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HOXB13 Mutations in a Population-Based, Case Control Study of Prostate Cancer

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Abstract

BACKGROUND—Prostate cancer (PC) is the most frequently diagnosed non-skin malignancy in men in the Western world, yet few disease-associated mutations have been found. Recently, a low frequency recurring mutation in the *HOXB13 gene* was reported among both hereditary PC families and men from the general population.

MATERIALS and METHODS—We determined the distribution and frequency of the G84E *HOXB13* variant in 1,310 incipient PC cases and 1,259 age-mated controls from a population-based, case control study of PC.

RESULTS—The G84E mutation was more frequent in cases than controls (1.3% versus 0.4%, respectively), and men with the *HOXB1*3 G84E variant had a 3.3-fold higher relative risk of PC compared with noncarriers (95% CI, 1.21–8.96). There was a stronger association between the G84E variant and PC among men with no first-degree relative with PC (OR, 4.04; 95% CI, 1.12–14.51) compared to men with a family history of PC (OR, 1.49; 95% CI, 0.30–7.50; p=0.36 for interaction). We observed some evidence of higher risk estimates associated with the variant for men with higher versus lower Gleason score (OR, 4.13; 95% CI, 1.38–12.38 vs OR, 2.71; 95% CI, 0.88–8.30), and advanced versus local stage (OR, 4.47; 95% CI, 1.28–15.57 vs OR, 2.98; 95% CI, 1.04–8.49), however these differences were not statistically different.

CONCLUSIONS—These results confirm the association of a rare *HOXB13* mutation with PC in the general population and suggest that this variant may be associated with features of more aggressive disease.

Keywords

polymorphism; prostate neoplasm; genetic susceptibility

Introduction

Prostate cancer (PC) is the most frequently diagnosed non skin malignancy in men in the Western world, with more than 241,740 new cases and approximately 28,170 PC-specific

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deaths estimated for 2012 (1). Among the most significant risk factors for PC is a positive family history. Men with a first-degree family history of PC have a two- to three-fold increase in risk relative to men without such a history (2). This is supported by twin studies, which estimate that inherited genetic factors account for approximately 42% of PC incidence (3). Family-based genome-wide linkage studies have identified over 20 loci that segregate with the disease in hereditary PC (HPC) families; however, only a few of these loci have been replicated in multiple independent sets of HPC families (4–6).

One locus, which has been replicated in multiple data sets is at 17q21-22, located at approximately 57–60 cM (7). Originally reported on the basis of a linkage study using 175 HPC pedigrees (8), initial linkage reports in this region showed only marginal evidence for linkage (LOD = 2.36), reflecting the modest size of the families (average of 2.6 affected men per family with DNA), locus heterogeneity associated with the disease, and presence of phenocopies. The strongest evidence was provided by pedigrees with four or more affected individuals (LOD = 3.17) (8). Interest in the region increased with replication of the original finding (9,10), and narrowing of the region to a 1-LOD support interval of about 10cM (11).

In 2012, the homeobox transcription factor *HOXB13* was proposed as a gene for PC risk from the 17q21-22 region (12). This was based on analysis of more than 200 genes. Specifically, in an analysis of HPC families, investigators reported that 18 affected men in four families carried a rare but recurring mutation in the second position in codon 84, resulting in a non-conservative replacement of a glutamic acid for a glycine (G84E). These findings were validated in an assessment of unrelated affected men of European descent, in which the mutation rate was 1.4%, compared to 0.1% found in controls. Among men with early onset familial PC, the carrier rate was 3.1%, compared to those with late-onset non-familial PC (0.6%; $p=2.0\times10^{-6}$). Replication studies have focused on clinical features of disease (13), studies of familial disease (14), particular ethnic groups (15,16), or hospital-based series (17).

None of the above analyses address the mutation frequency among men from the general population in the United States. Nor do they allow for stratification based on long-term follow up. Toward that end, we have investigated the frequency and distribution of *HOXB13* mutations among 1,457 cases and 1,342 controls from two Western Washington population-based, case- control studies of PC.

Methods

Study Population

We used data and biological specimens from two previous population-based case-control studies. As previously described (18,19), study subjects were Caucasian and African American residents of King County, Washington. Cases from the first study were 40–64 year old men diagnosed with histologically confirmed PC between 1993 and 1996. Cases from the second study were 35–74 year old men diagnosed with histologically confirmed PC between 2002 and 2005. For both studies, cases were identified from the metropolitan Seattle-Puget Sound population based tumor registry that is operated as part of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) program.

Controls were recruited by random-digit telephone dialing (20). In both studies, household census information was obtained, and men within the study age range and without a self-reported physician's diagnosis of PC and who agreed to receive information, were contacted. Household census information was obtained for 94% and 81% of residential telephone numbers contacted in the first and second studies respectively. For both studies, controls were recruited evenly throughout the ascertainment period for cases, and were

frequency matched to cases by 5-year age groups in both studies. Of eligible controls identified, 75% and 63% were interviewed in the first and second studies respectively.

Of 753 eligible interviewed cases in the first study, 631 (84%) provided blood samples yielding sufficient DNA for genotyping, and of 703 eligible interviewed controls, 565 (80%) provided blood samples. Of 1,001 eligible interviewed cases in the second study, we collected 827 (83%) blood samples for genotyping, and of 942 eligible interviewed controls, 787 (84%) provided blood samples. Out of a total of 230 genotyped African American men, there was only one case and no controls with the *HOXB13* variant. As such, we were unable to stratify by race and we therefore confined all further analyses to Caucasian men. We conducted analyses for the present study on a total of 1,310 cases and 1,259 controls with sufficient DNA for this specific study and who were successfully genotyped for the *HOXB13* variant, all Caucasian.

As previously described (18,19), we interviewed study participants using a structured questionnaire eliciting demographic characteristics, medical and lifestyle history. The Seattle–Puget Sound SEER cancer registry provided information on stage and Gleason score of PC at diagnosis for cases. Vital status and underlying cause of death were obtained through the cancer registry, which links quarterly to the Washington State Center for Health Statistics database, and death certificates were requested from the state to confirm cause of death. The Fred Hutchinson Cancer Research Center's Institutional Review Board approved the studies and the Internal Review Board of the National Human Genome Research Institute approved genotyping. Written informed consent was obtained from all study participants.

Single Nucleotide Polymorphism Genotyping

A custom designed TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA) was used to genotype the *HOXB13* G84E (rs138213197) SNP on the ABIPrism 7900HT sequence detection system according to the manufacturer's instructions. The 40 blind duplicates showed 100% concordance.

Statistical Analysis

Genotype frequencies for *HOXB13* (rs138213197) were consistent ($P > 0.05$) with Hardy-Weinberg equilibrium (HWE) among Caucasian controls. We estimated the odds ratio (OR) and 95% confidence intervals (CI) for the association between the *HOXB13* G84E variant and PC by fitting unconditional logistic regression models, adjusted for age.

To investigate whether an association between the *HOXB13* G84E mutation was modified by family history of PC, we stratified subjects by first-degree relative with PC. To statistically assess departures from multiplicative effects we included a product term in stratified analyses, and used a log-likelihood ratio test to compare logistic models with and without the product term.

We used polytomous logistic regression to explore differences by stage (local versus regional/distant) and Gleason score (2–7, including 3+4 versus 7–10, including 4+3). We grouped Gleason score 7 (4+3) tumors with Gleason scores 8–10 because outcomes such as PC-specific mortality for 4+3 tumors have been shown to be similar to 8–10 tumors (21). We additionally characterized tumors by a composite measure of aggressiveness and conducted polytomous logistic regression. Tumors were classified as "less aggressive" if they met the following criteria: local stage, Gleason score 2–6 or 7 (3 + 4), and diagnostic serum PSA <20 ng/mL. "More aggressive" cancer was defined by regional/distant stage, Gleason score 7 (4 + 3) or 8–10, or serum PSA ≥20 ng/mL.

To analyze risk of death from PC associated with the *HOXB13* G84E variant, we calculated PC-specific hazard ratios (HR) and 95% CIs by Cox proportional hazards regression, adjusting for age. Time to PC-specific mortality was defined as the time from diagnosis to death. The censoring date was the date of last vital status update from the cancer registry (July 1, 2012).

In addition, we calculated the adjusted HR associated with the variant for risk of recurrence/progression. Time to recurrence/progression was defined as time from diagnosis to first reported evidence of recurrence, as previously described (22). The censoring date was the date that the follow-up questionnaire was returned. For those who died of PC prior to administration of the follow-up survey, time to recurrence was imputed (22). We used Stata statistical software (version 11.0, Stata Corp.) for all analyses.

Results

Compared with controls, cases were more likely to report a first-degree family history of PC (Table 1). Cases were more likely to have had a Prostate-Specific Antigen (PSA) test in the last five years before reference date (date of diagnosis for cases and a randomly assigned date for controls that approximated the distribution of diagnosis dates for cases). The majority of cases had localized stage disease (78%), moderate Gleason score (79%), and less aggressive tumors (66%).

Overall in our study, the *HOXB13* G84E mutation was identified in 18 out of 1,457 cases and 5 out of 1,342 controls. Due to the limited numbers of African Americans with the *HOXB13* G84E variant (one case and no controls), we were unable to perform meaningful stratified analyses and therefore conducted all further analyses with the 1,310 Caucasian cases and 1,259 Caucasian controls. The *HOXB13* G84E mutation was more frequent in Caucasian cases than controls (1.3% in cases versus 0.4% in controls) and had a 3.3-fold higher risk of PC compared with noncarriers of G84E, adjusted for age (95% CI, 1.21–8.96; Table 2). There appeared to be a greater association between the *HOXB13* G84E variant and PC among men with no first-degree relative with PC (OR, 4.04; 95% CI, 1.12–14.51; Table 3) compared with men with a family history of PC (OR, 1.49; 95% CI, 0.30–7.50); however, this interaction was not significant (likelihood ratio p=0.36).

We did not observe meaningful heterogeneity by stage (p=0.45), Gleason score (p=0.39), or cancer aggressiveness (p=0.50). However, the extremely small number of men with the *HOXB13* G84E variant hindered interpretation of stratified analyses. We observed some indication of greater risk of PC for men with higher Gleason score (OR, 4.13; 95% CI, 1.38–12.38; Table 4) compared with lower Gleason score (OR, 2.71; 95% CI, 0.88–8.30). Similarly, associations appeared to be somewhat stronger for regional/distant disease (OR, 4.47; 95% CI, 1.28–15.57; Table 4) versus local disease (OR, 2.98; 95% CI, 1.04–8.49), and more aggressive PC (OR, 4.06; 95% CI, 1.28–12.86; Table 5) versus less aggressive cancer (OR, 2.91; 95% CI, 0.99–8.55).

There were 82 cases who died of PC in an average 11.6 years of follow-up. We did not observe increased risk of prostate-specific mortality associated with the *HOXB13* mutation (HR, 0.94; 95% CI, 0.13–7.01; results not shown). There were 290 events of recurrence/progression in an average 8.5 years of follow-up. We did not find statistically significant evidence of increased risk for recurrence/progression (HR, 1.26; 95% CI, 0.46–3.43; results not shown). However, these analyses were limited as only one man with the mutation died of PC and only four men experienced recurrence/progression.

Discussion

Our data revealed that 1.3% of cases and 0.4% of controls from this population-based, case control study of PC carried the *HOXB13* G84E mutation, conferring a risk to carriers of 3.30 (95% CI 1.21–8.96). These numbers are in the range of what has been previously reported. Recall that other studies are focused on either family-based cohorts, hospital-based series and isolated populations. The original report cited a population of 5,083 unrelated subjects of self-reported European descent with PC in which 1.4% were carriers, compared to one in 1,401 (0.1%) controls (12). The finding of unaffected controls with the recurring mutation in our data set is not unexpected. Ewing et al. reported the existence of one HPC family in which a 70-year-old G84E carrier had not been diagnosed with PC. This may reflect the fact that the mutation is not completely penetrant, or that there is age-dependent penetrance.

Akbari et al. studying a set of cases and controls, aged 40–94 from two tertiary care centers in Canada, reported that 10 of 1,525 cases (0.7%) and two of 1,757 controls (0.1%) carried the G84E mutation (17). In a case-control study of familial PC at Vanderbilt University, Breyer and colleagues found that 16 of 858 familial PC probands (1.9%) and two of 825 (0.2%) controls without a personal or family history of PC carried the G84E mutation (14). In a separate series of 268 probands of European descent, the carrier rate was 1.5% (14).

These studies demonstrate that the mutation is rare in both the general and familial PC populations. But it does confer a significant elevation in risk. In the Akbari study, carriers had an increased risk of 5.8 (95% CI 1.3–26.5, P = .01) (17), and in the Breyer study it was 7.9 (95%CI, 1.8–34.5, P = 0.0062) (14). In the original study of Ewing et al., analysis of 5,083 unrelated case subjects and 1,401 controls revealed an OR of 20.1 (95% CI, = 3.5–803.3, $P = 8.5\times10^{-7}$)(12). All of the studies found only heterozygous carriers, which is not unexpected given the rarity of the mutation.

It will be interesting to assess the frequency of the mutation in various populations. The original study included seven African American families and two Asian families, but these were not reported separately. In two population-based case control studies in Sweden, Karlsson and colleagues analyzed 15 *HOXB13* polymorphisms in 5,003 cases and 4,693 controls (16). They found that the *HOXB13* G84E mutation is more frequent in the Swedish population (1.3% in population controls), and is associated with a 3.5-fold increased risk of PC. The strongest association was reported for younger-onset cases (OR, 8.6; 95% CI, 5.1–14.0) and for familial PC cases (OR, 6.6; 95% CI, 3.3–12.0) (16).

Recently, Lin et al. assayed the gene in a set of study subjects from the Chinese Consortium for Prostate Cancer Genetics (Chinese PCa) (15). They screened an initial set of 96 individuals and found putative disease associated mutations. These, including the G84E mutation, were then assessed in a stage two study that included 671 PC patients and 1,536 controls. Data were collected on an additional 751 cases by imputing mutations from haplotypes. Interestingly, the G84E mutation was not found at all in that study, but a novel mutation, G135E was found once among the 96 patients, and in two of the 575 additional patients in stage two, yielding a P value of 0.027. It was also found in two of the patients from stage three for whom the data were imputed, but not validated. It was not observed within any of the 1,536 controls. These data suggest that other recurring mutations may be found by examination of distinct populations.

One question of intense interest is how HOXB13 protein functions in the prostate and how the mutations might perturb the protein to affect disease risk. The *HOX* genes are a subfamily of the larger homeobox superfamily that is responsible for pattern formation across many species. In humans, there are four HOX gene clusters that are expressed at

precise times during development with a set regulating the anterior and proximal regions and another set, expressed later in development, that regulates the posterior and distal regions of the animal (for review: (23,24)). The HOX genes in the 13 group are expressed in the posterior region of developing vertebrates, including the urogenital system. Interestingly, *HOXB13* is one of few genes in the family to maintain a high level of expression during the life of the individual, with high expression levels observed in the normal prostate well into adulthood. HOXB13 is known to play a role in prostate cancer. Specifically, in androgen receptor negative prostate cancer cells, *HOXB13* is suppressed and overexpression of *HOXB13* results in significant inhibition of cell growth. In addition, *HOXB13* has been shown to suppress androgen-stimulated androgen receptor activity by interacting with the receptor (25). This means that *HOXB13* could serve as an androgen receptor interacting repressor to modulate hormone-activated androgen receptor signals (25).

The gene, which is itself 70Kb upstream of the major HOXB gene cluster, features two MEIS (myeloid ectropic viral integration site) protein-binding domains. The two MEIS are highly conserved among vertebrates (26,27) and their role is to fine tune HOX functions by regulating the interaction of HOX proteins with specific DNA molecules and proteins (12,15,28). Interesting the two recurring mutations found to date are both within MEIS binding domains. G84E is within the first domain, while G135E is within the second (15).

While there is no evidence as to how the mutations affect protein function, *HOXB13* has been considered as a marker of recurrent PC by at least one group of investigators (13). In their study, immunohistochemistry was used to examine 57 organ-confined PC tumors collected after radical prostatectomy. While there was no significant correlation between *HOXB13* expression and most clinical or pathological measures, gene expression did correlate with Gleason score and there was a positive correlation with pre-operative prostate specific antigen (PSA) levels, suggesting avenues for further study and validation. Although we examined the association of the *HOXB13* variant with risk of recurrence in the present study (HR 1.26; 95% CI, 0.46–3.43), there were only four instances of recurrence among men with the variant, limiting interpretation of the results.

While not significantly different, we did observe a stronger association with Gleason score ≥7 (OR 4.13, 95% CI 1.38–12.38) compared with Gleason score ≤ 7 (OR, 2.71; 95% CI, 0.88–8.302), with regional/distant stage disease (OR, 4.47; 95% CI, 1.28–15.57) versus local disease (OR, 2.98; 95% CI, 1.04–8.49), and with more aggressive (OR, 4.06; 95% CI, 1.28–12.86) versus less aggressive PC (OR, 2.91; 95% CI, 0.99–8.55). These risk estimates, however, are based on small numbers and further investigation of these clinical features of PC in relation to *HOXB13* mutation status is needed. While Breyer et al also noted this trend (14), Ewing and colleagues found no evidence of significant differences in Gleason grade between carriers and non-carriers, even accounting for age at diagnosis (12), although they did not present data that would allow for assessment of trend. Similarly, this information was not presented by Karlsson et al., (16).

The small number of mutation carriers observed limits generalization of our findings. Although there were 230 African American men in the present study, only one case had the *HOXB13* mutation. It is notable that none of the mutations reported by Ewing were in men who self identified as African American (12). We also only tested for the G84E mutation, as have other studies. At least four additional mutations have been reported (12). One useful exercise would be to take our most at risk cases and scan the entire gene for those additional mutations. Finally, the size of our carrier pool, and the fact that the data were from a population-based study, and not HPC families, also prohibited us from doing any type of penetrance analysis.

In this population-based, case-control study of PC, we found that while rare, as reported in other studies, the *HOXB13* G85E mutation is strongly associated with PC risk. There was also some suggestion that the association may vary with Gleason score, disease aggressiveness and stage, but additional studies and meta-analyses will be needed to confirm this observation. In addition, other mutations recurring in the general population remain to be determined.

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Table I

Distributions for selected characteristics among Caucasian prostate cancer cases and controls, King County, Washington, 1993–1996 and 2002–2005

Characteristic	Cases (n=1,310)		Controls (n=1,259)	
	N	%	n	%
Age (years)				
< 50	102	7.8	105	8.3
50–59	522	39.9	519	41.2
60–69	542	41.4	491	39.0
70–74	144	11.0	144	11.4
Family history of prostate cancer				
No	1,027	78.4	1,118	88.8
Yes	283	21.6	141	11.2
Number of PSA tests[a]				
None	290	22.1	445	35.4
1–2	321	24.5	239	19.0
3–4	244	18.6	153	12.2
≥ 5	392	29.9	225	17.9
Missing	63	4.8	197	15.7
PSA				
0 – 3.9	179	13.7	1,169	92.9
4 – 9.9	721	55.0	74	5.9
10 – 19.9	189	14.4	14	1.1
20+	118	9.0	2	0.2
Missing	103	7.9		
Stage of disease				
Local	1,020	77.9		
Regional	257	19.6		
Distant	33	2.5		
Gleason score				
Low (2–4)	70	5.3		
Moderate [5–7 (3+4)]	1,034	78.9		
High [7 (4+3), 8–10]	202	15.4		
Missing	4	0.3		
Cancer aggressiveness				
Less aggressive [b]	870	66.4		
More aggressive [c]	440	33.6		

[a] PSA tests done in the 5 years before reference date.

[b] Local stage, and Gleason score 2–6 or 7 (3 + 4), and diagnostic serum PSA <20 ng/mL.

[c] Regional/distant stage, or Gleason score 7 (4 + 3) or 8–10, or serum PSA ≥20 ng/mL.

Table II

Genotype distribution and odds ratios (ORs; 95% CI) for associations between *HOXB13* genotypes and prostate cancer risk

	Cases (n=1,310)		Controls (n=1,259)			
Genotype	**n**	**%**	**n**	**%**	**OR[a]**	**95% CI**
GG	1,293	(98.7)	1,254	(99.6)	1.00	(reference)
GA	17	(1.3)	5	(0.4)	3.30	(1.21–8.96)

[a]Adjusted for age.

Table III

Genotype distribution and odds ratios (ORs; 95% CI) for associations between *HOXB13* genotypes and prostate cancer risk, stratified by family history of prostate cancer

	Cases (n=1,310)		Controls (n=1,259)			
Genotype	**n**	**%**	**n**	**%**	**OR[a,b]**	**95% CI**
First-degree relative with prostate cancer						
GG	277	(97.9)	139	(98.6)	1.00	(reference)
GA	6	(2.1)	2	(1.4)	1.49	(0.30–7.50)
No first-degree relative with prostate cancer						
GG	1,016	(98.9)	1,115	(99.7)	1.00	(reference)
GA	11	(1.1)	3	(0.3)	4.04	(1.12–14.51)

[a] Adjusted for age.

[b] Likelihood ratio test for interaction by family history: p=0.36.

Table IV

Genotype distribution and ORs (95% CI) for associations between HOXB13 genotypes and prostate cancer risk stratified by Gleason score and stage

	Cases (n=1,310)		Controls (n=1,259)			
Genotype	**n**	**%**	**n**	**%**	**OR[a,b,c]**	**95% CI**
Gleason score ≤ 7[d] (n=750)						
GG	742	(98.9)	1,254	(99.6)	1.00	(reference)
GA	8	(1.1)	5	(0.4)	2.71	(0.88–8.30)
Gleason score ≥ 7[e] (n=556)						
GG	547	(98.4)	1,254	(99.6)	1.00	(reference)
GA	9	(1.6)	5	(0.4)	4.13	(1.38–12.38)
Local stage (n=1,020)						
GG	1,008	(98.8)	1,254	(99.6)	1.00	(reference)
GA	12	(1.2)	5	(0.4)	2.98	(1.04–8.49)
Regional/distant stage (n=290)						
GG	285	(98.3)	1,254	(99.6)	1.00	(reference)
GA	5	(1.7)	5	(0.4)	4.47	(1.28–15.57)

[a] Adjusted for age.

[b] Heterogeneity by Gleason score: p=0.39.

[c] Heterogeneity by stage: p=0.45.

[d] Includes Gleason 3+4.

[e] Includes Gleason 4+3.

Table V

Genotype distribution and ORs (95% CI) for associations between HOXB13 genotypes and prostate cancer risk stratified by aggressiveness

	Cases (n=1,310)		Controls (n=1,259)			
Genotype	**n**	**%**	**n**	**%**	**OR[a,b]**	**95% CI**
Less aggressive (n=870)						
GG	860	(98.8)	1,254	(99.6)	1.00	(reference)
GA	10	(1.2)	5	(0.4)	2.91	(0.99–8.55)
More aggressive (n=440)						
GG	433	(98.4)	1,254	(99.6)	1.00	(reference)
GA	7	(1.6)	5	(0.4)	4.06	(1.28–12.86)

[a] Adjusted for age.

[b] Heterogeneity by aggressiveness: p=0.50.