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Testing for the recurrent *HOXB13* G84E germline mutation in men with clinical indications for prostate biopsy

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Abstract

Purpose—The G84E variant of *HOXB13* was recently found to be associated with a significantly increased risk of prostate cancer in a case control study. We estimated the prevalence of this mutation in a clinical population of men at risk for prostate cancer scheduled to undergo prostate biopsy.

Materials and Methods—We prospectively collected clinical information and DNA samples from men undergoing diagnostic prostate biopsy between June 2005 and October 2011. We genotyped samples for *HOXB13* G84E using the MassARRAY system. We determined the prevalence of the G84E variant in the overall cohort, among patients with positive family history (FH), and among men age 55 years or younger.

Results—1175 subjects underwent biopsy, of which 948 had a DNA sample for analysis. Four patients had the G84E variant detected [prevalence 0.42%, 95% confidence interval (CI) 0.12% – 1.12%], of which three had prostate cancer on biopsy. None of 301 patients with a positive FH (prevalence 0.00%, 95% CI 0.00% – 1.52%) and one of 226 subjects age 55 years or younger tested positive (prevalence 0.44%, 95% CI 0.01% – 2.44%).

Conclusion—The *HOXB13* G84E variant is rare in this cohort, even in those with a positive family history. Our findings question the utility of testing for this variant among unselected men presenting for a diagnostic prostate biopsy.

Introduction

The ability to detect germline mutations associated with a high risk of developing cancer has transformed the screening of patients with familial colon, breast, and ovarian cancer. For

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Conflicts of interest: None.

example, in colon cancer, patients with a germline mutation confirming familial adenomatous polyposis or Lynch syndrome are recommended to start screening colonoscopy at a young age of ten to twelve years or 20 to 25 years, respectively.¹ Similarly, detection of mutations in either of the breast cancer susceptibility proteins one or two (BRCA1 or BRCA2) significantly alters recommendations for screening for breast and ovarian cancer. Depending on the patient's age, carrying mutations in *BRCA1* increases the relative risk for breast cancer up to ~60-fold and for ovarian cancer up to ~40-fold.² Current guidelines recommend magnetic resonance imaging-based screening to improve early detection and even prophylactic mastectomy or salpingo-oophorectomy to avoid development of breast cancer for these patients.^{3,4}

Though germline mutations associated with prostate cancer have been identified, they were not found to be of similar clinical utility.^{5–8} In 2012, a germline mutation (G84E) in *HOXB13*, a gene encoding transcription factor homeobox B13, was found to be associated with a significantly increased risk of early-onset (age 55 years or less) and hereditary prostate cancer.⁹ *HOXB13* has been shown to physically interact with the androgen receptor,^{10,11} providing a compelling rationale for its role as an oncogene.⁹ The effect of this mutation on cell development and differentiation in prostate cancer is currently being explored. In a case-control study of 5083 subjects with prostate cancer and 1401 control subjects, men with prostate cancer had significantly higher odds [odds ratio (OR) of 20.1; 95% confidence interval [CI], 3.5 to 803.3] of carrying the *HOXB13* G84E mutation than those without prostate cancer.⁹ Specifically, the prevalence of the *HOXB13* G84E mutation was 1.4% among the cases compared with 0.1% among the controls.⁹ However, subjects with prostate cancer in this study were highly selected, having either familial or early-onset prostate cancer or having undergone prostatectomy for clinically localized prostate cancer at Johns Hopkins University.⁹ These results may therefore not be readily applicable to a more general population of men being evaluated for prostate cancer.

Family history of prostate cancer is an important risk factor for prostate cancer and many studies have shown that a positive family history is independently associated with a 2 to 2.5 times increased risk of cancer on biopsy.^{12–14} In fact, the Prostate Cancer Prevention Trial risk calculator as well as current nomograms incorporate family history as one of their important predictors.^{15–17} Family history can be easily obtained in everyday clinical practice, but may be unreliable. Conversely, germline DNA testing for the *HOXB13* G84E variant is highly accurate,¹⁸ but would be associated with additional costs for the laboratory (approximately \$200).

In order to assess the potential clinical utility of testing for the *HOXB13* G84E mutation, we analyzed DNA samples in a cohort of men at risk for prostate cancer who were scheduled to undergo prostate biopsy. Our objectives were (1) to estimate the prevalence of this mutation and (2) to determine its association with a positive family history of prostate cancer in a clinical urologic population.

Patients and Methods

Patient population and clinical data

We prospectively collected clinical risk factors, biopsy outcomes, and buffy coat DNA from men undergoing prostate biopsy between June 2005 and October 2011 at the University of Michigan in an Institutional Review Board approved database and tissue repository (n=1413). All patients had given written informed consent for data and tissue collection prior to biopsy. We prospectively collected age at the time of biopsy, race (African American, white, or other), family history of prostate cancer (any relatives with prostate cancer, number of first or second degree relatives with prostate cancer, number of relatives who died of prostate cancer), digital rectal exam findings, number of previous biopsies, indication for biopsy, and pathologic findings (positive versus negative biopsy, primary and secondary Gleason grade). Patients were excluded if the prostate biopsy was performed for reasons other than primary detection of prostate cancer (n=238, Figure). Of the 1175 remaining subjects, 99% had 12 or more cores taken on biopsy. Eight men were excluded because they did not consent for obtaining a DNA sample and 208 men did not have a blood sample drawn (Figure).

Genotyping of *HOXB13* Variants

Blinded DNA samples were processed by the laboratory as follows: DNA was extracted from blood using the ActivePure DNA kit (5 Prime, Gaithersburg, MD). We genotyped the samples for *HOXB13* G84E using the MassARRAY system (Sequenom, San Diego, CA). The Sequenom panel included 17 additional genomic variants (data not shown) resulting in a total of 18 single nucleotide polymorphisms (SNPs). Samples that did not have a call for at least 16/18 (88.9%) SNPs were excluded from analysis (n=11, Figure). Samples that achieved the 88.9% call rate yet had missing data for *HOXB13* G84E were genotyped by Sanger sequencing (n=11). All G84E variants were verified by duplication on the Sequenom platform as well as by Sanger sequencing. In addition, a randomly selected subset of 100 additional samples (11%) was subjected to Sanger sequencing in a blinded fashion. Sequencing confirmed the MassARRAY results for all 100 samples.

Statistical analyses

We used the t-test, Wilcoxon rank-sum test, and chi-squared test to compare baseline characteristics between subjects with and without a DNA sample as well as between subjects with and without prostate cancer family history. We calculated the rate of positive family history and the carrier rate of the *HOXB13* G84E variant with 95% confidence intervals assuming a binomial distribution.¹⁹ Because the association of the *HOXB13* G84E variant with prostate cancer was especially strong among subjects with early onset prostate cancer (age 55 years or less) or with a positive family history in the prior study,⁹ we specifically calculated carrier rates in these subgroups. We assessed the association of family history with a finding of prostate cancer on biopsy in bivariable logistic regression and in multivariable logistic regression, adjusting for age, race, digital rectal exam findings, PSA, and number of previous biopsies.

In addition, we describe the characteristics of the subjects with the *HOXB13* G84E variant. We performed all analyses using Stata version 12SE. All tests were 2-tailed and we set the probability of a Type 1 error at 0.05 or less.

Results

Among the 1175 subjects who underwent a diagnostic prostate biopsy, mean age was 62.0 years, 275 men (23%) were 55 years old or younger, median PSA was 5.5 ng/ml, 86% were Caucasian, 18% had a suspicious rectal exam finding, 32% had a positive family history, and 72% had no prior prostate biopsy. The prostate biopsy revealed cancer in 487 men (41%). There were no significant differences between subjects with and without a DNA sample (Table 1).

Of the 948 men with a DNA sample, 301 men [32%, 95% confidence interval (CI) 29% – 35%] reported that one or more first and/or second degree relatives had been diagnosed with prostate cancer. Men with positive family history were younger and had lower PSA values than men without a family history of prostate cancer (Table 2). Having a positive family history was significantly associated with a finding of prostate cancer on biopsy in both bivariable (OR 1.44, 95% CI 1.09 – 1.89) and multivariable analyses (OR 1.88, 95% CI 1.39 – 2.55).

Four of the 948 subjects tested positive for the *HOXB13* G84E variant for a carrier rate of 0.42% [95% CI 0.12% – 1.12%, Table 3]. None of the 301 patients with a positive family history [carrier rate 0.00% (95% CI 0.00% – 1.52%)] and one of the 226 subjects age 55 years or younger [carrier rate 0.44% (95% CI 0.01% – 2.44%)] tested positive for the *HOXB13* G84E variant. Of the four patients with the *HOXB13* G84E variant, three had prostate cancer detected on biopsy, for a positive predictive value of 75% (3/4, table 3). All four patients were Caucasian and the patient with negative biopsy findings had undergone one previous negative biopsy (Table 3).

Discussion

Our prior work suggested that presence of the *HOXB13* G84E mutation was rare (0.1–0.2% carrier frequency) in the general population but associated with a significantly increased risk of prostate cancer.⁹ However, its role more broadly in prostate cancer detection has been untested. In this report, we found a low (0.42%) *HOXB13* G84E carrier rate in a cohort of men presenting for prostate biopsy for clinical indications, although one would expect a greater prevalence of this variant in this clinical cohort of patients suspected to have prostate cancer than in the general screened population. The carrier rate was surprisingly low even in the subset of men with a positive FH. Given this low carrier rate, testing for *HOXB13* G84E may be of limited value among unselected men presenting for a diagnostic prostate biopsy.²⁰

The *HOXB13* G84E variant is a germline mutation and therefore passed from generation to generation and present in all cells from birth. Testing for this mutation may identify a carrier who is at risk for the disease, but it remains unknown if and/or when a carrier will actually develop prostate cancer (i.e. mutation penetrance). This can be contrasted with testing for tumor-specific mutations. For example, detection of the *TMPRSS2:ERG* fusion product in

the urine is specific for the presence of prostate cancer cells within the prostate.²¹ Elevated levels of the TMPRSS2:ERG fusion product in urine were associated with clinically significant cancer at biopsy and prostatectomy.²² Incorporating a TMPRSS2:ERG and PCA3 risk score into the Prostate Cancer Prevention Trial risk calculator significantly improved its predictive accuracy and resulted in net clinical benefit in decision curve analyses.²²

Given the association of *HOXB13* G84E with familial prostate cancer, we evaluated the importance of family history in this cohort. Having at least one relative with a diagnosis of prostate cancer increased the odds of a positive biopsy finding by 1.89 in multivariable analysis, which is consistent with previous studies.^{12,13} Due to the much greater prevalence rate of positive family history (32%) and the ease with which it can be obtained, family history presently is a more valuable indicator for prostate cancer in clinical practice compared to testing for the extremely rare *HOXB13* G84E variant.

Similar to our findings, a study evaluating the association of 16 other SNPs with prostate cancer was limited by the low prevalence of the mutations.⁶ Men with 5 or more of these factors had an odds ratio of 9.46 for prostate cancer.⁶ However, the prevalence of 5 or more factors was very low (0.3% in the control group and 1.4% in the prostate cancer group) and severely limited test accuracy and clinical usefulness.^{7,20} It has been speculated that combining even more SNPs in models to predict cancer may ultimately provide important risk stratification. However, these models are unlikely to yield high discriminatory accuracy and will likely be limited by high false positive rates, thus limiting their usefulness for screening.²³

Our study has several limitations. Not all subjects biopsied during the study period had an analyzable DNA sample available. However, we did not find any significant differences between subjects with and without a DNA sample. Although we included almost thousand subjects in this study, the confidence intervals around our carrier rates are relatively wide. However, even if the carrier rate for the *HOXB13* G84E variant were around one percent, this low prevalence would still preclude clinical use in general urologic practice.

In summary, this study reports on the prevalence of the germline mutation *HOXB13* G84E in a large clinical cohort of subjects scheduled for prostate biopsy. The low carrier rate of this mutation questions its clinical utility in everyday practice. However, despite low carrier rates, understanding the biological and clinical implications of this germline mutation in individuals with familial and/or early-onset prostate cancer remains important and a focus of continued investigation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure.
Cohort selection

Table 1

Comparison of subjects with and without a DNA sample

	Without DNA sample n=227	With DNA sample n=948	p value
Age, mean (SD)	63.0 (9.2)	62.1 (8.5)	0.15 [*]
Age 55 years or younger, n (%)	49 (22)	226 (24)	0.47 ^{**}
Race, n (%)			0.14 ^{**}
Caucasian	187 (82)	826 (87)	
African-American	26 (11)	73 (8)	
Other	14 (6)	48 (5)	
Suspicious DRE, n (%)	34 (15)	171 (18)	0.27 ^{**}
PSA, median (IQR)	5.6 (3.9 – 8.5)	5.4 (4.0 – 8.0)	0.93 [†]
Positive FH, n (%)	73 (32)	301 (32)	0.87 ^{**}
Previous biopsy, n (%)			0.62 ^{**}
0	158 (70)	688 (73)	
1	39 (17)	153 (16)	
>1	30 (13)	107 (11)	
Cancer found on biopsy	100 (44)	387 (41)	0.38^{**}

PSA = prostate specific antigen; IQR = interquartile range; DRE = digital rectal exam; FH = family history;

^{*} t-test;^{**} chi-squared test;[†] Wilcoxon rank-sum test

Table 2

Characteristics of the four patients with the HOXB13 G84E mutation

	Patient 1	Patient 2	Patient 3	Patient 4
Age	73	57	63	55
Race	Caucasian	Caucasian	Caucasian	Caucasian
Suspicious DRE	No	No	No	No
PSA	10.5	17.2	8.8	5.6
Positive FH	No	No	No	No
Previous biopsy, n	1	0	0	0
Biopsy finding	Negative	Gleason 4+5=9	Gleason 4+3=7	Gleason 3+3=6 also HGPIN

DRE: digital rectal exam; FH: family history; HGPIN: high grade prostatic intraepithelial neoplasia