Author's response to reviews

Title: Addressing the contribution of previously described genetic and epidemiological risk factors associated with increased prostate cancer risk and aggressive disease within men from South Africa

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Version: 2 Date: 2 October 2013

Author's response to reviews: see over
REVIEWER 1: Francis Chinegwundoh

General comment: An important paper as so little genetic linkage work done in black Africans. Evidence of collaboration between Internationally renowned institutions and more local institutions is welcomed. Although little in the way of positive findings (apart from well known confirmatory epidemiology), nonetheless a marker study in the field. Likely that there are different genetic changes to that, which pertains in white populations. This work provides basis for further study. The authors acknowledge greater subject numbers required.

Response: The authors greatly appreciate these comments and the realization of the dire need and the significance of putting this study together.

Major revision: Major compulsory revision - methodology refers to "Tindall et al submitted". Either this submitted work is published before this one and so can be referenced properly, or the full methods put into this paper as an annexe. I wished to know more about the methods but had no way of so doing.

Response: Unfortunately the accompanying paper (submitted with this publication originally BMC Urology Tindall et al submitted accompanying paper) which addressed the clinical presentation, significance and limitations of performing prostate cancer research within culturally distinct communities within Africa, the overall limitations of the field for Africa, as well as the population substructure for future genetic based studies was not reviewed. This paper has been redrafted and submitted to the British Journal of Cancer (Tindall et al., BJC, submitted). The following changes were made to the text to reflect this change.

The following sentence was removed;

• Introduction: The outline of the SAPCS is presented within this issue as an accompanying study protocol [Tindall et al., submitted] with further detail contained within the study website [www.SAPCS.Webs.com].

The following sentences were added to reflect the submission to British Journal of Cancer and the results from this study of relevance to the current publication:

Materials and Methods, Study design and inclusion:

• The population contributions and substructure of the SAPCS participants, the latter based on genome-wide genetic analysis, is defined elsewhere [Tindall et al., BJC, submitted]. We recognize a unique within Africa Southern Bantu population clustering.

• A clinical and demographic summation of patients and controls within the SAPCS has been presented elsewhere, including the evolution and limitations of establishing a prostate cancer study within the constraints of Africa [Tindall et al., BJC, submitted].

Results:

• We recently described the Southern Bantu population groups included in this study as forming a single genetic cluster distinct from other African populations including both Eastern and Western Bantu-derived populations, supporting the combined analysis of Southern Bantu men for genetic association studies, [Tindall et al, BJC, submitted].
Discussion:

- As presented elsewhere [Tindall et al., BJC, submitted], the SAPCS is biased towards a more aggressive prostate cancer phenotype, demonstrated by frequency of extreme serum PSA levels and Gleason scores, compared to White and Black prostate cancer sufferers within the USA. Lack of routine PSA testing, medical infrastructure and increased use of traditional healers all contribute to symptomatic presentation and bias towards aggressive disease (while controlling for age at presentation) compared to current studies based on western practices and indolent disease presentation. The SAPCS therefore provides a unique alternative resource to study the impact of known genetic and epidemiological factors driving aggressive prostate cancer disease within Africa.

REVIEWER 2: Rick Kittles

Comment: The study is extremely under powered and this should be stated in the paper.

Response: The lack of study power and the fact that this is a ‘pilot study’ is addressed throughout the context of the paper as outlined below;

- **Within the conclusion of the abstract the authors state:** Despite a clear increased prostate cancer risk associated with an African ancestry, experimental data is lacking within Africa. This **pilot study** is therefore a significant contribution to the field. While genetic risk factors (largely European-defined) show no evidence for disease prediction in the SAPCS, several epidemiological factors were associated with prostate cancer status. **We call for improved study power by building on the SAPCS resource**, further validation of associated factors in independent African-based resources, and genome-wide approaches to define African-specific risk alleles.

- **Within the discussion almost a paragraph is dedicated to addressing study power:** One must caution however that our power to detect statistical significance is hindered by a relatively small sample size, to between 32 and 44%. A case group >1,000 subjects would be required to achieve 80% power to detect a statistically significant (P-value<0.05) OR≥1.4 with MAF≥0.2 in single hypothesis testing. Difficulties related to achieving highly significant associations with GWAS defined prostate cancer risk alleles in African populations has however been discussed previously [33]. As well as requiring large sample sizes to detect significant associations in GWAS, low levels of linkage disequilibrium in African populations may contribute to weak associations between causal variants and SNPs that are genotyped on GWAS platforms. Regardless, further validation in a larger study is required to improve power and more confidently reject the null hypothesis.

- **And again in the last and concluding paragraph of the discussion:** These pilot analyses are directing further investigative efforts as the study leaders focus on increasing the study numbers to achieve optimal study power.
Comment: The authors tried to put too much information in a short paper without describing or presenting important details. For instance: who are the subjects, their ethnicity, demographic, and clinical characteristics. Without knowing their cultural and genetic differences, one cannot say, if any of the subjects should be analyzed separately or not.

Response: We agree with the reviewer that the information within this study is extensive and hence two papers were drafted. The first paper (originally submitted as a complimentary paper to the BMC Urology but not reviewed, see response above for reviewer 1) addresses the clinical presentation, genetic substructure, cultural aspects and study limitations of the SAPCS. This paper has been redirected to British Journal of Cancer and is under review. Tindall et al., submitted ‘Clinical presentation of prostate cancer in Black South Africans: establishing a unique study to evaluate global disparities.’ In contrast, the current paper addresses the impact of known genetic and epidemiological factors associated with prostate cancer risk within the SAPCS.

Comment: Did they do analyses pooling stage 1 and 2? Maybe, they could get a better estimate of OR.

Response: Stage 2 analysis was pooled. As stated in the Results, Stage 2: Follow-up Genotype and Genetic Risk Score Analysis, first sentence, ‘Six variants achieving an un-corrected P-value≤0.05 in Stage 1, were genotyped on additional stage 2 samples, for a combined study size of 503 (297 cases and 206 controls).’ ORs were reported based on the pooled analysis.

Comment: For case only study, why did they do the association test with PSA in cases, not in controls?

Response: Controls were added to genotype-phenotype associations (latter including not only PSA but also family history of PCa, and family history of any cancer) increasing the numbers previously presented in Table S2, while the case-only analysis was removed from the original presentation of results in Table 1 (to avoid repetition), cases were further assessed for genotype associations with Age at PCa presentation, Gleason score and Tumor grade. As a consequence Table S2 is now presented as Table 2 in the main text of the paper and not within the supplement. New Table 2 is titled, Genotype-phenotype association analysis for variants genotyped in stage 2 analysis.

Comment: How did they define tumor grade?

Response: Materials and Methods, Study design and inclusion: The following sentence has been updated to reflect clinical measurements (i.e. defining tumor grade or disease status). ‘Subjects were reviewed by local urologists, PSA testing performed, and prostate cancer status defined histopathologically by Gleason score and tumor grade (well, moderate and poor differentiation).’

Comment: For the analyses of AUC, they used up to 38 SNPs. They said predictability improved as they added more SNPs but most of the SNPs are not significantly associated with prostate cancer. They also should examine how adding SNPs to PSA, family history, etc. all the significant variables in stage 3 improve the predictability.
Response: Although we observed a slight increase in AUC with the addition of SNPs to the genetic risk models, as stated in the manuscript, this difference is only minor and only marginally significant when comparing 6 and 38 SNP combinations for two of the models tested. Refer to results, 'This difference was only significant for models 2 and 3 when comparing each the 6 SNP and 38 SNP combinations to 3 SNPs. For Model 2 the difference between AUC relative to 3 SNPs is 0.035 (95%CI 0.005-0.065, P-value=0.0208) and 0.065 (95%CI 0.015-0.116, P-value=0.0117) for 6 and 38 SNPs respectively. For Model 3, the difference between AUC relative to 3 SNPs is 0.047 (95%CI 0.005-0.088, P-value=0.0282) and 0.093 (95%CI 0.027-0.159, P-value=0.0061) for 6 and 38 SNPs respectively.' All SNPs (as they presented with the largest AUC) were tested against the most significant predictor for prostate cancer, namely PSA as presented in Figure 3. As per the results it is stated, 'Genetic risk Model 2 for 38 SNPs resulted in the largest AUC of all models tested (AUC=0.671) and was therefore evaluated against the most common marker of prostate cancer to date, serum PSA levels, to predict prostate cancer in this study population (Figure 3). A sentence was added to the discussion to highlight the lack of predictive impact of known genetic loci within the SAPCS. As no variants tested in this study remained independently predictive after adjusting for multiple testing and combined within a genetic risk model showed no improvement on the predictive capability of serum PSA testing, highlights the need for independent prostate cancer genetic marker identification within the context of Africa. And highlighted again in the concluding paragraph, 'In an attempt to use individual genetic profiles of the SAPCS population to determine prostate cancer occurrence, we failed to show a significant predictive power alone or improve correlation in combination with the current serum PSA testing method.' As the predictive capability of all genetic risk models failed to accurately define prostate cancer status (as mentioned in the text) and is further emphasized by the observation that the AUC is decreased when combined with PSA levels, currently the most accurate early predictor of prostate cancer, we believe the combined PSA-GRS model is sufficient to investigate the impact of these variants in enhancing the predictive capability of current markers of prostate cancer.

Comment: They could expand more about the Stage 3 epidemiological analysis. Their findings in this section are potentially important for explaining why African descent men have higher incidence of prostate cancer compared to other ethnic/racial groups. There is definitely a need for a more thorough analysis and description of methods and results. They need more detailed discussion to explain observed patterns, especially many of readers do not know about the socio-cultural conditions that these men live in, i.e., access to health care, etc.

Response: As requested by the reviewer this discussion has been expanded for the significant epidemiological findings of relevance to the study cohort and the region. The following text has been included in the discussion;

- Although there has thus far been limited success in identifying demographic, lifestyle or environmental influences on prostate cancer predisposition, the multi-faceted nature of this disease is indisputable. These factors have as yet not been explored within the context of Africa. We provide evidence in this study for potential drivers of prostate cancer risk within the SAPCS. While a family history of prostate cancer has been associated with increased risk for a positive diagnosis for prostate cancer within the USA [36], a familial link has previously been attributed to increased screening in men with a family history of the disease, which may contribute to this observed association. Alternatively, results from the REDUCE study, which boasts minimal screening bias,
reported a geographically-dependent association between prostate cancer and a family history of the disease, yet a significant association, regardless of geographic location, was observed between prostate cancer and a family history of prostate and/or breast cancer. In our study risk was similarly not specifically attributed to a family history of prostate cancer, but rather a history of any cancer [37]. Where an Australian-based study reported an association between prostate cancer and a vertex only balding pattern [38], the SAPCS showed a significant association with a combination of vertex and frontal balding, although age at onset was not a significant factor. While diabetes mellitus increases risk for most human cancers (reviewed in [39]), the impact on prostate cancer appears largely protective [40, 41], however, an increased over-all mortality rate has been observed in prostate cancer patients with diabetes compared to those without [42]. Further complicating the assessment of this interaction is a lack of differentiating type I and type II diabetes and the potential effect of diabetes medications on prostate cancer outcome [43]. In our study of aggressive prostate cancer disease we observed a significant increased risk associated with pre-existing diabetes. In line with an Australian-based study, which correlated increased ejaculation frequency (especially early in life), with reduced prostate cancer risk [44] we show a significant protective effect of increased sexual activity and an inverse correlation with erectile dysfunction in the SAPCS. Although no association with the presence of STDs was observed, we cannot exclude that increased ejaculation, associated with sexual activity (or inversely associated with erectile dysfunction), may not be driving protection as a result of pathogenic shedding, specifically within an environment where pathogenic diseases are significant health concerns. Compared to a recent report that suggests a decreased risk of prostate cancer associated with regular use of aspirin (but not with alternative non-steroidal anti-inflammatory drugs), [45], frequent aspirin use within the SAPCS was inversely correlated with prostate cancer. This disparity may be impacted by the generic employment of the term aspirin, often used to refer to any form of headache medicine, including paracetemol, which exhibits very minor anti-inflammatory activity. A unique aspect of the SAPCS is the inclusion of both rural and more urbanized clinic locations. The significance of the observed increased prostate cancer risk associated with men from the Venda ethnolinguistic classification requires further investigation based on genetic and/or environmental drivers. A potential significant environmental implication for the observed association may be based on almost 70 years of dichlorodiphenyltrichloroethane (DDT) spraying for malaria control in Venda households [46] and previous controversial association with urogenital birth defects [47]. Interestingly, men within a health or education related occupation were more likely to be diagnosed with prostate cancer. The latter could be a direct consequence of increased access and adoption of western medical practices.