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Genetic Factors influencing Prostate Cancer Risk in Norwegian Men

Lovise Mahle	Oslo
Eli Grindedaal	Oslo
J. Xu, S. Zheng, Haitao Chen	NorthShore Research Institute, Chicago
K. Cooney	Univ Utah, Salt Lake City
S. D. Fosså	Oslo University Hospital, Dep of Oncology and University of Oslo , Faculty of Medicine
K Axcrona	
Srdjan Djurovic	NORMENT, KG Jebsen Centre for Psychosis Research and Department of Medical Genetics, Oslo University Hospital, Oslo, Norway
Ole A. Andreassen	NORMENT, KG Jebsen Centre for Psychosis Research, Institute of Clinical Medicine, University of Oslo, Oslo, Norway, Division of Mental Health and Addiction, Oslo University Hospital, Oslo, Norway,
Ian G. Mills	Centre for Molecular Medicine Norway, Nordic European Molecular Biology Laboratory Partnership, Forskningsparken, University of Oslo, 21 0349 Oslo, Norway; Department of Molecular Oncology, Institute for Cancer Research, Oslo University Hospital, 0424 Oslo, Norway; PCUK Movember Centre of Excellence, Centre for Cancer Research and Cell Biology (CCRCB), Queen's University, Belfast BT7 1NN, Northern Ireland, UK.
WB Isaacs, CM Ewing, K Wiley	Brady Urological Inst, Johns Hopkins School of Med

Abstract: Norway has one of the highest rates of death due to prostate cancer (PCa) in the world. To assess the contribution of both common and rare single nucleotide variants (SNPs) to the prostate cancer burden in Norway, we assessed the frequency of the established prostate cancer susceptibility allele, *HOXB13 G84E*, as well as a series of validated, common PCa risk SNPs in a Norwegian PCa population of 779 patients. The *G84E* allele was observed in 2.3% of patients compared to 0.7% of control individuals, OR= 3.8, P=1x10-4. While there was a trend toward an earlier age at diagnosis, overall the clinicopathologic features of PCa were not significantly different in G84E carriers and non-carriers. Evaluation of 32

established common risk alleles revealed significant associations of risk alleles at 13 loci, including SNPs at *8q24*, and near *TET2*, *SLC22A3, NKX3-1*, *CASC8, MYC*, *DAP2IP, MSMB, HNF1B, PPP1R14A,* and *KLK2/3.* When the data for each SNP are combined into a genetic risk score (GRS), Norwegian men within the top decile of GRS have over 5 fold greater risk to be diagnosed with PCa than men with GRS in the lowest decile. These results indicate that risk alleles of *HOXB13* and common variant SNPs are important components of inherited PCa risk in the Norwegian population, although these factors appear to contribute little to the malignancy's aggressiveness.

Introduction

Despite being one of the countries with the highest health care expenditure per capita, Norway has the highest rate of prostate cancer (PCa)-related deaths in the western world, and ranks third internationally (1). The reasons for this excess mortality due to PCa in Norway are unknown.

One possible contributor to high Norwegian PCa mortality rates is an ancestry-associated difference in the burden of genetic risk factors, including variants which might interact with environmental factors to increase risk and possibly disease aggressiveness. Twin studies suggest that PCa exhibits a high degree of heritability which is more significant than other common cancers including breast and colon cancer (2). Genome-wide association studies (GWAS) have identified at least 100 single-nucleotide polymorphism loci or SNPs that are associated with increased risk of PCa, at least in men of European descent (3). Although the impact of each individual risk SNP is small, men who carry the top percentiles of inherited risk alleles are 4-6-fold more likely to be diagnosed with PCa (4, 5). Recent studies suggest PCa risk SNPs account for ~33% of familial PCa risk in men of European ancestry (3). An analysis of PCa risk SNPs in the Norwegian population has not been previously reported to our knowledge.

In early 2012 the identification of the first *bona fide* prostate specific cancer susceptibility gene, *HOXB13* was reported (6). This finding has been confirmed by many labs around the world (eg ref 7) as well as by researchers in the International Consortium for Prostate Cancer Genetics (ICPCG) (8). Through combined analyses of different study populations within the ICPCG, the observation was made that the most common mutation in *HOXB13* in US men, *G84E*, was found to be at the highest frequency in individuals of Nordic descent. Indeed, as many as 8 to 10% of Swedish (9) and Finnish (10) men with family history positive prostate cancer diagnosed at an early age carry a *G84E HOXB13* mutation, compared to ~1% or less in unaffected men. A critical additional finding was that all *G84E* mutation carriers shared a common haplotype (8), that is, they are all descended from a common founder, presumably of Nordic origin. Thus, in addition to more common variants

we were interested in determining the frequency of *G84E* in Norway, a population with the highest prostate cancer mortality rate in any country outside of the African diaspora.

Methods

Description of patient/control population

Blood DNA from 779 men treated with curative intent in Norway in 2009 was available for genetic studies as part of a national trial, which evaluated the incidence of side effects after curative treatment of PCa (11). All available clinical and pathologic information on these patients was obtained from the Norwegian Cancer Registry. Control DNA samples were obtained from 1643 Norwegian individuals who consented to provide samples for research purposes at the time of blood donation.

SNP and HOXB13 G84 Genotyping:

A total of 32 SNPs known to be associated with risk of being diagnosed with PCa were assessed in this study. Genotyping in cases was performed as previously described using mass spectrometry (5, 12). Genotyping in controls was obtained from GWAS data (do you know which array). The complete open reading frame for HOXB13 was sequenced using Sanger Sequencing. Correlation between sequence data obtained using Sanger sequencing and taqman technology, as previously described, was 100%. To compare allele frequencies between Norwegian PCa patients and Norwegian population, array data from n=4700 Norwegian blood donors were included.

Genetic risk assessment and Statistical analysis

Genetic risk assessment was used for the evaluation of individual disease risk. Risk alleles (R) and non-risk alleles (N) were counted at each locus. Genotype RR was counted as 2, RN as 1, and NN as 0. Then, the number of risk alleles was summed (5).

Student's *t*-tests (for normal variables) and Mann–Whitney U-tests (for non-normal variables) were used to compare the variables among groups in univariate analyses. In multivariate analyses, logistic regression was used to evaluate the three genetic risk assessment methods after adjusting for clinical variables. All statistical analyses were

performed using SPSS 22.0 (IBM Corporation, New York, USA). Two-tailed $P < 0.05$ was considered statistically significant.

Results

HOXB13:

To search for coding sequence variants including possible novel founder mutations in the Norwegian population, we sequenced the complete coding region of the *HOXB13* gene in 779 PCa cases. As summarized in Table 1, over half (52.8%) of these cases had cancers with clinical Gleason Score 7 or higher. In total, we found 18 carriers of the *G84E HOXB13* mutation, for a carrier frequency of 2.3%. This rate compares to 0.7% carrier rate observed in 1643 anonymous blood donors. The OR for PCa in *G84E* carriers in the Norwegian population is 3.8, P=0.001 (Table 2).

An evaluation of the clinicopathologic tumor characteristics in the *G84E* carriers showed that these cancers were similar to cancers seen in non-carriers. While, there is a trend towards younger age at diagnosis in *G84E* carriers undergoing radical surgery for PCa (60.5 years for *G84E* carriers vs. 62.6 years for non-carriers), the frequency of *G84E* carriers in men diagnosed with high grade disease (Gleason 7 and higher) is the same (2.36%) as in men with low grade disease (2.25%), suggesting that *G84E* does not predispose men to higher grade prostate cancer in the Norwegian population.

Other than the *G84E* variant, we found no other non-synonymous coding sequence mutations. The only other variants observed were two synonymous polymorphisms at Serines 122 and 127 (rs8556 and rs9900627, respectively).

PCa risk SNPs:

We genotyped a set of 32 validated PCa risk SNPs (12 13) in the same set of 779 PCa cases. These data are presented in Table 3. Of the 29 evaluable SNPs (no control data are

available for three SNPs), 13 showed a statistically significant association with PCa risk. With several exceptions, the remaining SNPs showed frequency differences in the same direction as previously reported (12), thus, it is likely that the relatively small sample size accounts for the lack of significance of many of these SNPs. Significant associations (P<0.05) were observed in the Norwegian PCa population for SNPs within or adjacent to: *TET2, SLC22A3, NKX3-1, CASC8, MYC, DAP2IP, MSMB, HNF1B, PPP1R14A*, and *KLK2/3*. The most significantly associated region of the genome in terms of harboring multiple risk alleles is *8q24*, with four independent SNPs showing significant associations. The SNP with the largest effect overall was *rs16901979* at 8q24, which showed an OR of 1.7.

SNP genotype frequencies were also compared between cases with high grade (Gleason 7 or higher) and all other study individuals (cases with Gleason 6 or less as well as all controls). As expected from previous studies (3), most SNPs were not associated with high grade disease. However, 5 SNPs demonstrated increased frequencies in high grade cases, reaching P values of 0.05 or less.

By combining SNP data, a genetic risk score (GRS) was calculated based on the number of risk alleles an individual inherits (5,13,14). PCa risk as a function of GRS in the Norwegian population was examined in Table 4. The median GRS for cases was significantly higher than that calculated for the control population (P=4.98E-27). An analysis of risk by decile of GRS indicates an ability to stratify risk over a 5-fold range, with individuals with the highest and lowest decile of GRS having a 2.5 and 0.5 fold risk of PCa, respectively, when compared to the average population risk.

There was no difference in the GRS in carriers vs. non-carriers of *HOXB13 G84E*, indicating the independent effects of these alleles on PCa risk (Suppl Table 2). Furthermore, GRS had no effect on risk of PCa death, although the number of events was low (Suppl Table 3).

Discussion

In a Norwegian population of men with PCa, we find that both *HOXB13 G84E* and at least 13 PCa risk SNPs are associated with risk for PCa. The significant association of *HOXB13 G84E* with PCa risk in the Norwegian population extends the number of study populations where such an association has been observed. While the *G84E* variant and some of the larger effect SNPs are present at higher frequencies than in European American study populations, the lack of association of these SNPs with clinical parameters suggests that these genetic factors are most likely not responsible for the increased burden of lethal PCa in Norway.

The SNP with the largest effect in this study was *rs16901979* at *8q24* near the gene *PRNCR1*, which showed an OR of 1.7. Interestingly, this allele is present at ~50% higher allele frequency in Norway as compared to the frequency observed in a US control population from the REDUCE trial population (12). *PRNCR1*, which codes for a long non-coding RNA, has been identified as a possible component in disease progression through the coordination of androgen receptor (AR) signaling (15), although this was not confirmed in another subsequent study (16). Another substantial difference is observed at *rs2660753*, at *3p12*, near the *CHMP2B* gene, involved in endosome sorting, where the OR observed in Norwegian PCa is ~1.5 vs ~1.1 in REDUCE (14).

One hallmark of *HOXB13* mutations in PCa is the presence of founder mutations in different study populations. The *G84E* allele is the result of a founder mutation thought to originate in the Nordic region within the last several hundred years, most likely in Finland or Sweden. We have previously observed a founder mutation (*G134E*) in Han Chinese (17) and a recent report describes unique missense changes (*A128D* and *F240L*) in Portuguese PCa patients (18) as well as Japanese PCa patients. A series of unique missense changes have also been observed in prostate cancer patients of African descent (unpublished observations, WBI). We have recently observed a Y87D mutation in a PCa patient coincident with a loss-of-function ATM mutation. Together with *L144P* and *Y88D* observed in different PCa cell lines (LNCaP and LAPC4 respectively (6)), this brings the total number of unique *HOXB13* mutations found to date to 14. Further study is required to understand the association (and function) of the variant alleles with PCa risk. In the current study we did not find any novel variants in the Norwegian population.

This study provides evidence to support the possible use of PCa risk SNPs to stratify risk in the Norwegian population. Indeed, calculation of a genetic risk score using the SNPs genotyped in this study allows for the stratification of risk for PCa across a 5 fold range, i.e. men who are in the upper 10% of risk allele carriers are ~5 fold more likely to be diagnosed with prostate cancer than men in the lowest 10%. This level of risk is sufficient to warrant consideration of genetic risk estimate calculations for targeted early screening in the general population (19). The use of SNPs to identify men in the Norwegian population who are at increased inherited risk for PCa and thus would benefit from early disease screening and monitoring could eventually result in significant declines in mortality and morbidity in these men.

While both *HOXB13 G84E* and PCa risk SNPs appear to contribute to the inherited risk for PCa in Norway, these variants most likely cannot explain the high rate of PCa-associated mortality in this population. Given the recent findings of how important deleterious germline mutations in *BRCA2* and potentially other DNA repair genes are in predisposing men to more aggressive PCa, it would be important to examine the frequency of deleterious mutations in these genes in this population. In particular, a survey of the frequency of Norwegian founder mutations in high penetrance genes like *BRCA1* (20), *ATM* (21), *PMS2* (22), and *CHEK2* (23) might provide some novel insight into this question.

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