Although we could replicate many known prostate cancer risk SNPs, the numbers replicated were fewer than expected by chance. More important, SNPs do not add to the accuracy of predictive models for prostate cancer risk either alone or in addition to PSA.

Appendix – Supplementary methods

1. Study cohort

Between 1991 and 1996, 11 063 men, born between 1923 and 1945 and living in the city of Malmö, Sweden, provided a blood sample. The Swedish Cancer Registry was used to identify men diagnosed with prostate cancer up to December 31, 2005. These diagnoses were based on standard clinical practice and not as a result of any biopsy or other screening in this study. Due to a 1-yr delay in registration, this information was obtained from the South Swedish Regional Tumor Registry for 2006.

2. Genotyping

Genotypes were determined using the Sequenom MassARRAY MALDI-TOF system. Assay design was made using the MassARRAY Assay Design v2.0 software (Sequenom, USA). Primers were obtained from Metabion GmbH, Germany, and all reactions were run under the same conditions except for the primer annealing temperature of the primary polymerase chain reaction (PCR). PCR reactions were performed in a total volume of 6 µl containing 2.5 ng of template DNA, 1.25X HotStarTaq PCR buffer, 0.15 units of HotStarTaq polymerase, 3.5 mM magnesium chloride, 0.5 mM deoxynucleotide triphosphates (dNTPs), and 100 nM of each primer. Amplifications were performed using GeneAmp 9700 machines with dual 384 heads as follows: 95°C for 15 min; 45 cycles at 95°C for 20 s; 56°C, 60°C, or 64°C for 30 s; 72°C for 60 s; and finally 72°C for 3 min. Dephosphorylation of unincorporated dNTPs was achieved using

shrimp alkaline phosphatase. Concentrations of individual hME primer pairs were adjusted to even out peak heights in the mass spectrum. The extension reactions were then made by mixing the adjusted MassEXTEND primer mix (containing approximately 1 μM of each primer) with hME EXTEND mix containing buffer and 50 μM each of dNTP mix and 1.25 units of Thermo Sequenase. PCR amplification of hME reactions was performed as follows: 94°C for 2 min and 99 cycles at 94°C for 5 s, 52°C for 5 s, and 72°C for 5 s. The samples were then manually desalted by using 6 mg of clean resin and a dimple plate and subsequently transferred to a 384-well SpectroCHIP using a nanodispenser.

3. Prostate-specific antigen levels

The levels of PSA in archived EDTA anticoagulated blood plasma obtained at baseline were determined using the dual-label DELFIA ProStatus assay (PerkinElmer, Turku, Finland) calibrated against the World Health Organization 96/670 [30]. The coefficient of variation was 9.1% at PSA levels of 0.43 ng/ml, 7.8% at PSA levels of 2.7 ng/ml, and 8.3% at PSA levels of 29 ng/ml.

4. Statistical analysis

For quality control we excluded participants with >20% missing data and computed per-SNP missing rates and departure from Hardy-Weinberg equilibrium in controls. The Cochran-Mantel-Haenszel test was performed to evaluate the association between SNPs and prostate cancer risk, with each strata consisting of one case along with up to three matched controls. To identify SNPs in LD that might not represent independent tests, we determined the LD measure r^2 for all pairs of SNPs on the same chromosome. For SNPs that were in LD (r^2 >0.2) and associated with prostate cancer, we tested for independence by asking if each SNP was significant when conditioned on the most significant SNP using the —chap command.