller JW, Pato CN, Pato MT, Petryshen TL, Kolonel LN, Lander ES, Sklar P, Henderson B, Hirschhorn JN, Altshuler D. Assessing the impact of population stratification on genetic association studies. *Nat Genet* 2004;**36**:388–93.
 - 28 Gryfe R, Di Nicola N, Gallinger S, Redston M. Somatic instability of the APC I1307K allele in colorectal neoplasia. *Cancer Res* 1998;**58**:4040–3.
 - 29 Laken SJ, Petersen GM, Gruber SB, Oddoux C, Ostrer H, Giardiello FM, Hamilton SR, Hampel H, Markowitz A, Klimstra D, Jhanwar S, Winawer S, Offit K, Luce MC, Kinzler KW, Vogelstein B. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. *Nat Genet* 1997;**17**:79–83.
 - 30 Parsons R, Myeroff LL, Liu B, Willson JK, Markowitz SD, Kinzler KW, Vogelstein B. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res* 1995;**55**:5548–50.
 - 31 Kittles RA, Young D, Weinrich S, Hudson J, Argyropoulos G, Ukoli F, Adams-Campbell L, Dunston GM. Extent of linkage disequilibrium between the androgen receptor gene CAG and GGC repeats in human populations: implications for prostate cancer risk. *Hum Genet* 2001;**109**:253–61.
 - 32 Sartor O, Zheng Q, Eastham JA. Androgen receptor gene CAG repeat length varies in a race-specific fashion in men without prostate cancer. *Urology* 1999;**53**:378–80.
 - 33 Reichardt JK, Makridakis N, Henderson BE, Yu MC, Pike MC, Ross RK. Genetic variability of the human SRD5A2 gene: implications for prostate cancer risk. *Cancer Res* 1995;**55**:3973–5.
 - 34 Chakraborty R, Weiss KM. Admixture as a tool for finding linked genes and detecting that difference from allelic association between loci. *Proc Natl Acad Sci USA* 1988;**85**:9119–23.
 - 35 Lander ES, Schork NJ. Genetic dissection of complex traits. *Science* 1994;**265**:2037–48.
 - 36 Pritchard JK, Rosenberg NA. Use of unlinked genetic markers to detect population stratification in association studies. *Am J Hum Genet* 1999;**65**:220–8.
 - 37 Devlin B, Roeder K. Genomic control for association studies. *Biometrics* 1999;**55**:997–1004.
 - 38 Schork NJ, Fallin D, Thiel B, Xu X, Broeckel U, Jacob HJ, Cohen D. The future of genetic case-control studies. *Adv Genet* 2001;**42**:191–212.
 - 39 Reich DE, Goldstein DB. Detecting association in a case-control study while correcting for population stratification. *Genet Epidemiol* 2001;**20**:4–16.
 - 40 Parra EJ, Kittles RA, Argyropoulos G, Pfaff CL, Hiester K, Bonilla C, Sylvester N, Parrish-Gause D, Garvey WT, Jin L, McKeigue PM, Kamboh MI, Ferrell RE, Pollitzer WS, Shriver MD. Ancestral proportions and admixture dynamics in geographically defined African Americans living in South Carolina. *Am J Phys Anthropol* 2001;**114**:18–29.
 - 41 Pfaff CL, Parra EJ, Bonilla C, Hiester K, McKeigue PM, Kamboh MI, Hutchinson RG, Ferrell RE, Boerwinkle E, Shriver MD. Population structure in admixed populations: effect of admixture dynamics on the pattern of linkage disequilibrium. *Am J Hum Genet* 2001;**68**:198–207.
 - 42 Wacholder S, Rothman N, Caporaso N. Counterpoint: bias from population stratification is not a major threat to the validity of conclusions from epidemiological studies of common polymorphisms and cancer. *Cancer Epidemiol Biomarkers Prev* 2002;**11**:513–20.
 - 43 Thomas DC, Witte JS. Point: population stratification: a problem for case-control studies of candidate-gene associations? *Cancer Epidemiol Biomarkers Prev* 2002;**11**:505–12.
 - 44 Wang Y, Localio R, Rebbeck TR. Evaluating bias due to population stratification in case-control association studies of admixed populations. *Genet Epidemiol* 2004;**27**:14–20.
 - 45 Helgason A, Yngvadottir B, Hrafnkelsson B, Gulcher J, Stefansson K. An Icelandic example of the impact of population structure on association studies. *Nat Genet* 2005;**37**:90–5.