Prevalence of the HOXB13 G84E prostate cancer risk allele in men treated with radical prostatectomy

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Objective
• To determine the prevalence and clinical correlates of the G84E mutation in the homeobox transcription factor, or HOXB13, gene using DNA samples from 9559 men with prostate cancer undergoing radical prostatectomy.

Patients and Methods
• DNA samples from men treated with radical prostatectomy at the University of Michigan and John Hopkins University were genotyped for G84E and this was confirmed by Sanger sequencing.
• The frequency and distribution of this allele was determined according to specific patient characteristics (family history, age at diagnosis, pathological Gleason grade and stage).

Results
• Of 9559 patients, 128 (1.3%) were heterozygous carriers of G84E.
• Patients who possessed the variant were more likely to have a family history of prostate cancer than those who did not (46.0 vs 35.4%; P = 0.006).
• G84E carriers were also more likely to be diagnosed at a younger age than non-carriers (55.2 years vs 58.1 years; P < 0.001).
• No difference in the proportion of patients diagnosed with high grade or advanced stage tumours according to carrier status was observed.

Conclusions
• In the present study, carriers of the rare G84E variant in HOXB13 were both younger at the time of diagnosis and more likely to have a family history of prostate cancer compared with homozygotes for the wild-type allele.
• No significant differences in allele frequency were detected according to selected clinical characteristics of prostate cancer.
• Further investigation is required to evaluate the role of HOXB13 in prostate carcinogenesis.

Keywords
HOXB13, prostate cancer, family history, genetic epidemiology

Introduction
The findings from epidemiological studies indicate that there is a familial component to prostate cancer. Family history, particularly among first-degree relatives, is a strong risk factor for prostate cancer with an ~2- to 2.5-fold increase in risk associated with a positive history of disease. Risk has been shown to increase with the number of affected relatives and is inversely related to the age at diagnosis among those relatives [1]; however, the specific genes that explain the observed associations have largely eluded researchers for decades.

Recently, we discovered a rare, recurrent mutation in the HOXB13 gene, G84E, which was associated with a significant increase (~10- to 20-fold) in the risk of prostate cancer. The frequency of the variant was 1.4% in all men with prostate cancer in the study and increased to 3.1% among men diagnosed with early-onset and familial prostate cancer [2].

The homeobox transcription factor gene HOXB13, located on the long arm of chromosome 17 (17q21), belongs to a superfamily of genes considered critical to animal embryonic development and characterized by a highly conserved
DNA-binding domain. *HOXB13* is thought to play a role in the development of the prostate gland; however, the expression of its protein remains elevated into adulthood. The mechanism whereby *HOXB13* influences prostate carcinogenesis is currently unknown, but it is certain to be an area of intense investigation.

A number of studies have subsequently confirmed the presence of the G84E mutation and its association with familial and hereditary prostate cancer [3–8]. In the present investigation, we set out to determine the prevalence of G84E in a large case series of men with prostate cancer undergoing radical prostatectomy at one of two institutions, representing a population of men with both sporadic and familial disease. Because of the relative rarity of the mutation, this large sample of patients enables us to examine clinical characteristics that influence survival by carrier status, which may provide valuable clues as to the function of the gene in prostate cancer.

**Patients and Methods**

**Study Subjects**

Eligible patients were diagnosed at any age with primary prostate cancer, histologically confirmed, and treated with radical prostatectomy at either the University of Michigan Medical Center (UM) (*n* = 1511) between 1999 and 2012 or Johns Hopkins University Hospital (JHU) (*n* = 8048) between 1993 and 2012. Note that a proportion (39%) of these patients was also included in our initial report describing the *HOXB13* G84E mutation in men with prostate cancer [2]. Demographic and clinical information collected on each patient included date of and age at diagnosis (UM), date of and age at radical prostatectomy (UM and JHU), race, family history of prostate cancer, pathological Gleason grade, pathological (TNM) stage, and surgical margin status. Patients were followed passively for disease recurrence, additional treatment(s), and vital status through 2012. The protocol and consent documents were approved by the institutional review boards at each institution.

**Genotyping Methods**

DNA was extracted from whole blood using standard methods. Samples were genotyped using the MassARRAY system (Sequenom) or Taqman assay (rs138213197; Applied Biosystems, Foster City, CA, USA). All G84E mutation carriers who were identified on either platform and a randomly selected subset of samples (~10%) were subjected to duplicate genotyping using Sanger sequencing in a blinded fashion, with 100% concordance among duplicate samples.

**Statistical Analysis**

All analyses were conducted using SAS software (SAS Inc. v.9.2, Cary, NC, USA). The genotype frequencies for G84E were tested for and consistent with Hardy–Weinberg equilibrium (*P* > 0.05). We calculated the distribution of categorical variables among all subjects and the median and range for all continuously measured variables of interest. As there were no homozygotes for the variant allele, the frequency of carriers was determined among all subjects. Simple chi-squared tests were used to compare the frequencies for select characteristics between carriers and non-carriers of G84E. Breslow–Day chi-squared tests were used to evaluate the potential for effect modification by age at diagnosis on the association between genotype and both family history and Gleason sum. *P* values < 0.05 were considered to indicate statistical significance.

**Results**

Selected characteristics and the genotype frequencies for G84E of the 9559 patients with prostate cancer included in our investigation are shown in Table 1. Age at diagnosis was available for 98% of UM patients; the median (range) age was 59 (38–77) years. For the remaining 2% of patients with missing information, we used age at surgery to obtain an approximate age at diagnosis as there was a mean difference of just 4 months between age at diagnosis and age at surgery in the UM series. Age of surgery was available for JHU patients; the age distribution was similar to that of the UM series (median [range] 58 [33–77] years). Approximately 85% of UM patients and 88% of JHU patients were white, and ~36% of patients at each institution had a documented family history of prostate cancer in a first- or second-degree relative. A greater proportion of UM patients had high (4 + 3 and higher) Gleason grade tumours, and elevated pre-treatment PSA (≥10 ng/mL) compared with JHU patients. Conversely, a greater proportion of JHU patients were diagnosed with non-organ-confined disease (≥T3a) compared with UM patients. The G84E carrier rate was 1.3% among all patients, with no appreciable difference in the proportion of patients possessing the risk allele between the JHU (1.4%) and the UM group (1.1%). No homozygous carriers were detected. As mutation carriers were almost exclusively white (two patients of unknown race were carriers), the remaining analysis was restricted to the 8341 white patients in the study.

Table 2 shows the associations between the G84E genotype and selected clinical characteristics at the time of diagnosis. G84E carriers in the present study were more likely to be diagnosed at a younger age compared with non-carriers (55.2 vs 58.1 years; *P* < 0.001). Patients who possessed the variant were also more likely to have a family history of prostate cancer (46.0 vs 35.4%; *P* = 0.006). Regression model analysis of the probability of being diagnosed with early-onset prostate cancer (≤60 years), mutually adjusting for both G84E genotype and family history, we observed a nearly twofold increase in the odds of early-onset disease associated with the mutation (odds ratio [OR] 1.90; 95% CI 1.23–2.94), which was slightly higher than the OR detected for family history (OR 1.45; 95%
CI 1.32–1.59). No difference was observed in the proportion of patients with high grade tumours by carrier status (16.7% of carriers vs 17.5% of non-carriers had tumours with Gleason sum ≥ 4 + 3; P = 0.95). Likewise, there was no difference in the proportion of patients diagnosed with advanced stage (≥pT3a) tumours by carrier status (24.6 vs 27.5%; P = 0.52). Finally, our analysis of the various relationships between carrier status and clinical measures of interest did not provide evidence for effect modification between G84E and family history of disease on age at diagnosis, tumour stage or Gleason sum.

### Discussion

The results of the present study confirm the presence of the prostate cancer susceptibility allele HOXB13 G84E variant in a subset of men with prostate cancer. The observed prevalence of the risk allele (1.3%) among all patients was consistent with previous estimates [4,6], including our own [2]. G84E carriers were more likely to have been diagnosed at a younger age and have a positive family history of prostate cancer compared with non-carriers; however, we did not observe any difference in the distribution of Gleason grade or tumour stage between carriers and non-carriers of the G84E mutation.

Our research team was the first to report the association between HOXB13 and prostate cancer [2] with the initial discovery of the G84E variant as a result of targeted sequencing of 202 genes in a 15.5 Mb candidate region on 17q21–22. This region was identified in a linkage analysis of pedigree data from 175 families with hereditary prostate cancer participating in the University of Michigan Prostate Cancer Genetics Project (PCGP) [9]. The probands (the youngest case with DNA) from four families (three from PCGP and one from JHU) with the strongest evidence for linkage in this region were all observed to harbour a substitution G→A in the second position of codon 84 resulting in the replacement of glycine by glutamic acid. Subsequent genotyping of family members observed near complete cosegregation of the mutation with disease among affected relatives with just one unaffected carrier of G84E. Finally, an OR of 20.1 (P = 8.5 × 10⁻⁷), and a carrier frequency of 1.4% among cases, was reported in a case–control study of PCGP and JHU [2].

In our initial report examining clinical characteristics and their relationship to G84E carrier status, we included all cases available to us, including a large number of cases from the hereditary prostate cancer studies at each of our institutions. In the present report, we included men presenting for radical prostatectomy, not specifically selected for early-onset and/or family history of disease.

The International Consortium for Prostate Cancer Genetics (ICPCG) confirmed the presence of G84E in 112 (4.6%) of the 2443 families (all of European descent) participating in the consortium. After exclusion of PCGP and JHU participants, an OR for prostate cancer of 4.3 (95% CI 2.32, 7.96) was reported among G84E carriers in the remaining ICPCG families [5]. Several studies have reported measures of association of prostate cancer with G84E ranging from 3.3 to 8.8 [3.4, 6–8, 10, 11] and, with one exception [4], stronger estimates among men with early-onset, familial and hereditary prostate cancer. Stott-Miller et al. [4], observed a fourfold increase in
the likelihood of diagnosis associated with G84E among men with no family history of prostate cancer, but only a 1.5-fold increase among men with a positive family history in a first-degree relative. The same investigation suggested the risk allele was also associated with higher grade and advanced stage disease, a finding not replicated by others including the present study [10,12]. A recently published meta-analysis of G84E and prostate cancer risk in European Americans (including 24 213 cases and 73 631 controls) reported a pooled OR of 4.07 (95% CI 3.05–5.45) with an overall carrier frequency of 1.4% [10,11]. Akbari et al. [7] observed a significant difference in the frequency of G84E between prostate cancer cases and controls in a large Canadian study; the prevalence was <1% in both groups (0.7 and 0.1%, respectively) in that study. Work from the ICPCG identified a common haplotype (GG) associated with prostate cancer among Chinese men [17]. In our previous investigation of G84E in a prostate biopsy series at the UM, the carrier rate was lower than expected: just 4/948 men (0.42%). Despite the fact that 3/4 men were subsequently diagnosed with prostate cancer (positive predictive value of 0.75%), we suggested there would be limited utility in testing for G84E, because of its rarity in the general population [14].

Studies have also examined the association between the G84E mutation and the risk of breast and colon cancer, with some inconsistency in their findings. While Akbari et al. [15] observed no association between the variant and breast cancer (positive predictive value of 0.75%), we suggested there would be limited utility in testing for G84E, because of its rarity in the general population [14].

In addition to G84E, additional rare HOXB13 mutations have been detected by our group [2] and others [7]. Recently, a novel rare mutation in HOXB13 (G135E) was reported to be associated with prostate cancer among Chinese men [17]. In that case series (n = 96), the entire coding region of the gene was sequenced and a single patient was observed to have the mutation. A subsequent case–control study of 671 cases and 1536 controls identified two additional carriers of G135E, both cases. The variant, observed in the second of two highly conserved myeloid ecotropic viral integration site (or MEIS)-binding domains on exon 1 of the gene (G84E is

Table 2 Comparison of Hoxb13 gene carriers with non-carriers according to selected prostate cancer characteristics.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>UM</th>
<th>JHU</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG, n (%)</td>
<td>GA, n (%)</td>
<td><strong>P</strong></td>
</tr>
<tr>
<td>Total no. of patients**</td>
<td>1274</td>
<td>15</td>
<td>0.43</td>
</tr>
<tr>
<td>Age at diagnosis/surgery ≤60 years</td>
<td>721 (56.6)</td>
<td>10 (66.7)</td>
<td>0.0004</td>
</tr>
<tr>
<td>&gt;60 years</td>
<td>553 (43.4)</td>
<td>5 (33.3)</td>
<td>0.28</td>
</tr>
<tr>
<td>Family history</td>
<td>No</td>
<td>767 (60.2)</td>
<td>7 (46.7)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>465 (36.5)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>42 (3.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Pathological Gleason score ≤7</td>
<td>324 (25.4)</td>
<td>2 (13.3)</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>7 (3 + 4)</td>
<td>610 (47.9)</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td></td>
<td>≥7 (4 + 3)</td>
<td>298 (23.4)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>42 (3.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Pathological T stage ≤T2/N0</td>
<td>1051 (82.5)</td>
<td>13 (86.7)</td>
<td>1.0</td>
</tr>
<tr>
<td>≥T3 or any T/N1</td>
<td>218 (17.1)</td>
<td>2 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (0.4)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
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*Chi-squared test or Fisher's exact test for comparisons with small cells (excludes missing data). **Whites only.
located in the first), was predicted to have a deleterious effect on HOXB13 protein function using the POLYPHEN software program [17].

HOXB genes are thought to regulate a number of processes that influence cancer initiation and progression, and it appears that there is some organ specificity in the function of different genes within this family in tumorigenesis [18]. HOXB13 was first identified in 1996 [19], and is involved in early embryonic development of the gland and prostate cell differentiation [20]. HOXB13 has been shown previously to regulate transcriptional activity of the androgen receptor [21] critical to prostate tumour growth. Furthermore, Norris et al. [22] suggest a more complicated interaction between HOXB13 and the androgen receptor, whereby HOXB13 can either act directly on the androgen receptor to regulate the activity of some genes or as a coregulator with the androgen receptor on others.

There are limitations to the present investigation which need to be considered when interpreting its findings. Despite the fact that we were able to assemble a large case series for this investigation, the low frequency of the mutation in the population limited our ability to generate precise estimates of the association between G84E and the characteristics under investigation while also controlling for important covariates. Nevertheless, our analysis stratifying associations of interest by age at diagnosis (≤60 vs >60 years) suggests that a family history of prostate cancer was more common among those with the mutation, and more so among those diagnosed at an earlier age. G84E carriers diagnosed at an earlier age were also slightly more likely to have high grade disease (16.3%) than non-carriers (14.0%), with the reverse relationship observed among carriers diagnosed after age 60 years where they were less likely to be diagnosed with high grade disease (17.9%) than non-carriers (23.7%). These differences, however, were not significant.

In addition, despite the compatibility of our prevalence estimates with previous reports, our findings may not be generalizable to all men diagnosed with prostate cancer. The fact that these men were appropriate candidates for surgery would necessarily exclude men known to have advanced disease at the time of diagnosis; however, the demographic (age at diagnosis and race) and clinical (Gleason grade and pathological tumour stage) characteristics of the cases participating in the present investigation are similar to other large cohorts of men with prostate cancer treated with radical prostatectomy [23–25]. The relatively high proportion of the patient population with a family history of prostate cancer (∼36%) may reflect the referral patterns in the regions served by the academic institutions as well as the educational level of the patient population. Furthermore, it is likely that men with a family history of disease are screened more frequently and therefore may be more likely to be diagnosed with less advanced stage disease. Our data indicate that ~25% of patients with a positive family history were diagnosed with advanced (2pT3a) stage disease as opposed to 28% of patients without a family history ($p = 0.001$). Likewise, 15% of patients with a positive family history were diagnosed with high (≥4+3) grade disease as opposed to 19.5% of patients without a family history ($p < 0.001$); therefore, the fact that this mutation was associated with family history may potentially have biased the findings related to stage toward the null and may explain the absence of any association between G84E with either grade or stage. Our analyses stratified on family history, however, do not support this notion. The mutation frequency among men with both high grade disease and a positive family history was 1.3% compared with 1.4% among men with high grade disease without a family history ($p_{\text{Breslow–Day}} = 0.19$). Similar results were observed with respect to tumour stage (data not shown).

In conclusion, the results of the present study confirm the presence of the G84E mutation in the HOXB13 gene in prostate cancer and estimate its prevalence to be just under 2% of patients with prostate cancer. While no significant association was observed between G84E and tumour pathological features, the increase in the frequency of this mutation in patients with a positive family history and earlier-onset disease reinforces the importance of uncommon, but highly penetrant genes in the genetic epidemiology of prostate cancer. Further investigation is clearly warranted to reveal the underlying biological mechanism to explain this relationship.

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Conflict of Interest


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