Published in final edited form as:
Prostate. 2013 January ; 73(2): 169–175. doi:10.1002/pros.22552.

A Novel Germline Mutation in *HOXB13* Is Associated With Prostate Cancer Risk in Chinese Men

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Abstract

BACKGROUND—A rare mutation G84E in *HOXB13* was recently identified to be associated with prostate cancer (PCa) in Caucasians. The goal of this study is to test association between *HOXB13* genetic variants and PCa risk in Chinese men.

METHODS—All study subjects were part of the Chinese Consortium for Prostate Cancer Genetics (China PCa). In the first stage, we screened for mutations by sequencing the *HOXB13* coding region in 96 unrelated PCa patients. In stage 2, G84E and novel mutations found in stage 1 were genotyped in 671 PCa patients and 1,536 controls. In stage 3, mutation status in 751 additional PCa patients was imputed via haplotype.

RESULTS—The G84E mutation was not detected in this study. However, a novel mutation, G135E, was identified among 96 patients in stage 1. It was also observed twice in 575 additional PCa patients but not in 1,536 control subjects of stage 2. The frequency of G135E was significantly different between cases and controls, with a *P*-value of 0.027, based on Fisher's exact test. Haplotype estimation showed that G135E mutation carriers shared a unique haplotype that was not observed in other subjects. In stage 3, two more PCa patients were predicted to carry the G135E mutation.

CONCLUSIONS—We identified a novel rare mutation in the *HOXB13* gene, G135E, which appears to be a founder mutation. This mutation is associated with increased PCa risk in Chinese men. Consistent with a previous report, our findings provide further evidence that rare mutations in *HOXB13* contribute to PCa risk. *Prostate 73: 169–175, 2013.*

Keywords

HOXB13; G135E; G84E; prostate cancer; Chinese; rare mutation

INTRODUCTION

Prostate cancer (PCa) is the most common noncutaneous cancer and second leading cause of cancer death among men in the United States [1]. In China, although the incidence of PCa is much lower than that in the U.S., the incidence is increasing rapidly, especially in metropolitan areas [2]. With increasing life expectancy, PCa is becoming a major public health issue in China.

Despite the unclear etiology of PCa, the contributions of hereditary factors are well established. In recent years, genome-wide association studies (GWAS) have identified over 40 independent SNPs that are consistently associated with PCa risk in Caucasians [3–15]. Some of these SNPs are also associated with PCa risk in the Chinese population [16,17]. However, most of the common SNPs identified by GWAS confer a relatively small-to-moderate effect, and the cumulative effect of risk SNPs can only account for a portion of PCa risk [18]. Other genetic variants, such as rare SNPs and copy number variants (CNV), are hypothesized to account for a substantial portion of the remaining genetic risk for PCa.

By sequencing over 200 genes in a PCa linkage region at 17q21-22 among 94 probands of PCa families, a rare but recurrent mutation, G84E (rs138213197), in the *HOXB13* gene was found in four probands. The mutation co-segregated with PCa in these four families [19]. In a subsequent analysis of unrelated subjects, G84E frequency was observed to be significantly higher in PCa patients (1.4%) than in controls (0.1%; $P = 8.5E{-}7$), especially among PCa patients who had early age at diagnosis and a positive family history (3.1%) [19]. This mutation is located in a conserved functional domain of HOXB13, and the alteration from glycine to glutamic acid is predicted to affect protein function.

To investigate potential role of *HOXB13* in PCa among Chinese men, we sequenced this gene in Chinese PCa patients and performed an association study between genetic variants of *HOXB13* and PCa risk in unrelated Chinese PCa patients and controls.

MATERIALS AND METHODS

Subjects

All of the participants in our study were part of a Chinese Consortium for Prostate Cancer Genetics (China PCa), which was initiated in 2010 and designed to investigate the genetic determinants of PCa in Chinese men. Cases were recruited from 18 hospitals in Eastern and Southern China. PCa diagnosis was verified histologically for each participant. Cancer-free controls were collected from April 2010 to November 2010 in Shanghai, which is located in Southeastern China. A random cluster sampling method was used to select four districts and counties of Shanghai. For each selected district or county, one or two communities were selected. Inclusion criteria for controls were as follows: (1) age 40–79 years old, fully independent self-care, and has been a resident in Shanghai for more than 1 year; and (2) able to provide informed consent and provide a blood sample and complete a medical examination.

In stage 1, 96 PCa patients were randomly selected from China PCa for sequencing of the coding regions of the *HOXB13* gene. In stage 2, the 96 subjects that were initially sequenced, as well as 575 PCa patients and 1,536 controls from China PCa were studied for

G84E and novel mutations found in stage 1. In stage 3, additional 751 PCa patients from China PCa with GWAS data were studied. Characteristics of subjects in each stage are summarized in Table I.

This study was approved by the Institutional Review Board at Fudan University, as well as each participating hospital. Written consent was obtained from all participants.

Sequencing and Genotyping

Sequencing of *HOXB13* exon regions in stage 1 was conducted using the ABI 3730XL DNA analyzer. First, gene-specific primers were designed and PCR reactions were performed in a 10 μl volume consisting of 30 ng genomic DNA, 0.2 μM of each primer, 0.2 nM/L of each deoxynucleotide triphosphate, 1.5 mM/L $MgCl_2$, 20 mM/L Tris–HCl, and 0.5 U Taq polymerase (Invitrogen Corporation, Carlsbad, CA). Second, each PCR product was purified using the QuickStep PCR Purification kit (Edge Biosystems, Gaitherbury, MD). Third, all sequencing reactions were performed using dye terminator chemistry (BigDye, ABI, Foster City, CA) and then precipitated using 65% ethanol. Finally, samples were loaded onto an ABI 3730XL DNA analyzer after adding 8 μl formamide. The results were analyzed using Sequencher software (Gene Codes Corp., Ann Arbor, MI).

G84E mutation of *HOXB13* and other novel mutations identified in stage 1 were genotyped among subjects in stage 2 using the iPLEX MassARRAY system (Sequenom, Inc., San Diego, CA). Primers were designed using Spectro Designer software (Sequenom) and experiments were carried out according to the standard manufacturer's protocol. The overall genotyping call rate was 96.9%. Laboratory technicians were blinded to case–control status.

Haplotype Estimation and Statistical Analysis

Based on GWAS data that were available for all the subjects in this study, we performed a haplotype analysis in the region of *HOXB13*. A 70,698 bp region (44,112,044–44,182,742, build 36) was identified based on gene structure and recombination hot spots. Eighteen SNPs in the region were selected as haplotype tagging SNPs (ht-SNPs) based on Haploview software version 4.2 [20] (Daly Lab at the Broad Institute, Cambridge, MA). Haplotypes of these 18 ht-SNPs for each subject were estimated by PLINK software [21]. All of the SNPs used for estimating haplotypes passed quality control criteria: $P > 0.001$ for the Hardy–Weinberg equilibrium test, minor allele frequency (MAF) >0.01, and genotype call rate >95%. A list of these SNPs is presented in Supplementary Table I.

Association analysis of HOXB13 mutations with PCa risk was performed using Fisher's exact test by PLINK [21].

RESULTS

In stage 1, we sequenced the entire *HOXB13* coding region in 96 unrelated PCa patients. We did not find any subjects carrying the G84E mutation, however, a novel nonsynonymous mutation was discovered at the second position of codon 135 (GCA to GAA, bp: 44,160,551, build 36), leading to an amino acid substitution from glycine to glutamic acid during translation (G135E). This new mutation was not observed in any ethnicity within public databases, such as dbSNP or the latest release of the NCBI 1000 Genomes Sequencing Project. In addition, we found two synonymous variants in Chinese men, S122S (rs8556) and S171S (rs9900627), with MAF of 3.6% and 19.4%, respectively.

To further explore the potential association of the G135E mutation with PCa in a Chinese population, we genotyped this mutation in 671 unrelated PCa patients (including 96 cases in

stage 1) and 1,536 controls in stage 2. In addition to confirming the G135E carrier status of the subject detected using sequencing method in stage 1, we also found two more G135E carriers amongst the remaining PCa patients, while no control subjects carried this mutation. All three G135E carriers are heterozygous. The frequency of the G135E mutation between all PCa patients and cancer-free controls from stages 1 and 2 was statistically significant, with a Fisher's exact test *P*-value 0.027 (Table II).

To investigate the potential founder effect of the G135E mutation, we evaluated haplotypes based on 18 ht-SNPs among 666 PCa cases and 1,006 controls in stage 2 whose GWAS data were available. We identified a haplotype "G-T-C-G-A-C-C-T-G-G-G-A-T-G-G-T-A-G" that was present only in three subjects carrying the G135E mutation but not in any other subjects. This result suggested that G135E is likely a founder mutation and individuals with this haplotype likely carry the G135E mutation.

We also estimated haplotypes based on the 18 ht-SNPs for subjects in stage 3. Two out of 751 PCa patients carried the haplotype that harbors the G135E mutation. Therefore, these two patients likely carry the G135E mutation. Characteristics of all five subjects carrying G135E mutation are presented in Table III. A wide range of PSA levels at diagnosis, Gleason scores, and age at diagnosis were observed.

DISCUSSION

In this study, the G84E mutation of *HOXB13* that was previously identified in Caucasians was not detected in the Chinese population. However, a novel rare mutation in *HOXB13*, G135E, was discovered in Chinese PCa patients. This mutation was associated with elevated PCa risk in Chinese men. Haplotype estimation showed that all G135E mutation carriers share the same haplotype, and this unique haplotype was only observed in the three G135E mutation carriers, indicating a potential founder effect. Moreover, in another independent study population, we found two additional PCa patients that most likely carry the G135E mutation because they carry the unique haplotype harboring the G135E mutation.

HOXB13 is located in 17q21.3, about 70 kb upstream of the *HOXB* gene cluster. It is composed of two exons and one intron. Exon 1 includes two MEIS (myeloid ecotropic viral integration site) binding domains, while exon 2 contains a homeodomain. These domains are highly conserved among vertebrates [22–24]. The G84E rare mutation in the germ-line of Caucasians is located in the first MEIS interacting domain, while L144P, another rare mutation that was identified from a cell line, is located in the second MEIS interacting domain [19,24]. By comparing HOXB13 protein sequences among species, we found that the G135E mutation resides in the second MEIS interacting domain. The MEIS interacting domains are suspected to fine tune HOX function by regulating the interaction of HOX proteins with other DNAs and proteins [19,25], indicating a potential mechanism through which the G135E mutation affects HOXB13 function.

Given the important location of the G135E mutation, we then assessed the possible impact of the G135E mutation on the structure and function of the HOXB13 protein using PolyPhen software version 2 [26], which uses both sequence-based and structure-based information for prediction. Two datasets in PolyPhen-2 were used for estimating potentially deleterious effects. One dataset is HumDiv which contains 3,155 damaging alleles annoted in the UniProt database, while the other one, HumVar, contains 13,032 human disease-causing mutations from the UniProt database [27]. Results from each of these datasets suggested the *HOXB13* G135E mutation is likely to have a deleterious effect on protein function, with a probability of 0.98 by HumDiv and 0.65 by HumVar.

Based on the location, functional prediction, and haplotype analysis, we hypothesize the *HOXB13* G135E mutation is descended from a single ancestral mutation event and the deleterious effect of this mutation may contribute to elevated PCa risk among carriers in the Chinese population by altering the structure of the MEIS-binding site in the HOXB13 protein. This hypothesis should be tested by functional studies in the future.

The discovery of G135E from this Chinese population, together with the original finding of G84E mutation in Caucasian populations, suggests that rare mutations may play an important role in PCa etiology. Although few of these PCa risk-associated rare mutations have been identified to date, this was likely due to limited technology and information available in the past for assessing rare variants in the genome. It is expected that additional mutations such as this will be identified through the use of next-generation sequencing approaches and Exome SNP arrays. Rare and highly penetrant mutations such as those in *HOXB13* may potentially account for the so-called “missing” heritability, a phenomenon in which only a small percentage of genetic susceptibility is accounted for by ~40 common PCa risk-associated variants discovered to date [3–15]. As we reconsider the common disease common variant hypothesis and expand it also to include rare variants, this should improve our understanding of the genetic architecture underlying PCa and other complex diseases.

There are several limitations in this study. First, sample size is one of the most concerning issues for association studies of rare mutations. The statistically significant association between G135E and PCa risk was based on three carriers in 671 cases and zero carriers in 1,536 controls. A larger sample size is warranted to confirm the association. Second, although the association was further supported by two more likely carriers of the G135E mutation among 751 additional PCa cases, this was based on imputation from a haplotype analysis. Further confirmation of the carrier status for these two patients by direct genotyping is needed. Finally, we were not able to test for familial aggregation of the G135E mutation because there are few families with multiple affected members available in China. The lack of familial aggregation of PCa in China likely reflects the low detection rate of PCa in China in the past, due to the low adoption rate of PSA screening. However, it has been noted that more families with multiple PCa patients have been observed in recent years; these PCa families would allow us to identify additional PCa risk-associated variants.

CONCLUSION

We identified a novel rare mutation (G135E) of the *HOXB13* gene that is associated with increased PCa risk in Chinese men. Together with a previous finding of the G84E mutation in the same gene in European descent, this new finding provides further evidence that *HOXB13* plays an important role in PCa etiology. These findings also provide evidence that rare and high-penetrant mutations are an important component of genetic alterations for PCa.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank all of the subjects included in this study. This work was partially funded by the National Key Basic Research Program Grant 973 (2012CB518300) to Y.S. and J.X., the Key Project of the National Natural Science Foundation of China (81130047) to J.X., intramural grants from Fudan University “Thousand Talents Program” and Huashan Hospital to J.X., the National Institutes of Health (NCI CA129684) to J.X.

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TABLE I

Characteristics of Subjects in Each Stage

	Number		Age[a]		Gleason score[b]			
Stage	**Case**	**Control**	**Case**	**Control**	**<7**	**≥7**	**Missing**	**PSA[c]**
1	96	NA	72.4 ± 7.9	NA	20	76	NA	32.5 (17.0, 56.0)
2	671	1536	71.9 ± 7.9	61.1 ± 9.0	175	430	66	26.7 (12.9, 78.5)
3	751	NA	70.7 ± 8.2	NA	180	565	6	25.2 (13.0, 86.6)

[a] Age (year) is expressed as mean ± SD. Age for cases is age of diagnosis while age for controls is age of enrollment.

[b] Number of subjects with Gleason score <7, ≥7, or with missing data.

[c] PSA (ng/ml) is expressed as median (Q1, Q3).

TABLE II

Number of *HOXB13* G135E Mutation Carriers in PCa Cases and Controls

G135E	PCa cases	Controls	Fisher's exact *P*-value
Carrier	3	0	0.027
Noncarrier	639	1,491	

TABLE III

Characteristics of Subjects Found to Carry G135E at *HOXB13*

Subjects	G135E	Age[a]	PSA	Gleason score	TNM stage	PCa
1	Genotyped	72	54.0	7	T3N0M0	Yes
2	Genotyped	81	15.0	6	T2cN0M0	Yes
3	Genotyped	75	15.4	7	T3aN0M0	Yes
4	Imputed[b]	67	15.7	6	NA	Yes
5	Imputed[b]	65	>100	7	TxN0M1	Yes

[a]Age of diagnosis.

[b]The haplotype tagging genotype for G135E is “G-T-C-G-A-C-C-T-G-G-G-A-T-G-G-T-A-G” for SNPs listed in the Supplementary Table I.