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European Association of Urology

Letter to the Editor

Reply to Xiaoling Lin, Brian T. Helfand, and Jianfeng Xu's Letter to the Editor re: Daniel A. Leongamornlert, Edward J. Saunders, Sarah Wakerell, et al. Germline DNA Repair Gene Mutations in Young-onset Prostate Cancer Cases in the UK: Evidence for a More Extensive Genetic Panel. Eur Urol 2019;76:329–37

As Lin et al. note, at present the panel of genes for which germline pathogenic mutations are believed to contribute to a higher risk of prostate cancer (PCa) or aggressive disease is limited, and even for these putative candidates the supporting statistical evidence remains modest because of the rare nature of these variants. Owing to these inherent limitations regarding study power, we agree that additional PCa susceptibility genes remain to be determined and that the current gene panels are not entirely sufficient for clinical utility. The pertinent question therefore is the identity of any additional genes that could inform future clinical care pathways. Through our analyses [1] we have observed evidence to suggest that additional DNA repair genes (DRGs) beyond those currently regarded as the most strongly supported candidates (*ATM*, *BRCA1*, *BRCA2*, *CHEK2*) may contribute to PCa risk, and identified several specific candidates for further follow-up. As noted by Lin et al. and ourselves, the ADA (adaptive combination of *p* values) methodology means that the gene set identified in our study represents initial rather than definitive evidence for a role of any individual gene or variant in PCa risk, unless and until they have been validated externally. Accordingly, we carefully described this necessity in our discussion (eg, "Whilst the novel genes we have identified represent exciting candidate moderate penetrance PCa risk genes, these findings nonetheless require additional validation in independent cohorts. In particular, we note that the optimal *p* value truncation thresholds used by ADA are tuned towards greater sensitivity than specificity to maximise power for rare variant discovery in sequencing study sample sizes, and no suitable replication set was available for confirmation of our findings." and "We believe that these genes warrant evaluation by the wider scientific and clinical communities in larger prospective studies or meta-analyses.").

The aim of our study was to evaluate a substantially wider panel of DRGs in a larger case–control cohort than had been performed previously to report our preliminary findings on whether, and for which genes, any higher risk of PCa and/or poor-prognosis disease appeared to be observed. Owing to the previously described nature of the methodology, we would therefore not necessarily expect that each of the genes selected via ADA will subsequently be replicated; instead, these simply represent the strongest candidates for further follow-up. Lin et al. further note that sequencing panels offered by commercial companies contain only a small number of DRGs, with only *ATM*, *BRCA1*, *BRCA2*, and *CHEK2* regularly present, therefore additional DRGs are not widely evaluated at present. These sequencing panels are however necessarily derived from scholarly evidence that is inevitably incomplete and continually evolving, or in many instances derived from studies with comparatively small numbers of genes and/or samples examined. Indeed, comparing the 23 candidate genes for which we observed provisional evidence of a contribution to PCa risk through our combined analyses against several leading commercial panels available at the time our study was conducted, the Illumina TruSight Cancer Panel (94 genes) only includes 11 of the 23 genes we identified; the Agilent Clearseq Comprehensive Cancer panel (151 genes) and Qiagen Human Prostate Cancer GeneRead DNAseq Gene Panel (20 genes) only contain *ATM*, *BRCA1*, and *BRCA2*; the GeneDx Hereditary Prostate Cancer Panel (16 genes) contains only 6 in our list; and the 66-gene BROCA Cancer Risk Panel Version 7 contains 9 of these genes. This issue similarly affects custom panels, as almost all panel sequencing studies for PCa reported to date have only considered a small number of genes derived from pre-existing knowledge and are limited in their ability to comprehensively evaluate wider gene sets, including many of the novel candidate genes we identified in our study. Accordingly, opportunities for external validation or refutation of the observations reported by ourselves and others would be missed, potentially hindering progress towards the identification of a definitive panel of clinically informative PCa risk genes.

While the general statement by Lin et al. is accurate that after exclusion of the 20 prospective risk genes selected by

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ADA in our case–control analysis, the frequency of mutations subsequently becomes greater among controls than cases across the total group of remaining genes (9.0% vs 6.5%, $p_{ADA} = 0.02$; odds ratio [OR] 0.70, 95% confidence interval [CI] 0.52–0.95), we must consider their conclusion that "It is apparent that we would not conclude that PTVs [protein-truncating variants] in the remaining (144) DRGs are protective" to be overly simplistic and devoid of the nuance evident from the underlying data. While we restricted our report to focus solely on genes associated with higher PCa risk, considering those as being of greater importance for immediate validation, a two-sided ADA analysis for detection of both risk and protective genes selected only 10 genes as being associated with a protective effect against PCa. Exclusion of these 10 prospective protective genes alongside the 20 selected by ADA as being associated with higher risk leaves a PTV rate across the remaining 67 DRGs in which at least one PTV had been observed of 6.7% in controls and 6.0% in cases ($p_{ADA} = 0.51$; OR 0.89, 95% CI 0.64–1.24), with no PTVs observed among either cases or controls within our cohort for the final 67 DRGs. Therefore, in contrast to the deductions of Lin et al., we did not observe a protective effect for 144 DRGs in the study panel; according to a two-sided ADA, 20 genes were implicated as potentially increasing the risk of PCa and 10 genes as potentially reducing the risk, while no supporting evidence for an effect in either direction was observed in our data for the remaining 134 genes. As Lin et al. conclude, while it is self-evident that one would not consider PTVs in all of the 144 remaining genes not selected as associated with higher PCa risk via the one-sided ADA to confer a protective effect, and would further hypothesise that mutations in DRGs would be likely to generally confer a pathogenic rather than a protective effect, it remains inherently plausible that mutations within a small subset of specific genes acting through very specific processes could indeed serve to confer lower risk. Therefore, we consider that our results demonstrate the potential utility of the ADA approach for selection of prospective candidate genes in currently feasible sample sizes for sequencing studies, with the important caveats previously noted that the ADA methodology is likely to overselect rather than underselect individual genes, and consequently these findings must be replicated before application of this candidate gene panel in clinical practice. We hope that our findings will be of use to the scientific community in informing the composition of future sequencing panels, with the ultimate goal of expanding the panel of clinically meaningful PCa genes, and in turn that any potential future benefit to patients that might be derived through these novel candidate PCa risk genes would be achieved in the shortest possible timeframe.

Conflicts of interest: The authors have nothing to disclose.

Reference

[1] Leongamornlert DA, Saunders EJ, Wakerell S, et al. Germline DNA repair gene mutations in young-onset prostate cancer cases in the UK: evidence for a more extensive genetic panel. Eur Urol 2019;76:329–37.

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