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Short Research Communication

Synonymous Polymorphisms in HOXB13 as a Protective Factor for Prostate Cancer

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Abstract

Background: Genomic association and linkage studies, as well as epidemiological data have implicated both the HOXB13 gene and single nucleotide polymorphisms (SNPs) in the development of prostate cancer (PCa). The recent association between the G84E polymorphism in the HOXB13 gene and PCa has been shown to result in a more aggressive cancer with an earlier onset of the disease. We examined the frequency of this mutation and other recurrent HOXB13 SNPs in patients with PCa and those with benign prostatic hyperplasia (BPH) or no cancer.

Methods: Reverse transcriptase-polymerase chain reaction (RT-PCR) was performed on exons 1 and 2 of HOXB13 gene, followed by bidirectional Sanger Sequencing on peripheral blood from 232 PCa (age 46-92) and 110 BPH (age 45-84) patients. Statistical analysis was used to correlate between recurrent SNPs and PCa.

Results: The G84E mutation was found at a low frequency in randomly selected PCa and BPH (both 0.9%). Two recurrent, synonymous SNPs, rs8556 and rs900627, were also detected. rs8556 was detected in 48 PCa (20.7%) and 26 BPH (23.6%) subjects; rs9900627was detected in 27 PCa (11.6%) and 19 BPH (17.3%) subjects. Having both rs8556 and rs9900627 or being homozygous for either one was associated with being 2.9 times less likely to develop PCa (p=0.05).

Conclusions: Although a larger study in order to confirm our findings, our data suggests a significant negative correlation between two SNPs, rs8556 and rs9900627, and the presence of PCa.

Key words: prostate cancer; HOXB13; polymorphism; SNP; benign prostatic hyperplasia; G84E

Introduction

Prostate cancer (PCa) has risen in incidence due to increased male longevity, making it the second most common cancer, with 899,000 new cases reported annually, as well as a leading death of men worldwide [1,2]. Men have a one in six risk of developing PCa during their lifetime, however its clinical course differs from indolent in some to rapidly fatal in others [3]. PCa is a complicated disease due to the absence of clear risk factors such as lifestyles, environmental factors, or infectious agents [4]. Due to the high incidence of PCa, the poor prognosis for metastatic disease, and the increase in male longevity, there has been pressure to understand the causes of PCa, as well as developing better prevention and detection strategies. PCa is a heritable cancer, as family history, along with age and race, has been shown to put a patient at higher risk for the development of PCa [5]. Epidemiological data has also implicated genetics in the causation of PCa [6,7]. Genome-wide association studies (GWAS) have been used to investigate single nucleotide polymorphisms (SNPs) and their incidence in PCa, resulting in the discovery of several SNPs with a biological relevance to PCa [6,8]. Two SNPs have been discovered to be associated with

PCa, such as rs10993994 on chromosome ten near the MSMB gene and rs12653946 near the IRX4 gene on chromosome five [4]. In addition to GWAS, linkage studies have correlated HOXB13 and a rare, recurrent germline, mutation in the gene with familial PCa risk, early onset of disease and a more rapid progression [9,10]. This nonsynonymous SNP, rs138213197 (a transition of guanine to adenosine, c.251G→A), causes an amino acid change in the 84th amino acid from a glycine to a glutamate. A recent meta-analysis by Huang et al. [11] that included 120,167 participants from 11 different studies showed that G84E carrier frequency ranged from 0.1-4.9% in patients with PCa as compared to 0-1.4% in control subjects. The results showed that men harboring the G84E variant had a 4.51 fold higher relative risk for the development of PCa. We sought to examine the frequency of the G84E mutation, as well as other clinically significant SNPs in the HOXB13 gene in our own patient cohort.

Methods

We performed reverse transcriptase-polymerase chain reaction (RT-PCR) followed by bidirectional Sanger Sequencing of the mutation hotspots in the two HOXB13 coding exons using total nucleic acid extracted from the peripheral blood of 342 subjects (232 with biopsy-confirmed PCa and 110 with biopsy-confirmed benign prostatic hyperplasia [BPH] or no cancer). These patients were referred to urologists due to prostate complaints and elevated PSA or abnormal digital rectal exam (DRE) and were determined to need a prostate biopsy. The median age of patients with PCa was 68 (range 46 to 92) and the median age of the BPH patients was 65 (range 45 to 84). Forward and reverse primers for the RT-PCR reaction were as follows: 5′-tgt aaa acg acg gcc agt AAC TAT GCC CCC TTG GAT CT-3′ and 5′- cag gaa aca gct atg acc GAG ATC TTG CGC CTC TTG TC-3′ (uppercase indicates the actual sequences and lowercase represents the M13 linker used for sequencing). RNA sequencing was chosen for efficiency purposes, as this allowed both exon 1 and 2 to be sequenced in one reaction. The PCR products were purified and then sequenced in both directions using the M13 universal primers (forward and reverse) on an ABI 3730xl Genetic Analyzer. Sequencing data are base-called by sequencing analysis software (v5.4) and assembled by the ABI SeqScape software version 2.7 (Applied Biosystems, Foster City, California).

Results

Recurrent SNPs found in the HOXB13 gene are shown in Table 1. The G84E polymorphism was detected in two individuals with PCa (0.9%) and in one patient with biopsy-confirmed BPH (0.9%). Two SNPs, rs8556 and rs900627 were also detected in both the PCa and BPH cohorts. SNP rs8556 was detected in 48 individuals with PCa (20.7%) and 26 with non-cancer (23.6%); rs9900627 was detected in 27 PCa patients (11.6%) and 19 BPH patients (17.3%). Using Wilcoxon Rank Sum tests, there was no significant difference between patients with PCa and biopsy-confirmed non-cancer patients when each of the polymorphism was considered separately. However, there was significant negative correlation between PCa patients and subjects without cancer only when patients were homozygous for rs8556 or rs9900267 or harbored both polymorphisms (P=0.05). Patients who had both rs8556 and rs9900627 polymorphisms or were homozygous for either one were 2.9 times less likely to have prostate cancer than those without these SNPs (Relative risk=0.359; 95% CI=0.116 to 1.11). Both rs8556 and rs9900627 polymorphisms are silent; they do not result in an amino acid change (Table 1.).

Table 1. HOXB13 SNPs and their prevalence in Prostate Cancer/Benign Prostatic Hyperplasia.

SNP	rs8556	rs9900627	rs138213197	Co-existence of rs8556 & rs9900627
Nucleic Acid Change	366C>T,	513T>C	251G>A	
Amino Acid Change	S122S (Synonymous)	S171S (Synonymous)	G84E	
Prevalence in PCa				
Total	48/232 (20.7%)	27/232 (11.6%)	2/232 (0.9%)	3/232 (1.3%)
Heterozygous	47/232 (20.3%)	26/232 (11.2%)	1/232 (0.4%)	
Homozygous	1/232 (0.4%)	1/232 (0.4%)	1/232 (0.4%)	
Prevalence in non-cancer				
Total	26/110 (23.6%)	19/110 (17.3%)	1/110 (0.9%)	3/110 (2.7%)
Heterozygous	23/110 (20.9%)	18/110 (16.4%)	1/110 (0.9%)	
Homozygous	3/110 (2.7%)	1/110 (0.9%)	0/110 (0.0%)	

Discussion

To the best of our knowledge, there have been no reports published to date showing genetic variation in the HOXB13 gene that is associated with a reduced susceptibility for PCa. While investigating the occurrence of the G84E mutation, as well as other clinically significant SNPs in our 342 patient cohort, we discovered two SNPs (rs8556 and rs9900627) in HOXB13 that had a negative correlation with the presence of PCa when present in a homozygous state or when both coexist in the same individual. Patients who are homozygous at either rs8556 or rs9900627, or who harbor both of these SNPs together are associated with a 2.9 times lower relative risk for the development of PCa as compared to those who do not have the variations (p=0.05).

The HOX genes belong to the homeobox superfamily of transcription factors containing a highly conserved homeodomain. HOXB13 is part of a 200kb span of HOXB genes on chromosome 17 that contains one of the four HOX clusters. Expression of the HOX genes is necessary for the proper development of the animal body [9]. The HOX genes are all expressed during the development of the embryo, however, HOXB13 is highly expressed in a constant and androgen-independent manner into adulthood in the prostate [12].

HOXB13 has been shown to physically interact with the androgen receptor and that it suppresses hormone-mediated androgen receptor activity in a dose dependent manner [13]. Unfortunately, the biology underlying the difference in susceptibility to having cancer cannot be explained by a difference in oncogenic properties of the HOX13 with the polymorphisms because both polymorphisms are synonymous. However, synonymous polymorphisms have been reported to play a role in protein translation efficiency, in the levels of the translated protein, as well as affecting splicing events, mRNA stability, microRNA binding, and nucleosome formation [14]. Different codons have different speeds or accuracy of translation mainly due to the abundance of their respective transfer RNAs (tRNAs) in the ribosome [15]. In addition, the change in translation rate and efficiency can affect protein folding, as most protein folding is performed during translation [16]. The demonstration that the effects are relevant only when present in homozygous state or when both polymorphisms co-exist, suggests that dosage of the abnormality is important. It is possible that the effect of each polymorphism on the level of protein is small and both alleles need to be affected to achieve significant change in protein levels. Further studies and protein analysis are needed to confirm this hypothesis.

In Summary, although our patient cohort is small and a larger study is needed for validation, our data suggest that there is a significant negative correlation between the presence of PCa and the two SNPs, rs8556 and rs9900627. The data presented here shows that men with these SNPs are 2.9 times less likely develop PCa.

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Competing Interests

See Acknowledgements.

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