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SNPs in the kallikrein gene region associated with prostate cancer risk: true cause or association by design?

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We recently conducted a genome-wide association study (GWAS) using data from 1,854 PrCa cases with clinically detected (not PSA screened) PrCa diagnosed at <60 years or with a family history of the disease, and 1,894 population screened controls with a prostate specific antigen (PSA) of <0.5ng/ml (Eeles et al. 2008). These were analysed for 541,129 SNPs using the Illumina Infinium platform. Putative associations were evaluated using a further 3,268 cases and 3,366 controls. After these two stages, associations at seven loci, on chromosomes 2,3,6,7,10,11,19 and X, reached genome-wide levels of significance ($p=2.7\times10^{-8}$ to $p=8.7\times10^{-29}$). The SNP rs2735839 on chromosome 19 lies between two kallikreins, PSA (*KLK3*) and hK2 (*KLK2*). It was associated with a per allele OR for PrCa of 0.83 (95%CI 0.75-0.91; ptrend in stage 2 = 0.0002; ptrend overall = 2×10^{-18}). We also showed that rs2735839 was strongly associated with PSA level, in the direction consistent with the disease association (per allele rise in geometric mean PSA 1.12., $p=6\times10^{-8}$).

Ahn et al (this issue of *Nature Genetics*) analysed 24 tagSNPs in the kallikrein region on chromosome 19 (to include *KLK1, KLK2, KLK3* and KLK15) in five studies and found that none showed a significant association with PrCa risk. They also confirm the association between several SNPs, including rs2735839, and PrCa risk. They raise the possibility that the association found with PrCa risk in our study may reflect the selection of subjects based on PSA levels rather than a causal relationship with PrCa risk.

It is clear that the selection of controls in stage 1 of our study for low PSA levels did influence the association in stage 1. This is reflected in the minor allele frequency for rs2735839, which is 21.1% in the stage 1 controls, compared with 14%-15% in the UK 1958 birth cohort and the CGEMS study (males and females). However, the controls in stage 2 were not highly selected for PSA level. The only selection was to exclude controls with PSA levels of >10, and to require a negative prostatic biopsy if the PSA was >4. The MAF in the stage 2 controls (15.2%) is similar to that in other control populations and indicates that any selection bias at this stage was minimal.

To further evaluate the evidence for this association, we have undertaken an analysis of rs2735839 (together with SNPs at the other loci identified in our GWAS) in 13 further case-control studies as part of The PRACTICAL consortium. These studies comprise 7,370 PrCa cases and 5,342 controls. The estimated per allele OR for PrCa associated with rs2735839

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was 0.89 (95% CI 0.83-0.95; p=.0007), very close to our original estimate (Kote-Jarai, CEBP in press, 2008, cited with permission). There was no evidence of heterogeneity in the OR estimates among studies (see figure 1). We also note that when data from the five CGEMS studies are combined, the per allele OR is also remarkably similar (per allele OR 0.90, 0.83-0.90; P=.01), although this was not formally significant using the 4 degree freedom test given by Ahn et al (2008). If the results from our stage 2, PRACTICAL and CGEMS are combined, the overall evidence of association reaches genome-wide levels of significance ($p<10^{-8}$), demonstrating that, even disregarding our stage 1 result, the association is unlikely to be due to chance. The overall effect size, while modest, is comparable to that seen for other cancer associated loci.

None of the control series used in PRACTICAL, nor in CGEMS, involved selection for PSA level, and for this reason and those given above, the association appears unlikely to be driven purely by control selection. Selection bias related to case ascertainment is an alternative possible explanation. We excluded from our GWAS any cases identified through PSA screening, and several of the studies included in PRACTICAL are drawn from populations where PSA screening has not been used (e.g. the study from Finland). Thus, the association is unlikely to be due to PSA screening for asymptomatic disease. PSA testing is, however, also used in the process of diagnosis of symptomatic disease. This raises the possibility of a more subtle bias, in that some cases may have raised PSA related to the genotype but not related to their disease. Whether or not this potential bias is significant could be resolved using genotyping in studies based on biopsy of whole populations not driven by the PSA level (e.g. the Prostate Cancer Prevention Trial; Thompson et al., 2007), or studies where mortality is the endpoint.

Conversely, there are plausible biological grounds for believing that the association with *KLK* polymorphisms may be causal. For example, polymorphisms in the promoter of *KLK3* are associated with alterations in androgen receptor binding (e.g. Lai et al., 2007). Moreover, it is known that PSA level is a long-term predictor of prostate cancer risk (Lilja et al, 2007), and it is plausible that determinants of PSA level, including genetic determinants, may influence prostate cancer risk.

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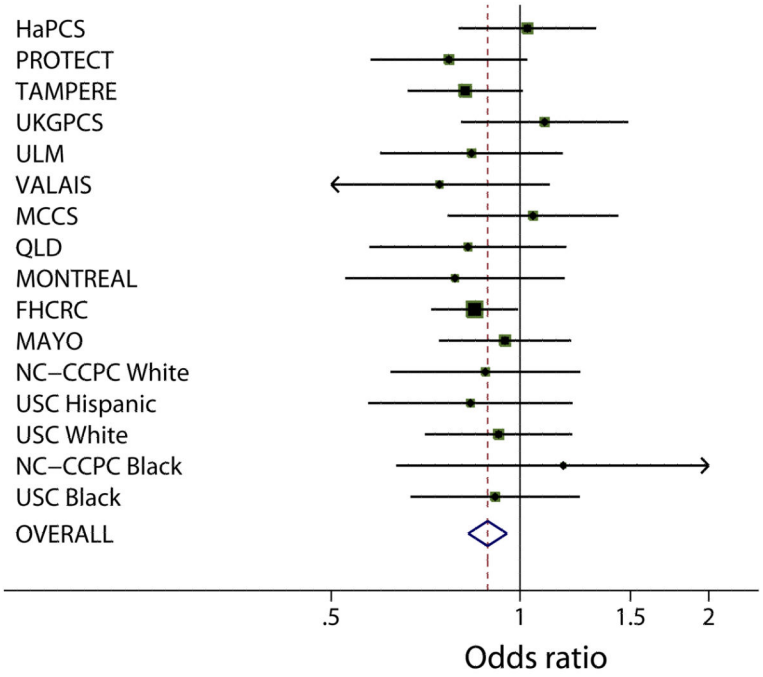

figure 1.

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