Detection of G640T SNP as a Marker of Risk for Prostate Cancer

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Introduction

Single nucleotide polymorphisms (SNPs) are the most common type of genetic variation. A SNP occurs when a single nucleotide is replaced by a different nucleotide. Its presence could alter gene expression, leading to increased susceptibility to diseases and adverse health effects. The N-acetyltransferase 1 gene is a phase II enzyme metabolizing gene responsible for the detoxification and elimination of toxins in the body. SNPs in this gene are known to compromise the functioning of NAT1.

A number of SNPs have been reported in the NAT1 gene, including the SNP at locus 640 with a G to T transformation in 20% of the population. In men, the presence of the G640T mutation increases the risk of prostate cancer when exposed to heterocyclic amines which are often found in burnt meat. The purpose of this study is to determine the presence of the G640T SNP using a new approach in conjunction with qPCR. High resolution curve (HRM) analysis along with qPCR helps to determine the presence or absence of the SNP.

Objective

The main focus of this study is to determine the presence of SNP (G640T) among prostate cancer cases and matched controls.

1. The first objective is to develop an appropriate protocol to determine the presence of SNPs in DNA obtained from cases and controls.
2. The second objective is to determine the frequency of the presence of the SNP among cases and compare it to the literature value of 20%.

Methodology

1. DNA Extraction from Prostate Blood Samples

   1. Precipitate nuclear pellets + centrifuge
   2. Add extraction buffer and extract aqueous DNA layer + centrifuge
   3. Precipitate pure DNA
   4. Re-dissolve DNA

2. Quantifying DNA Concentration

   Mean concentration of DNA = 34.5µM/ng

3. Primer Design

   NAT1 640 Primers:
   F = TAGCTCTAGAATACATGAGA
   R = AAGCCCAACACAGTAA

   House Keeping Gene Primer (GAPDH):
   F = TGGTCGACAGTACGCCATCTTC
   R = GGTGACCGAGGCCAATACG

4. qPCR and HRM analysis

Results

The following 4 figures were obtained from HRM analysis using Bio-Rad Precision Melt Analysis software, following qPCR. 6 prostate and 6 control samples were used and their data was then compiled onto the figures presented. These figures can be used to qualitatively detect a SNP at locus 640 by comparing the melt curves of control DNA samples previously identified as having the SNP with the unknown prostate samples. A greater resemblance of the samples would suggest the presence of the SNP at locus 640. This data was then compared to HRM results of synthetic DNA designed to have the SNP G640T and another designed to be wild type, in order to confirm concordance.

Summary

QPCR and HRM analysis proved to be a successful means to detect the presence or absence of a SNP at locus 640. Despite other techniques such as DNA sequencing, mass spectrometry, RFLP analysis etc. that are currently being used to detect SNPs, HRM analysis provides a quicker and more efficient method from which further analysis can be done.[7] Of the 6 prostate samples, samples 147, 149 and 150 are likely to contain the G640T SNP. Thus the prevalence found was 50% as compared to the literature value of 20%.[8] Evidently, a larger sample population is likely to lower the experimental prevalence obtained.

Future Work and Acknowledgements

• The confirmed samples should be genotyped to validate the results.
• Further analysis should be done on synthetic DNA samples to confirm presence of the SNP in cases and controls.

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References