Research Article

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The *HOXB13* G84E Mutation Is Associated with an Increased Risk for Prostate Cancer and Other Malignancies

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Abstract

Background: A rare nonconservative substitution (G84E) in the *HOXB13* gene has been shown to be associated with risk of prostate cancer. DNA samples from male patients included in the Mayo Clinic Biobank (MCB) were genotyped to determine the frequency of the G84E mutation and its association with various cancers.

Methods: Subjects were genotyped using a custom TaqMan (Applied Biosystems) assay for G84E (rs138213197). In addition to donating a blood specimen, all MCB participants completed a baseline questionnaire to collect information on medical history and family history of cancer.

Results: Forty-nine of 9,012 male patients were carriers of G84E (0.5%). Thirty-one percent ($n = 2{,}595$) of participants had been diagnosed with cancer, including 51.1% of G84E carriers compared with just 30.6% of noncarriers ($P = 0.004$). G84E was most frequently observed among men with prostate cancer compared with men without cancer ($P < 0.0001$). However, the mutation was also more commonly observed in men with bladder cancer ($P = 0.06$) and leukemia ($P = 0.01$). G84E carriers were more likely to have a positive family history of prostate cancer in a first-degree relative compared to noncarriers (36.2% vs. 16.0%, $P = 0.0003$).

Conclusions: Our study confirms the association between the *HOXB13* G84E variant and prostate cancer and suggests a novel association between G84E and leukemia and a suggestive association with bladder cancer. Future investigation is warranted to confirm these associations in order to improve our understanding of the role of germline *HOXB13* mutations in human cancer.

Impact: The associations between *HOXB13* and prostate, leukemia, and bladder suggest that this gene is important in carcinogenesis. *Cancer Epidemiol Biomarkers Prev; 24(9); 1366–72. ©2015 AACR.*

Introduction

The *HOX* gene family belongs to a larger homeobox superfamily of transcription factors characterized by a highly conserved DNA-binding domain, or homeodomain. The 39 *HOX* genes are subdivided into four clusters (A, B, C, D) located on chromosomes 7 (7p15), 17 (17q21) 12 (12q13), and 2 (2q31), respectively, where each gene assigned to one of 13 paralog groups defined by their homeodomain. *HOX* genes play an important role in human embryonic development, and have been shown to promote proliferation of stem and progenitor cells (1). A number of the *HOX* genes have demonstrated proangiogenic properties directly and indirectly via increased expression of *FGF2*, *VEGFA*, *IL8*, and *CXCL1* suggesting a role in tumor progression; however, their exact role in the carcinogenic process is not well understood (2).

In 2012, a rare nonconservative mutation in the *HOXB13* gene was first reported to be associated with an increase in risk of prostate cancer (3). The mutation, a substitution of adenine for guanine in the second position of codon 84, results in the replacement of glutamic acid for glycine (G84E). The initial discovery was based on 94 families with hereditary prostate cancer (3+ first-degree relatives with confirmed disease) and evidence for linkage on 17q21-22 based on prior linkage analyses. In each of these 94 families, targeted sequencing of 202 genes in the region with the strongest evidence for linkage was performed in the youngest man with prostate cancer revealing near complete cosegregation between the G84E mutation with disease in four families. Subsequent genotyping of G84E (rs138213197) in a unique Caucasian sample of 5,011 men with prostate cancer and in 1,401 men without cancer determined an overall carrier frequency of 1.4% and that cases were 20 times more likely than controls to possess the mutation (OR, 20.1; $P = 8.5 \times 10^{-7}$). Numerous subsequent studies have confirmed this association and suggest furthermore that the mutation is more common among men with early-onset and familial or hereditary prostate cancer (4–13).

The function of *HOXB13* in prostate cancer is not well understood. The protein is known to be important in embryonic development of the prostate gland and is expressed in normal prostate tissue into adulthood. There is evidence that *HOX* genes

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may act as both an oncogene and a tumor suppressor gene in prostate as well as other cancers (14, 15), and *HOXB13* in particular has been shown to interact with the androgen receptor influencing both proliferation and differentiation of normal prostate and cancer cells (16).

The goal of the current investigation is to determine the overall carrier frequency of the G84E mutation in *HOXB13* and its association with prostate and other cancers among male participants of the Mayo Clinic Biobank (MCB.) The MCB is a large repository containing biospecimens, clinical, and questionnaire data from over 42,000 Mayo Clinic (Rochester, MN) patients. Because of the rarity of the *HOXB13* mutation, large studies like these are critical in estimating the contribution of this gene mutation to cancer risk.

Materials and Methods

Male patients enrolled in the MCB between April 2009 and March 2012 ($n = 9{,}012$) were included in the study, from a total of over 22,000 participants at that time (17). Participation in the MCB is voluntary and all participants provide written informed consent and a blood sample, and if applicable, archived tissue samples. Patients also completed a baseline questionnaire intended to collect information on demographics, personal and family medical history, health behaviors (including smoking, diet, and physical activity), environmental exposures, and self-assessed well-being. The MCB goal is to recruit 50,000 patients. Mayo Clinic patients aged 18 years and older, English-speaking, and U.S. residents are eligible to participate. The primary method of recruitment is by mailed invitation to any patient scheduled for an appointment in any of the following departments of the Mayo Clinic (general medicine, internal medicine, family medicine, preventive medicine, obstetrics and gynecology, and executive health) chosen specifically to represent a wide range of medical issues and conditions. As a major source of data for the MCB is patient medical records, all MCB participants also give permission to obtain electronic medical records (EMR) and paper records for approved studies. The EMR system has been in place since 1994 and the institution has been completely paperless since 2004, with uniform paper medical records as far back as 1907.

Each of the 9,012 men in the study provided information in the baseline self-administered survey on personal history of cancer as well as a family history of cancer among first-degree relatives (parents, siblings, and children). EMRs maintained by the Mayo Clinic Cancer Registry were used to both confirm self-reported cancers as well as identify incident cancer cases diagnosed after the baseline questionnaire. The Mayo Clinic Cancer Registry is primarily responsible for identifying all new invasive cancer cases diagnosed within the Mayo Clinic Rochester Health System through diagnostic (ICD9) billing codes. The MCB links with the cancer registry approximately every 6 months for identification of new cancer cases. For this study, incident cancer cases were identified through December 31, 2012. Approximately 85% of cancers in this study were diagnosed before MCB enrollment and for these patients, we relied on self-reported diagnoses; however, 95% of cancers reported were confirmed with EMR data. Our reliance on self-report any diagnosed without EMR confirmation allowed for the possibility of a cancer diagnosis either outside of the Mayo Clinic or before the present paperless system. Blood samples were obtained by venipuncture at any Mayo Clinic outpatient laboratory. DNA was extracted from whole blood on a STAR DNA extraction machine (Autogen Inc.). The samples were then genotyped for the G84E mutation in *HOXB13* using a custom TaqMan Assay (rs138213197). Both duplicate samples and empty wells were included on each plate and the concordance rate among duplicates was 100% with all empty wells giving no calls. Heterozygotes were confirmed using Sanger sequencing and Sequenom assays as described in Ewing and colleagues (3).

Statistical analysis

All analyses were conducted using Statistical Analysis Systems software (SAS Inc. v.9.2). The genotype frequencies for G84E were tested for and consistent with Hardy–Weinberg equilibrium ($P > 0.05$). We calculated the distribution of categorical parameters among all subjects and the median and range for all continuously measured parameters of interest. Carrier frequency was determined among all subjects. Fisher exact tests were used to compare the frequencies for select cancers between carriers and noncarriers of G84E, as there were small counts recorded for most cancers among carriers of the rare mutation. Exact logistic regression models were used to estimate the ORs associated with (i) personal history of cancer by tumor type and (ii) family history of cancer, both adjusted for age and excluding MCB participants who reported that they were adopted ($N = 168$; 1.9%) from analyses related to family history. ORs and *P* values were produced for only those cancers with at least one G84E affected mutation carrier (i.e., all cell sizes ≥ 1) and included leukemia, bladder, prostate, sarcoma, and testicular cancer for analyses examining a personal history of cancer and included leukemia, bladder, breast, colon, kidney, liver, lung, melanoma, pancreatic, prostate, stomach, thyroid cancer for analyses examining cancer family history. *P* values less than 0.05 were considered statistically significant.

Results

The 9,012 men in this study represent approximately 42.5% of the total patient population recruited during the first 3 years of the MCB. Key characteristics of the MCB male patient samples are presented in Table 1. The median age at time of enrollment and

Table 1. Demographic characteristics of the MCB male participants by *HOXB13* carrier status

Characteristic	***HOXB13* carrier (G84E) *N* (%)[a]**	**Noncarrier (WT) *N* (%)**
Total	49	8,963
Age at recruitment (survey)		
20–29	0 (0.0)	196 (2.2)
30–39	2 (4.1)	365 (4.1)
40–49	4 (8.2)	766 (8.5)
50–59	11 (22.4)	1,804 (20.1)
60–69	13 (26.5)	2,412 (26.9)
70–79	12 (24.5)	2,371 (26.5)
≥ 80	7 (14.3)	1,049 (11.7)
Race		
White	48 (98.0)	8,511 (95.0)
Black	0 (0.0)	53 (0.6)
Other/unknown	1 (2.0)	399 (4.5)
Adoption status		
Yes	1 (2.0)	167 (1.9)
No	47 (95.9)	8,609 (96.1)
Unknown	1 (2.0)	187 (2.1)
	Median (range)	**Median (range)**
Age	65 (31–90)	66 (20–100)

Abbreviation: WT, wild-type.

[a]Percentages may not sum to 100 due to rounding.

Table 2. Cancer diagnoses among MCB male participants ($N = 9{,}012$) by *HOXB13* carrier status

Tumor type	***HOXB13* G84E carriers ($n = 49$) *N* (%)[c]**	***HOXB13* G84E noncarriers ($n = 8{,}963$) *N* (%)**	***P*[a]**	**OR[b] (95% CI)**
Any cancer				
Yes	24 (51.1)	2,587 (30.6)	**0.004**	**1.60 (1.14–2.24)**
No	23 (48.9)	5,875 (69.4)		
Missing[d]	2	501		
Any cancer (excluding prostate)				
Yes	5 (17.9)	1,198 (16.9)	0.80	1.08 (0.58–1.81)
No	23 (82.1)	5,875 (83.1)		
Missing	21	1,890		
Bladder				
Yes	3 (11.5)	205 (3.4)	0.06	1.99 (0.84–3.86)
No	23 (88.5)	5,875 (96.6)		
Missing	23	2,883		
Leukemia				
Yes	3 (11.5)	86 (1.4)	**0.01**	**3.17 (1.35–6.03)**
No	23 (88.5)	5,875 (98.6)		
Missing	23	3,002		
Prostate				
Yes	19 (45.2)	1,343 (18.6)	**<0.0001**	**1.99 (1.37–2.90)**
No	23 (54.8)	5,875 (81.4)		
Missing	7	1,745		
Sarcoma				
Yes	1 (4.2)	123 (2.1)	0.4	1.48 (0.23–3.80)
No	23 (95.8)	5,875 (97.9)		
Missing	25	2,965		
Testis				
Yes	1 (4.0)	49 (0.8)	0.18	2.31 (0.36–5.86)
No	24 (96.0)	5,888 (99.2)		
Missing	24	3,026		

[a]*P* value from Fisher's exact method.
[b]Estimated OR adjusted by age and calculated for only cancers where all cell sizes ≥ 1 using exact logistic regression.
[c]Percentages may not sum to 100 due to rounding.
[d]Missing = excluded or unknown values: Most missing data are individuals excluded from particular analyses because of diagnosis of another cancer (other than the cancer of interest).

baseline survey was 66 years (range 20–100 years). The majority of study participants were of European ancestry (95%), reflective of the Mayo Clinic patient population. Other demographic and behavioral characteristics of MCB patient population not considered in present analysis have been previously reported (17). Forty-nine men were discovered to be carriers of the G84E mutation, representing 0.5% of the total population and was found almost exclusively among patients of known European ancestry ($N = 48$). A single carrier was of unknown ancestry; however, given the composition of the patient population, it may be inferred that this patient was also European.

Thirty-one percent of participants had been diagnosed with cancer ($N = 2{,}611$). Excluding nonmelanoma skin cancer, the five most frequent cancers diagnosed were prostate ($n = 1{,}362$ or 52.2% of all invasive cancers diagnosed), melanoma ($n = 496$; 19.2%), bladder ($n = 208$; 8%), Hodgkin disease or non-Hodgkin lymphoma [$n = 160$ or (6.2%)], and kidney cancer ($n = 137$; 5.3%). *HOXB13* carriers were significantly more likely to have a personal history of invasive cancer (51.1%) compared with noncarriers (30.6%; OR, 1.60; 95% CI, 1.14–2.24; Table 2). This association was largely driven by its association with prostate cancer. When prostate cancers are excluded from the analyses, we observed no association between the mutation and all other (nonprostate cancers; OR, 1.08; 95% CI, 0.58–1.81).

The G84E mutation was most frequently observed among men with prostate cancer with a carrier frequency of 1.4% compared with 0.4% of men with no history of any invasive cancer ($P < 0.0001$) and an elevated OR of 1.99 (95% CI, 1.37–2.90). Furthermore, among the prostate cancer cases, there was some suggestion that mutation carriers were more likely diagnosed with earlier-onset disease (<60 years at diagnosis) and more likely to have a positive family history of prostate cancer. However, due to the small number of mutation carriers, tests of statistical significance were not performed (Table 3). The G84E mutation was also significantly more common among patients diagnosed with leukemia (OR, 3.17; 95% CI, 1.35–6.03) and there was some suggestion of an association with bladder cancer; (OR, 1.99; 95% CI, 0.84–3.86; Table 2). In addition, we found no association between the mutation and the occurrence of multiple primary cancers in these men (OR, 1.13; 95% CI, 0.48–2.16). The only significant association observed between family history of cancer among first-degree relatives and the G84E mutation was for prostate cancer, as 36.2% mutation carriers had a positive family history in a father, brother, or son compared with just 16.0% of noncarriers ($P = 0.0003$; Table 4).

Discussion

Our results indicate that the G84E mutation in the *HOXB13* gene occurs in approximately 0.5% in a population primarily of European ancestry and confirms earlier reports that the mutation is associated with prostate cancer. We also report that leukemia and bladder cancer were more commonly diagnosed in G84E mutation carriers compared with noncarriers, both of these

Table 3. Select clinical characteristics among MCB *HOXB13* carriers and noncarriers diagnosed with prostate cancer

Characteristic	***HOXB13* G84E carriers ($n = 49$) *N* (%)**	***HOXB13* G84E noncarriers ($n = 8{,}963$) *N* (%)**
Total # cases	17	1,073
Age at diagnosis		
<60	4 (23.5)	108 (10.1)
≥60	13 (76.5)	965 (89.9)
Family history[a]		
Yes	5 (31.3)	296 (28.7)
No	9 (56.3)	555 (53.9)
Unknown	2 (12.5)	179 (17.4)
Stage		
Localized	14 (82.4)	857 (79.9)
Regional	2 (11.8)	160 (14.9)
Distant	0 (0.0)	5 (0.5)
Unknown	1 (5.9)	51 (4.8)
Grade differentiation		
Well/moderate	10 (58.8)	708 (66.0)
Poor/undifferentiated	6 (35.3)	343 (32.0)
Unknown	1 (5.9)	22 (2.1)
Pathologic tumor stage		
≤T2	13 (76.5)	563 (52.5)
≥T3	2 (11.8)	126 (11.7)
Unknown	2 (11.8)	384 (35.8)

[a]Among any first-degree family member (father, brother, and son).

findings deserve confirmation in an independent population and further investigation to improve our understanding of the role of *HOXB13* in carcinogenesis.

With the discovery of the G84E variant as a potential prostate cancer susceptibility gene in early 2012 (3), 16 studies have been published which confirm and attempt to quantify its contribution to risk of prostate cancer (4–13, 18–23). The reported estimates of overall risk vary between these studies from 3.3 to 8.8, and with one exception (5), studies report higher estimates for early-onset and familial prostate cancer. A recent meta-analysis of 11 of these studies reported that carriers of the G84E mutation are approximately 4.5 times more likely to be diagnosed with prostate cancer compared with noncarriers with higher risks among those with early-onset (OR, 9.73; 95% CI, 6.57–14.39), familial (OR, 7.27; 95% CI, 4.02–13.15), and aggressive disease (OR, 5.81; 95% CI, 3.72–9.08; ref. 23). However, other studies provide mixed evidence that the mutation is preferentially associated with aggressive clinical features (9, 10, 13, 21). Furthermore, the mutation frequency reported in this patient population for both prostate cancer cases and men without cancer is in line with prior reports which range from 0.1% to 1.4% in men without cancer and from 0.1% to 4.6% in prostate cancer cases (3–6, 10, 11, 13). The G84E mutation is suspected to be moderately penetrant with variable estimates of cumulative risk of prostate cancer (4, 20). An analysis by MacInnis and colleagues suggests that for a man with the *HOXB13* G84E mutation born in 1950, the cumulative risk of prostate cancer is 19% (95% CI, 5%–46%) by the time he reaches age 60 and 60% (95% CI, 30%–85%) by age 80 (20). The G84E mutation has been observed almost exclusively in men of Northern and Western European ancestry and has been predicted to have occurred between the middle of the 18th century and early 19th century in Northern Europe (11).

Unlike prostate cancer, the association between the G84E mutation and other tumor types has not been well established. In a case–control study conducted by Laitinen and colleagues, an association between the G84E variant and breast cancer of borderline significance (OR, 3.2; 95% CI, 0.9–11.9) was reported in a subset of 86 familial cases that tested negative for *BRCA1/2* mutations. In this same study, no relationship was reported between G84E and colorectal cancer (9). The association with familial breast cancer is supported by the results of Alanee and colleagues reporting an OR of 5.7 (95% CI, 1.0–40.7) among women of non-Ashkenazi ancestry with no mutation in *BRCA1/2* (24). However, studies by Akbari and colleagues suggest that the mutation is significantly associated with colorectal cancer (OR, 2.8; 95% CI, 1.2–6.8), but not breast cancer (OR, 1.2; 95% CI, 0.3–4.1; refs. 25, 26). The fact that the latter investigation's case group was comprised of nearly 75% of women considered to have sporadic breast cancer may explain this discrepancy (26). *HOXB13* has been shown to be overexpressed in breast cancer tissue and in an environment with low expression of *IL17BR* has been predicted to impair estrogen receptor (ER) signaling and resistance to tamoxifen among women with ER^+ breast cancer (27, 28). Finally, a recent large study in the Kaiser Permanente Research Program on Genes, Environment and Health (RPGEH) investigating the role of G84E in *HOXB13* with risk of cancer further confirmed that the mutation was associated with prostate cancer (OR, 3.63; 95% CI, 2.48–5.85) as well as a number of other tumors, including bladder (OR, 2.84; 95% CI, 0.70–8.94). The investigators relied upon imputation of the G84E variant using data from multiple sources. Imputation is an established approach for more common genetic variants, but has been used less frequently for rare variants. Internal validation in a subset of 3,462 men genotyped for G84E suggested moderate correlation between the imputed value and genotype ($r^2 = 0.57$; ref. 18).

Our novel observation of an association between the G84E mutation in *HOXB13* with leukemia as well as the association with bladder cancer in this study and others (18) deserve further examination and confirmation, yet there are biologic data to support a role for the gene with both tumors. A number of *HOX* genes are expressed hematopoetic stem and progenitor cells and generally result to an expansion of such cells without differentiation (1). In addition, while *HOXB13* has not been specifically implicated in the development of acute leukemia, a number of *HOX* genes, most notably *HOXA9* and *HOXB3*, are overexpressed or dysregulated in acute myeloid leukemia (AML) and acute lymphoid leukemia (1, 29). Indeed, overexpression of *HOXA9* in murine bone marrow results in AML, a process that is accelerated if the HOX cofactor, MEIS1, is also overexpressed. In bladder cancer, *HOXB13* is overexpressed compared with normal bladder tissue and higher still in transitional cell tumors with muscle invasion compared with nonmuscle invasive tumors (30). *HOXB13* overexpression is also associated with shorter disease-free survival in bladder cancer (30, 31). Interestingly, bladder and prostate are derived from the same embryologic structure, the urogenital sinus (32).

The strengths of the current investigation primarily lie in the MCB resource. The large patient population allowed for the investigation of this rare mutation with cancer. This project was limited to male participants in an effort to confirm the G84E association with prostate cancer. However, future studies will include females from the biobank to further examine the more novel findings with bladder and leukemia as well as address the inconsistency of findings in breast cancer. In addition, we used EMR data to classify the outcome and validate self-reported cancer diagnoses. More than 50% participants have more than 15 years of EMR data (17). The overall concordance between self-report

Table 4. Family history of cancer among first-degree relatives in MCB male participants by *HOXB13* carrier status

Characteristic	***HOXB13* G84E carriers (*n* = 47)** ***N* (%)[c]**	***HOXB13* G84E noncarriers (*n* = 8,609)** ***N* (%)**	***P*[a]**	**OR[b] (95% CI)**
Any cancer				
Yes	32 (68.1)	5,314 (61.7)	0.15	1.30 (0.89–1.98)
No	10 (21.3)	2,829 (32.9)		
Unknown	5 (10.6)	466 (5.4)		
Bladder				
Yes	2 (4.3)	278 (3.2)	0.67	1.13 (0.38–2.24)
No	39 (83.0)	7,113 (82.6)		
Unknown	6 (12.8)	1,218 (14.1)		
Breast				
Yes	11 (23.4)	1,423 (16.5)	0.16	1.26 (0.85–1.82)
No	29 (61.7)	6,174 (71.7)		
Unknown	7 (14.9)	1,012 (11.8)		
Colon				
Yes	2 (4.3)	1,037 (12.0)	0.16	0.58 (0.20–1.14)
No	36 (76.6)	6,464 (75.1)		
Unknown	9 (19.1)	1,108 (12.9)		
Kidney				
Yes	1 (2.1)	226 (2.6)	1.0	0.91 (0.14–2.21)
No	38 (80.9)	7,185 (83.5)		
Unknown	8 (17.0)	1,198 (13.9)		
Leukemia				
Yes	1 (2.1)	348 (4.0)	1.0	0.71 (0.11–1.73)
No	39 (83.0)	7,136 (82.9)		
Unknown	7 (14.9)	1,125 (13.1)		
Liver				
Yes	2 (4.3)	391 (4.5)	1.0	0.97 (0.33–1.91)
No	37 (78.7)	7,025 (81.6)		
Unknown	8 (17.0)	1,193 (13.9)		
Lung				
Yes	6 (12.8)	1,038 (12.1)	0.82	1.02 (0.60–1.58)
No	35 (74.5)	6,511 (75.6)		
Unknown	6 (12.8)	1,060 (12.3)		
Melanoma				
Yes	3 (6.4)	631 (7.3)	1.0	0.97 (0.43–1.73)
No	34 (72.3)	6,640 (77.1)		
Unknown	10 (21.3)	1,338 (15.5)		
Pancreas				
Yes	1 (2.1)	365 (4.2)	1.0	0.96 (0.11–1.70)
No	39 (83.0)	7,124 (82.8)		
Unknown	7 (14.9)	1,120 (13.0)		
Prostate				
Yes	17 (36.2)	1,381 (16.0)	**0.0003**	**1.82 (1.28–2.56)**
No	22 (46.8)	6,039 (70.1)		
Unknown	8 (17.0)	1,189 (13.8)		
Stomach				
Yes	1 (2.1)	341 (4.0)	1.0	0.71 (0.11–1.74)
No	39 (83.0)	7,117 (82.7)		
Unknown	7 (14.9)	1,151 (13.4)		
Thyroid				
Yes	1 (2.1)	215 (2.5)	1.0	0.92 (0.14–2.24)
No	39 (83.0)	7,198 (83.6)		
Unknown	7 (14.9)	1,196 (13.9)		
Any other cancer				
Yes	2 (4.3)	629 (7.3)	0.77	0.77 (0.26–1.53)
No	34 (72.3)	6,588 (76.5)		
Unknown	11 (23.4)	1,392 (16.2)		

[a]*P* value from Fisher's exact method and excluding unknown values.
[b]Estimated OR adjusted by age and calculated only for tumors where all cell sizes $\geq$1 using exact logistic regression.
[c]Percentages may not equal 100 due to rounding.

and medical record data was high. However, there was some discordance, as 5.8% of patients reported a cancer diagnosis that was not confirmed by EMR. We allowed for this because of the possibility that a patient may have been diagnosed either outside of the Mayo Clinic Health System or may have been diagnosed before available EMR and paper records were not reviewed for this study. Furthermore, the data gathered from the baseline questionnaire were reviewed for logic errors and omissions and any questionnaire with 10+ of such errors were returned to the participant for correction. All blood specimens were processed

in a central laboratory with a uniform protocol for DNA extraction and storage. Limitations of the study include the fact that the MCB is not population based and likely not representative of the general U.S. adult population. In fact, an analysis of characteristics of the MCB data compared with the Behavioral Risk Factor and Surveillance Survey (BRFSS) data of U.S. adults suggests that MCB participants are older, heavier, more educated, and have a higher proportion of patients of European ancestry than the general population (17). Furthermore, the response rate among all those invited to participate was 29%, with 15% refusing to participate and the remaining approximately 55% unresponsive to the invitation. Ridgeway and colleagues reported that age, gender, region of residence, and race/ethnicity were all significant predictors of participation. It is unclear whether and how these differences might affect the results of the current study (33). There is also potential for individual participants to be biologically related to one another and relatedness among MCB participants would be difficult to determine from data provided by participants. Relatedness among participants would have the effect of overestimating the overall prevalence of variants in the population, but less likely to have an impact on any associations with cancer, particularly given the rarity of the mutation and the relative rarity of the outcomes under investigation. Moreover, 9.1% of the sample did not report cancer on their baseline survey, but had evidence of a cancer diagnosis from EMR data. However, it is entirely possible that these diagnoses could have occurred post-baseline interview and therefore are not likely to be errors of omission. We relied on self-reported cancer family history for this report and there is a possibility of misclassification bias produced by differential recall of family history in men with a history of cancer compared men without cancer, yet evidence suggests that self-reported cancer family history is generally accurate, particularly among first-degree relatives (34). Because family history of cancer was assessed only at baseline, any changes in cancer family history were not captured in this analysis. Finally, for less common tumors, the study was limited in its ability to generate precise estimates of their association with *HOXB13*.

Conclusions

Our study confirms prior reports of the association between the rare missense mutation G84E in the *HOXB13* gene and prostate cancer in a large, clinical sample of men with and without cancer. Novel associations were discovered with both bladder cancer and leukemia reinforcing the importance of this gene in carcinogenesis, but need to be replicated. This may be accomplished in future meta-analyses for rarer tumors with companion bench studies to understand the contribution of *HOXB13* to human cancer.

Disclosure of Potential Conflicts of Interest

W.B. Isaacs has ownership interest in a patent on HOXB13 as a genetic marker of prostate cancer risk, owned by Johns Hopkins and University of Michigan. K.A. Cooney has ownership interest in a patent application. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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