Association of variants in genes related to the immune response and obesity with BPH in CLUE II

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BACKGROUND: Chronic inflammation and obesity may contribute to the genesis or progression of BPH and BPH-associated lower urinary tract symptoms (LUTS). The influence of variants in genes related to these states on BPH has not been studied extensively. Thus, we evaluated the association of 17 single-nucleotide polymorphisms (SNPs) in immune response genes (IL1B, IL6, IL8, IL10, TNF, CRP, TLR4 and RNASEL) and genes involved in obesity, including insulin regulation (LEP, ADIPOQ, PPARG and TCF7L2), with BPH.

METHODS: BPH cases (N = 568) and age-frequency matched controls (N = 568) were selected from among adult male CLUE II cohort participants who responded in 2000 to a mailed questionnaire. BPH was defined as BPH surgery, use of BPH medications or symptomatic BPH (American Urological Association Symptom Index Score ⩾ 15). Controls were men who had not had BPH surgery, did not use BPH medications and whose symptom score was ⩽ 7. Age-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using logistic regression.

RESULTS: None of the candidate SNPs was statistically significantly associated with BPH. However, we could not rule out possible weak associations for CRP rs1205 (1082C>T), ADIPOQ rs1501299 (276C>A), PPARG rs1801282 (-49C>G) and TCF7L2 rs7903146 (47833T>C). After summing risk alleles, men with ⩾ 4 had an increased BPH risk compared with those with ⩽ 1 (OR, 1.78; 95% CI, 1.10–2.89; P trend = 0.006).

CONCLUSIONS: SNPs related to immune response and obesity, especially in combination, may be associated with BPH.

INTRODUCTION

Dietary and lifestyle factors likely contribute to the development and progression of BPH and BPH-associated lower urinary tract symptoms (LUTS) in older men.1 The precise mechanisms by which these factors may influence this complex condition are not well-understood, but several lines of evidence suggest that the risk of BPH and LUTS could be increased by chronic inflammation and obesity-associated perturbations in energy and insulin regulation.3 More specifically, cytokines and reactive species elaborated during a chronic inflammatory state may damage prostate cell membranes and DNA leading to increased cellular replication to replaced damaged cells, and thus increasing the risk of hyperplasia. Obesity is also a state of increased oxidative stress and the metabolic perturbations that accompany obesity tend to be growth promoting, again possibly leading to hyperplasia and increased risk of BPH.4,6

In addition to modifiable factors, genetics likely has a role in BPH. A twin study estimated that genetic factors contributed 72% to high-moderate/severe LUTS risk.7 Because the genetic immune response and energy regulation are influenced by genetic variation, we hypothesized that single-nucleotide polymorphisms (SNPs) in genes involved in these pathways would influence BPH. Candidates include genes encoding pro- inflammatory cytokines (IL1B, IL6, IL8, IL10, TNF, CRP, TLR4 and RNASEL) and genes involved in obesity, including insulin regulation (LEP, ADIPOQ, PPARG and TCF7L2) with BPH in a case–control study nested in the community-based CLUE II cohort.

MATERIALS AND METHODS

Study population

Men in this study were participants in the CLUE II cohort, established in May 1989. In all, 32,894 volunteers were recruited in Washington County, E-629, Houston, 77030 TX, USA. E-mail: david.s.lopez@uth.tmc.edu

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Genes related to immune response and obesity and BPH

DS Lopez et al

Maryland and neighboring areas. At baseline, a brief medical history, blood pressure, a food frequency questionnaire,14 and 20 ml of blood were collected. Heparinized blood was collected, chilled until centrifuged, aliquotted into plasma, red blood cells and buffy coat, and frozen at –70 °C. Participants updated lifestyle, medical and exposure histories by mailed questionnaire in 1996, 1998 and 2000. Men were eligible for the BPH study if they responded to the 2000 follow-up questionnaire (on which we assessed LUTS and BPH medications), did not have a cancer diagnosis before 2000 (except possibly non-melanoma skin cancer) and had not undergone a TURP or prostatectomy before 1989. On the basis of these criteria 4086 men aged ≥40 years formed the source population. The Institutional Review Board at the Johns Hopkins Bloomberg School of Public Health approved the study.

BPH case and control selection

On the 2000 questionnaire men were asked whether they ever had a TURP (and the date), or whether they had regularly taken medications to treat an enlarged prostate or to treat the urinary symptoms of BPH (for example, finasteride and alpha-blockers). Among those who had not had a TURP and who were not using BPH medications, we identified men with LUTS using a slightly modified version (to fit the constraints of our questionnaire) of the AUA (American Urological Association) Symptom Index.15 Using the AUA Symptom Index algorithm, we assigned 0–5 points to the 0–100% frequency of symptoms and the 0–5+ mmHg that a man needed to urinate. We summed points across all symptoms and nocturia to obtain a score ranging from 0 to 35. For this study, we considered men with scores of 0–7 to be asymptomatic, 8–14 to have low-moderate symptoms, 15–19 to have high-moderate symptoms and 20–35 to have severe symptoms. Three case groups were identified for the 568 BPH cases: Group 1—Surgery for BPH since 1989 irrespective of BPH medication use in the past 2 years or current symptoms (N = 102); Group 2—Use of BPH medications in the past 2 years by men who never had BPH surgery and irrespective of current symptoms (N = 310); and Group 3—High-moderate to severe symptoms on the AUA Symptom Index in men who never had BPH surgery and who did not use BPH medications (N = 156). We frequency matched 568 controls to the 568 cases on baseline age (±5 years). Controls were defined as men who never had BPH surgery, did not use BPH medications in the past 2 years and currently had no or low symptoms (<7).

SNP assessment

Buffy coat DNA was extracted using the PurePure DNA analyzer from Qiagen (Valencia, CA, USA). Genotyping was performed using the Applied Biosystems TaqMan 5’ exonuclease assays, TaqMan Universal PCR Master Mix, No AmpErase UNG and 2.5 ng of genomic DNA. The thermal cycling conditions consisted of an initial hold at 95 °C denaturing step and a 1 min 60 °C annealing and extension step. The ABI Prism 7900HT Sequence Detection System was used to detect the nucleic acids and the Sequence Detection Software v2.2 was used to discriminate alleles and call genotypes (Applied Biosystems, Foster City, CA, USA). Laboratory personnel was masked to case–control status.

We performed SNP selection in two stages, candidate gene and follow-up haplotype tagging SNPs (tagSNPs). In stage 1, we genotyped 17 candidate SNPs in 12 genes (IL1B, IL6, IL8, IL10, TNF, CRP, TLR4, TNFR1, NFKBIA, ADIPOQ, PPARG, and TCF7L2). The majority of the selected SNPs were located in the gene promoter region.16 Candidates were selected based on allele frequency (≥5% minor allele frequency in whites) and functional data, including to gene expression or association with health conditions. Three SNPs—TLR4 rs4986790 (9896G>A, Gsp99pgly), NFKBIA rs469607 (−1385G>A, Arg462Gln) and PPARG rs1801282 (−49G>C, Pro12Ala)—were non-synonymous. Genotyping was successful for 93–99% of the men for each candidate SNP.

After observing possible associations for SNPs in IL10, CRP, and TLR4 for prostate17 and colorectal18 cancers in this cohort, in stage 2 we selected tagSNPs for these genes. TagSNPs were selected using Tagger to cover most of the variation in these genes (http://www.broad.mit.edu/mpg/tagger/tagger/server.html). The targeted regions encompassed 10 kb before the transcription start site to 5 kb after the transcription end site based on the National Center for Biotechnology Information NCBI Build 35 and the phased HapMap release 21 CEU population panel. The selection criteria were a pair-wise r2 of ≥0.8 and a minor allele frequency of ≥5%. Seven tagSNPs were chosen for IL10, four for CRP and eight for TLR4. Genotyping was successful for >95% of IL10 and CRP tagSNPs, but success was lower for TLR4 tagSNPs.

Covariate assessment

Self-reported age, race, marital status, education, weight, height, cigarette smoking and treatment for high blood pressure and high cholesterol were collected at baseline. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Participants were asked whether they had used any medications in the 48 h before blood donation. We classified sulfonlurea, other glucose-lowering medications, and insulin as BPH medications. We classified over-the-counter and prescription aspirin, ibuprofen and other non-aspirin non-steroidal anti-inflammatory agents (NSAIDs) as NSAI Ds. Blood pressure was measured three times by a study nurse with a blood pressure cuff while the participant was in a seated position; the third blood pressure value was recorded. Hypertension was defined as a systolic blood pressure of ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg or report of treatment for high blood pressure. Plasma total cholesterol concentration in the non-fasting blood specimens was measured previously using an enzymatic method.18

Statistical analysis

Baseline characteristics were compared between cases and controls using the chi-square test (categorical) and t test (continuous). Hardy–Weinberg equilibrium was tested among controls using the chi-square test. 

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Post hoc analyses, we summed number of risk alleles for the subset of SNPs for which there were possible associations with BPH. Then, we estimated the association between number of risk alleles and BPH using indicator variables with ≤1 risk alleles as the reference group. Analyses were conducted stratifying by level of potentially modifying factors (obesity, hypertension and NSAIDs use). Tests for interaction were done by entering into the model an ordinal variable with values corresponding to the number of variant alleles; its coefficient was evaluated by the Wald test. In post hoc analyses, we summed number of risk alleles for the subset of SNPs for which there were possible associations with BPH. Then, we estimated the association between number of risk alleles and BPH using indicator variables with ≤1 risk alleles as the reference group. Analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CIs). ORs were estimated assuming a co-dominant or a dominant model of inheritance. Tests for trend were conducted by entering into the model an ordinal variable with values corresponding to the number of variant alleles; its coefficient was evaluated by the Wald test.

In post hoc analyses, we summed number of risk alleles for the subset of SNPs for which there were possible associations with BPH. Then, we estimated the association between number of risk alleles and BPH using indicator variables with ≤1 risk alleles as the reference group. Analyses were conducted stratifying by level of potentially modifying factors (obesity, hypertension and NSAIDs use). Tests for interaction were done by entering into the model an ordinal variable with values corresponding to the number of variant alleles; its coefficient was evaluated by the Wald test. Analyses were performed using SAS version 9.1 (SAS Institute). P-values are from two-sided tests.

Haplotype frequencies were estimated using the Expectation-Maximization algorithm.19 We used a global score test for differences in haplotype frequency distribution between cases and controls20 permuted P-values were calculated from an empirical distribution created from a random 10 000 permuted data set. The association between each haplotype and BPH was estimated by regression substitution assuming additive association in Haplo Stat.

RESULTS

BPH cases and controls did not differ significantly on their characteristics, with the possible exception of cases having a higher prevalence of NSAIDs use (Table 1). In controls, all genotypes were distributed in accordance with Hardy–Weinberg equilibrium, except for two candidate SNPs (TLR4 rs115386889 (11381G>C), P = 0.05; IL10 rs1800896 (-1082A>G), P = 0.02) and one tagSNP rs3024496 (IL10 7951C>T), P = 0.01. We retained these SNPs because the deviations from the expected genotype frequencies did not appear great.

Candidate SNPs

All four of the candidate SNPs was statistically significantly associated with total BPH (Table 2). Possible weak, non-statistically significant associations were observed for CRP rs1205 (1082C>T), ADIPOQ rs1501299 (276C>A), PPARG rs1801282 (−49G>C) and TCF7L2 rs7903146 (47833T>C). These patterns were generally
similar across the BPH case definitions (data not shown). Men with ≥4 risk alleles had a statistically significant 78% higher risk of BPH when compared with those with ≤1, and risk increased across number of risk alleles \( (P_{\text{trend}} = 0.006; \text{Table 3}) \). Similar associations were observed for each BPH case definition (Table 3).

Effect modification
Among obese men (BMI ≥ 30 kg m\(^{-2}\)), \( IL10 \) rs1800896 (-1082G \( \rightarrow \) A) was positively associated with total BPH (vs A/A, A/G+G/G; OR, 1.83; 95% CI, 1.03–3.25), whereas in non-obese men (BMI < 30 kg m\(^{-2}\)) the association was inverse (OR, 0.73; 95% CI, 0.53–1.00; \( P_{\text{interaction}} = 0.01 \)). In hypertensive men, \( PPARG \) rs1801282 (-49C \( \rightarrow \) G) was inversely associated with total BPH (vs C/C, C/G+G/G; OR, 0.53; 95% CI, 0.34–0.81), but in men without hypertension this association was null (OR, 1.10; 95% CI, 0.74–1.63; \( P_{\text{interaction}} = 0.04 \)). NSAIDs use did not modify any associations (data not shown).

Haplotype analyses
The tagSNPs were not associated with total BPH or the three case definitions (data not shown). We observed five common haplotypes (≥5%) for \( IL10 \), four for \( CRP \) and four for \( TLR4 \). However, neither the distributions of haplotypes between cases and controls \( (P_{\text{global}} = 0.20, 0.57 \) and 0.76, respectively), nor individual haplotypes (vs the most common) were associated with total BPH (Table 4).

**DISCUSSION**
In this case–control study nested in CLUE II, none of 17 candidate SNPs in 12 genes involved in the immune response and obesity was statistically significantly associated with total BPH. However, when we combined risk alleles for four SNPs that were possibly weakly associated with total BPH (\( CRP \) rs1205 (1082C \( \rightarrow \) T), \( ADIPOQ \) rs1501299 (276C \( \rightarrow \) A), \( PPARG \) rs1801282 (-49C \( \rightarrow \) G) and \( TCF7L2 \) rs7903146 (4783T \( \rightarrow \) G)), we found that the greater the number of risk alleles carried, the greater the BPH risk. The 19 tagSNPs and their haplotypes did not provide any additional information about the association of \( IL10, CRP \) and \( TLR4 \) with BPH. Our findings suggest that variation in genes related to the immune response and obesity, especially in combination, may be associated with BPH.

No prior studies have evaluated \( CRP \) rs1205 (1082C \( \rightarrow \) T), \( ADIPOQ \) rs1501299 (276C \( \rightarrow \) A), \( PPARG \) rs1801282 (-49C \( \rightarrow \) G) and \( TCF7L2 \) rs7903146 (4783T \( \rightarrow \) G) with BPH. Some studies have investigated circulating concentrations of C-reactive protein and adiponectin with BPH. Three large studies have reported that higher levels of C-reactive protein, a non-specific inflammatory marker whose circulating levels are influenced by \( CRP \) variants,\(^{21} \) were positively associated with BPH/LUTS.\(^{22–24} \) Adiponectin, an insulin-sensitizing cytokine secreted by adipocytes, was inversely associated with incident BPH in a nested case–control study,\(^{25} \) but not significantly associated with BPH in a small Greek case–control study.\(^{26} \) Although there is no direct evidence that \( PPARG \)\(^{27} \) and \( TCF7L2 \) influence risk of BPH, variants in these genes are associated with diabetes,\(^{28,29} \) a purported risk factor for BPH.\(^{30} \)

The remaining candidate SNPs in genes involved in the immune response (\( IL1B, IL6, IL8, IL10, TNF, TLR4 \) and \( RNASEL \)) and obesity (\( LEP \)) were not associated with total BPH. Our results are largely consistent with a community-based prospective study that showed no significant association of \( IL10 \) (rs1800896) or \( IL8 \) (rs4073) (or an SNP in \( IL1B \) (rs16944)) that we did not study with clinical measures of BPH.\(^{31} \) In that study, the AA genotype of \( TNF \)
Table 2. Odds ratios and 95% confidence intervals of BPH for 17 candidate single-nucleotide polymorphisms in the CLUE II cohort of Washington County, Maryland

| Genotype | Cases, N | Controls, N | OR (95% CI)* |
|----------|----------|-------------|--------------|
| IL10     |          |             |              |
| -926C > A (rs1800872) |  |  |  |
| C/C      | 306      | 298         | 1.00 (Reference) |
| A/C      | 194      | 209         | 0.89 (0.50–1.61) |
| A/A      | 23       | 25          | 0.90 (0.71–1.15) |
| PN tend** | 0.43     |             |              |
| C-carrier| 217      | 234         | 1.00 (Reference) |
| A-carrier| 356      | 359         | 1.08 (0.83–1.38) |
| -1082A > G (rs1800896) |  |  |  |
| A/A      | 147      | 138         | 1.00 (Reference) |
| A/G      | 285      | 303         | 0.88 (0.66–1.17) |
| G/G      | 116      | 110         | 0.99 (0.69–1.40) |
| PN tend** | 0.88     |             |              |
| G-carrier| 401      | 413         | 0.91 (0.69–1.19) |
| CRP      |          |             |              |
| 1082C > T (rs1205) |  |  |  |
| C/C      | 250      | 273         | 1.00 (Reference) |
| C/T      | 237      | 225         | 1.15 (0.89–1.47) |
| P tend** | 1.01     |             |              |
| T-carrier| 295      | 272         | 1.18 (0.93–1.50) |
| 1059G > C (rs1800947) |  |  |  |
| C/G      | 500      | 495         | 1.00 (Reference) |
| C/C      | 52       | 50          | 1.03 (0.68–1.54) |
| PN tend** | 0.96     |             |              |
| C-carrier| 52       | 51          | 1.01 (0.67–1.51) |
| TLR4     |          |             |              |
| 11381G > C (rs11356889) |  |  |  |
| G/G      | 396      | 399         | 1.00 (Reference) |
| C/G      | 134      | 128         | 1.05 (0.79–1.39) |
| C/C      | 15       | 18          | 0.84 (0.41–1.69) |
| PN tend** | 0.99     |             |              |
| C-carrier| 149      | 146         | 1.02 (0.78–1.34) |
| 89864 > G [Asp299Gly] (rs4986790) |  |  |  |
| A/A      | 489      | 491         | 1.00 (Reference) |
| A/G      | 62       | 58          | 1.07 (0.73–1.56) |
| G/G      | 2        | 3           | 0.67 (0.41–1.36) |
| PN tend** | 0.86     |             |              |
| G-carrier| 64       | 61          | 1.05 (0.72–1.52) |
| IL6      |          |             |              |
| -174G > C (rs1800795) |  |  |  |
| G/G      | 177      | 177         | 1.00 (Reference) |
| C/G      | 254      | 269         | 0.94 (0.72–1.23) |
| C/C      | 99       | 86          | 1.15 (0.80–1.64) |
| PN tend** | 0.58     |             |              |
| C-carrier| 353      | 355         | 1.00 (0.77–1.28) |
| -572G > C (rs1800796) |  |  |  |
| G/G      | 482      | 478         | 1.00 (Reference) |
| C/G      | 43       | 57          | 0.74 (0.49–1.13) |
| C/C      | 4        | 2           | 1.00 (0.13–7.07) |
| PN tend** | 0.21     |             |              |
| C-carrier| 45       | 59          | 0.75 (0.50–1.13) |
| -597G > A (rs1800797) |  |  |  |
| G/G      | 182      | 189         | 1.00 (Reference) |
| A/G      | 261      | 266         | 1.02 (0.78–1.32) |
| A/A      | 98       | 80          | 1.27 (0.88–1.82) |
| PN tend** | 0.25     |             |              |
| A-carrier| 359      | 346         | 1.08 (0.83–1.38) |

Abbreviations: CI, confidence interval; OR, odds ratio. *From a logistic regression model adjusting for age. Cases and controls frequency matched on age. **From a logistic regression model with number of variant alleles entered as an ordinal variable.
association between the PPARG (rs1801282) -49G allele and diabetes,12 in hypertensive men, we found an inverse association for the G allele and BPH, but a positive association in men without hypertension. Although we did not find effect modification by NSAIDs use, our ability to detect interaction may have been limited by our assessment of NSAIDs use only for the 48 h before blood donation.

Aspects of our study warrant discussion. We sampled cases and controls from a community-based cohort and doing so helped to ensure that allele frequencies in controls reflected those in the source population. We used several BPH definitions and the results were generally consistent, which helps to support that the associations that we observed were capturing the same underlying complex condition. Our BPH case definition was based on symptoms, including treatment for symptoms, and our control definition was based on lack of symptoms, a parallel comparison. However, we cannot rule out that some controls and cases may have had an enlarged prostate that did not lead to symptoms or was not the cause of their symptoms, respectively. The BPH surgery cases were incident; that is, the men had their TURPs some time before their surgery. We asked the men to report whether they had a TURP, but not other far less common surgery cases were incident; that is, the men had their TURPs was not the cause of their symptoms, respectively. The BPH definitions and the results were generally consistent, which helps to support that the associations that we observed were capturing the same underlying complex condition. Our BPH case definition was based on symptoms, including treatment for symptoms, and our control definition was based on lack of symptoms, a parallel comparison. However, we cannot rule out that some controls and cases may have had an enlarged prostate that did not lead to symptoms or was not the cause of their symptoms, respectively. The BPH surgery cases were incident; that is, the men had their TURPs months to years after the donation of blood used for genotyping. However, these men likely had symptomatic enlarged prostate for some time before their surgery. We asked the men to report whether they had a TURP, but not other far less common procedures to treat BPH; thus, we could have missed some BPH cases. The cases defined based on BPH medications use or LUTS were prevalent. We collected LUTS information only once, thus, we could not study whether SNPs are associated with LUTS progression.

We used a hypothesis-driven approach to select the genes for study. We chose SNPs based on known or suspected functionality. For three genes, we inferred haplotypes based on tagSNPs. We did not correct for multiple testing for either the candidate SNPs or tagSNPs because none of their main effects was statistically significant. The evaluation of the association for number of risk alleles was conducted post hoc, that is, the SNPs we included for summing of risk alleles were those for which we noted minimal evidence for an association with BPH. For this post hoc analysis and using the prevalence of the number of risk alleles that we observed in the controls, we had 70% power for a two-sided test with $\alpha = 0.05$ to detect a statistically significant trend across number of risk alleles when the OR comparing $\geq 4$ to $\leq 1$ risk alleles was 1.78 or greater. These findings from this analysis require evaluation in other studies.

In conclusion, our findings suggest that polymorphisms in genes related to the immune response and obesity weakly influence BPH risk. That we found an increasing odds of BPH with increasing number of risk alleles suggests that multiple genes and/or pathways together may affect the development of BPH.
CONFLICT OF INTEREST
The authors declare no conflict of interest.

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REFERENCES
1 Espinosa G. Nutrition and benign prostatic hyperplasia. *Curr Opin Urol* 2013; **23**: 38–41.
2 Bostani Y, Kazzazi A, Montahn S, Laze J, Djavan B. Correlation between benign prostatic hyperplasia and inflammation. *Curr Opin Urol* 2013; **23**: 5–10.
3 Parsons JK, Sarma AV, McVary K, Wei JT. Obesity and benign prostatic hyperplasia: clinical connections, emerging etiologic paradigms and future directions. *J Urol* 2013; **189**(1 Suppl): S102–S106.
4 Untergerasser G, Madersbacher S, Berger P. Benign prostatic hyperplasia: age-related tissue-remodeling. *Exp Gerontol* 2005; **40**: 121–128.
5 Kristal AR, Arnold KB, Schenk JM, Neuhausser ML, Weiss N, Goodman P et al. Race/ethnicity, obesity, health related behaviors and the risk of symptomatic benign prostatic hyperplasia: results from the prostate cancer prevention trial. *J Urol* 2007; **177**: 1395–1400.
6 Rohrmann S, Smit E, Giovannucci E, Platz EA. Associations of obesity with lower urinary tract symptoms and noncancer prostate surgery in the Third National Health and Nutrition Examination Survey. *Am J Epidemiol* 2004; **159**: 390–397.
7 Rohrmann S, Fallin MD, Page WF, Reed T, Partin AW, Walsh PC et al. Concordance rates and modifiable risk factors for lower urinary tract symptoms in twins. *Epidemiology* 2006; **17**: 419–427.
8 Dranoff G. Cytokines in cancer pathogenesis and cancer therapy. *Nat Rev Cancer* 2004; **4**: 11–22.
9 Tsan MF. Toll-like receptors, inflammation and cancer. *Semin Cancer Biol* 2006; **16**: 32–37.
10 Casey G, Neville PJ, Plummer SJ, Xiang Y, Krumroy LM, Klein EA et al. RNASEL Arg462Gln variant is implicated in up to 13% of prostate cancer cases. *Nat Genet* 2002; **32**: 1–2.
11 Ahima RS, Osei SY. Adipokines in obesity. *Front Horm Res* 2008; **36**: 182–197.
12 Altschuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 2000; **26**: 76–80.
13 Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006; **38**: 320–323.
14 Block G, Hartman AM, Naughton D. A reduced dietary questionnaire: development and validation. *Epidemiology* 1990; **1**: 58–64.
15 Barry MJ, Fowler FJ, D’Leary MP, Bruskewitz RC, Holtgrewe HL, Mebstu WK et al. The American Urological Association symptom index for benign prostatic hyperplasia. *J Urol* 1992; **148**: 1549–1557.
16 Tsilidis KK, Helzlsouer KJ, Smith MW, Grinberg V, Hoffman-Bolton J, Clipp SL et al. Association of common polymorphisms in IL10, and in other genes related to inflammatory response and obesity with colorectal cancer. *Cancer Causes Control* 2009; **20**: 1739–1751.
17 Wang MH, Helzlsouer KJ, Smith MW, Hoffman-Bolton JA, Hoffman SC, Grinberg V et al. Association of IL10 and other immune response- and obesity-related genes with prostate cancer in CLUE II. *Prostate* 2009; **69**: 874–885.
18 Kviterovich P, White S, Forte T, Bachorik P, Smith H, Nsiderman A. Hyper-apobetalipoproteinemia in a kindred with familial combined hyperlipidemia and familial hypercholesterolemia. *Atherosclerosis* 1987; **7**: 211–225.
19 Long J, Williams R, Urbanek M. An E-M algorithm and testing strategy for multiple-locus haplotypes. *Am J Human Genet* 1995; **56**: 799–810.
20 Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am J Hum Genet* 2002; **70**: 425–434.
21 Carlson CS, Aldred SF, Lee PK, Tracy RP, Schwartz SM, Biedner M et al. Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. *Am J Hum Genet* 2005; **77**: 64–77.
22 Kupelian V, McVary KT, Barry MJ, Link CL, Rosen RC, Ayer LP et al. Association of C-reactive protein and lower urinary tract symptoms in men and women: results from Boston Area Community Health survey. *Urology* 2009; **73**: 950–957.
23 StSauver JL, Sarma AV, Jacobson DJ, McGree ME, Lieder MM, Girman CJ et al. Associations between C-reactive protein and benign prostatic hyperplasia/lower urinary tract symptom outcomes in a population-based cohort. *Am J Epidemiol* 2009; **169**: 1281–1290.
24 Schenk JM, Kristal AR, Neuhausser ML, Tangen CM, White E, Lin DW et al. Biomarkers of systemic inflammation and risk of incident, symptomatic benign prostatic hyperplasia: results from the prostate cancer prevention trial. *Am J Epidemiol* 2010; **171**: 571–582.
25 Schenk JM, Kristal AR, Neuhausser ML, Tangen CM, White E, Lin DW et al. Serum adiponectin, C-peptide and leptin and risk of symptomatic benign prostatic hyperplasia: results from the Prostate Cancer Prevention Trial. *Prostate* 2009; **69**: 1303–1311.
26 Michalakis K, Williams CJ, Mitsiades N, Blakenman J, Balafouta-Tselenis S, Giannopoulos A et al. Serum adiponectin concentrations and tissue expression of adiponectin receptors are reduced in patients with prostate cancer: a case control study. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 308–313.
27 Jiang M, Strand DW, Franco OE, Clark PE, Hayward SW. PPARgamma: a molecular link between systemic metabolic disease and benign prostate hyperplasia. *Differentiation* 2011; **82**: 220–236.
28 Willson TM, Lambert MH, Kliwer SA. Peroxisome proliferator-activated receptor gamma and metabolic disease. *Annu Rev Biochem* 2001; **70**: 341–367.
29 Flores JC, Jablonski KA, Bayley N, Pollin TL, de Bakker PI, Shuldiner AR et al. TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 2006; **355**: 241–250.
30 Sarma AV, Kellogg Parsons J. Diabetes and benign prostatic hyperplasia: emerging clinical connections. *Curr Urol Rep* 2009; **10**: 267–275.
31 Mullan RJ, Bergstrahl EJ, Farmer SA, Jacobson DJ, Hebbirg SJ, Cunningham JM et al. Growth factor, cytokine, and vitamin D receptor polymorphisms and risk of benign prostatic hyperplasia in a community-based cohort of men. *Urology* 2006; **67**: 300–305.
32 Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet* 1997; **24**: 1–8.