Analysis of SNPs in NAT1 gene as a risk factor for prostate cancer

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ABSTRACT

Prostate cancer is the most commonly diagnosed cancer in the male population and has a high survival rate. Consequently, a large number of men entering their older years need to adjust their lifestyle according to their condition. A review of the current research literature indicates an increased association between prostate cancer and certain genotypes of the human arylamine N-acetyltransferase 1 gene (NAT1). NAT1 encodes enzymes essential to the metabolism of toxic drugs and environmental chemicals; this gene plays a key role in the metabolic activation and detoxification of procarcinogens. The gene is highly polymorphic and single nucleotide polymorphisms (SNPs) have been reported at loci C190T, G445A, C559T, and T640G. These mutations and others at loci T1088A and C1095A are reported to increase prostate cancer risk. We believe that determination of these mutations in individuals can help establish the risk of developing prostate cancer. Hence, we are developing a novel approach to determine the presence of SNPs using real-time quantitative polymerase chain reactions (RT-qPCR) and high-resolution melting (HRM) analysis. Although this technique has been described in the literature, it has not yet been adopted for the detection of mutations. As we move forward, we will confirm these specific HRM profiles for the mutations.

OBJECTIVE

To develop an approach to detect NAT1 SNPs in individuals and establish risk of prostate cancer development using RT-qPCR and HRM analysis.

INTRODUCTION

NAT1, located on chromosome 8 at 8p21-22, is one of the two N-acetyltransferase genes in the human genome.(3) Although it comprises at least 9 exons covering approximately 53kb, the entire coding sequence of 870bp is contained within exon 9 only.(2) NAT1 has long been known for its key role in drug metabolism. Today, its highly polymorphic character has shifted the focus on its role in the activation and detoxification of environmental carcinogens.(1)

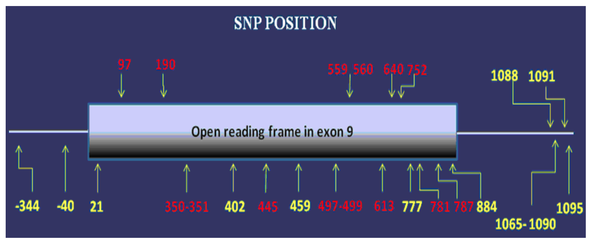

Figure 1. NAT1 is a highly polymorphic gene. The different SNPs present in the coding region, exon 9, of the gene are shown here.(4)

Single-nucleotide polymorphisms (SNPs) constitute the most common type of genetic variation, each representing a difference in a nucleotide.(5) Many NAT1 SNPs have been reported to increase predisposition to prostate cancer in different proportions of the population: C190T (64%), G445A (64%), C559T (64%), T640G (20%), T1088A (7%), and C1095A (20%).

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METHODOLOGY

Primer design

✧SNPs located at:
- *rs56379106* → *C190T*
- *rs4987076* → *G445A*
- *rs5030839* → *C559T*
- *rs4986783* → *T640G*

✧Forward and reverse primers designed using ***IDT PrimerQuest*** and ***OligoAnalyzer.***

RT-qPCR

✧iTaq Universal SYBR Green Supermix from Bio-Rad was used as a non-specific fluorescent dye.

✧***Protocol:*** 1X mix, 400nM of each primer, 100ng of genomic DNA, and variable amounts of Rnase-free water → total reaction volume of 10µL.

✧***Optimized cycling conditions:*** 95.0°C for 5:00, 95.0°C for 0:10, 60.0°C for 0:45 + plate read, GOTO 2. 45 more times, 95.0°C for 0:05, melt curve 65.0°C to 95.0°C increment 0.5°C for 0:05 + plate read, 72.0°C for 1:00, and 25.0°C for 2:00

HRM

Reference
=
NTC
No template control

RESULTS

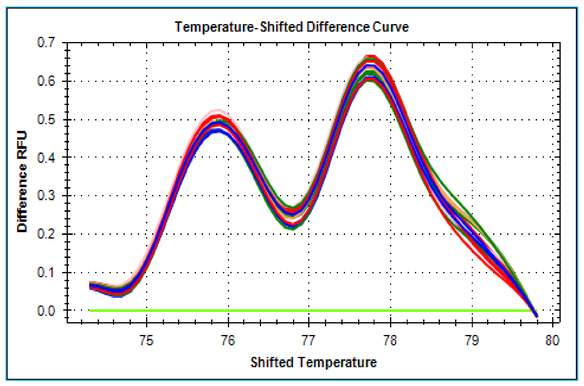

Figure 2. HRM graph representing numerous samples screened for NAT1 SNP C190T.

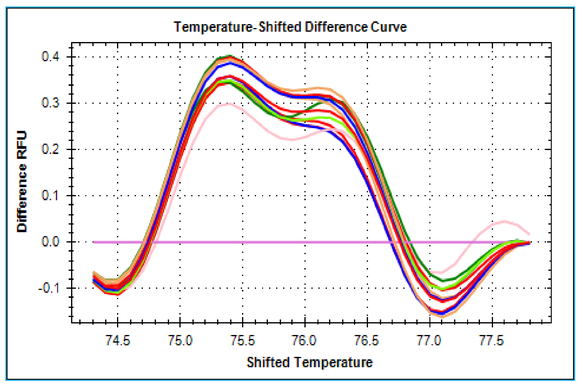

Figure 3. HRM graph representing numerous samples screened for NAT1 SNP G445A.

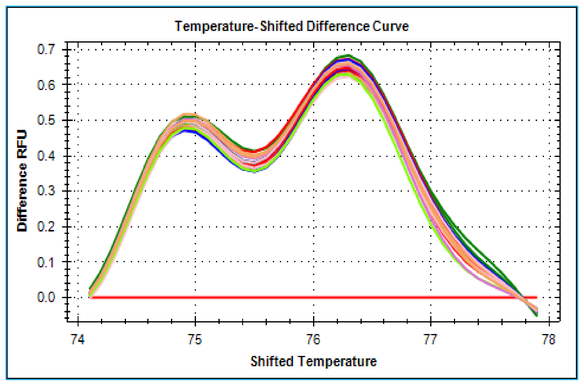

Figure 4. HRM graph representing numerous samples screened for NAT1 SNP C559T.

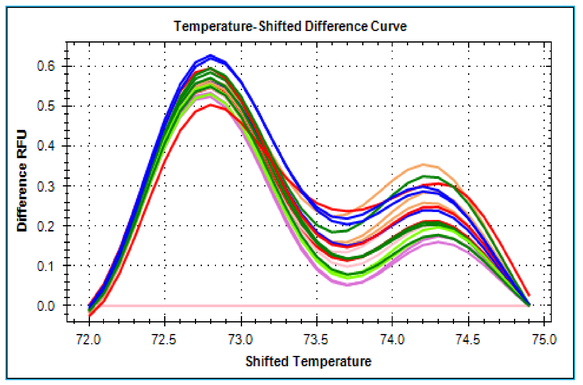

Figure 5. HRM graph representing numerous samples screened for NAT1 SNP T640G.

DISCUSSION

Further investigation is required in order to accurately determine the specific HRM profiles corresponding to each distinct SNP. As a comparison technique, a mutagenesis protocol needs to be implemented to create controls and distinguish cases. Later, gene sequencing may be required to support the findings.

CONCLUSION

RT-qPCR and HRM analysis can be used as detection techniques in order to establish the risk of developing prostate cancer in humans.. However, further investigation is essential to support the findings.

I would like to thank Dr. Gomes and my colleagues for making this experience so rewarding and guiding me through each step of the process, as well as UROP for giving me this opportunity in the first place. – M.E.

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