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.

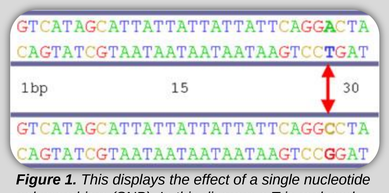


Figure 1. *This displays the effect of a single nucleotide polymorphism (SNP). In this diagram a T is replaced with a C through which the DNA sequence has been altered.* [1]

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.

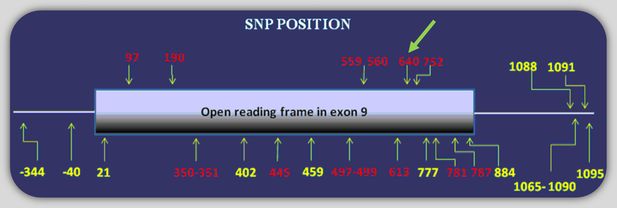


Figure 2. *This shows the various loci at which SNPs can be found on the NAT1 gene. The vast number of loci for SNPs gives NAT1 its highly polymorphic character. Locus 640 is indicated with the green arrow.* [2]

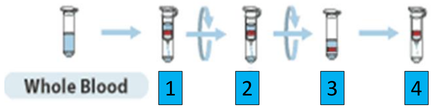
Objective

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 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

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ATCAGAAGGGAACAGTACATTCCAAATGAAGAATTTCTTCATTCTGAT
CTCCTAGAAGACAGCAAATACCGAAAAATCTACTCCTTTACTCTTAAG
CCTCGAACAATTGAAGATTTTGAGTCTATGAATACATACCTGCAGACA
TCTCCATCATCTGTGTTTACTAGTAAATCATTTTGTTCCTTGCAGACC
CCAGATGGGGTTCACTGTTTGGTGGGCTTCACCCTCACCCATAGGAGA
TTCAATTATAAGGACAAACAGATCTAATAGAGTTCAAGACTCTGAGTG
AGGAAGAAATAGAAAAAGTGCTGAAAAATATATTTAATATTTCCTTGC
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NAT1 640 Primers:

F=TGAGTCTATGAATACATACCTGCAGA

R=AAGCCCACCAAACAGTGAA

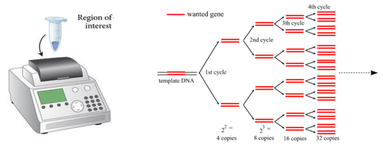
House Keeping Gene Primer (GAPDH)

F=TGTTCGACAGTCAGCCGCATCTTC

R=GGTGACCAGGCGCCAATACG

4. qPCR and HRM analysis

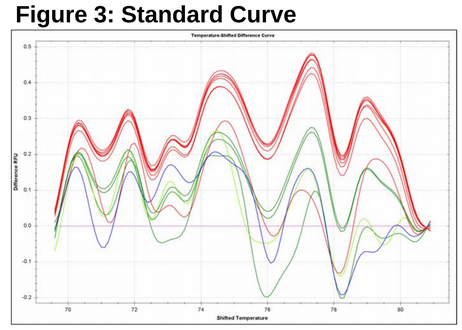
	1	2	3	4	5	6	7	8
A	109	125	150	148	147	149	HKG	NPC
B	109D	125D	150D	148D	147D	149D	HKG	NTC
C								
D								



Results

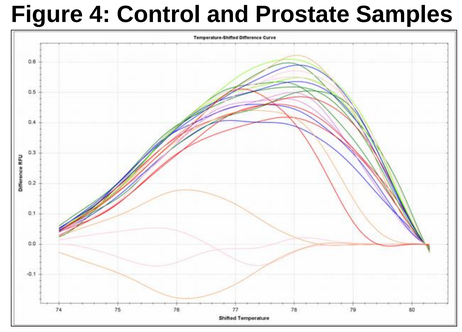
The following 4 figures were obtained from HRM analysis using Bio-Rad Precision Melt Analysis software, following qPCR. 6 prostate and 6 control samples were used and their data was then compiled onto the figures presented. These figures can be used to qualitatively detect a SNP at locus 640 by comparing 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.

Figure 3: Standard Curve



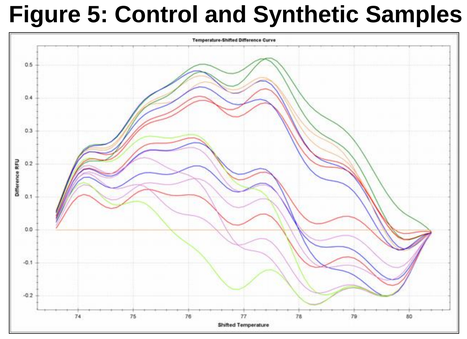
= Serial Dilutions of sample 124
=No template Control
=Control 1319
=No template Control
=Control 165
=Negative Control

Figure 4: Control and Prostate Samples



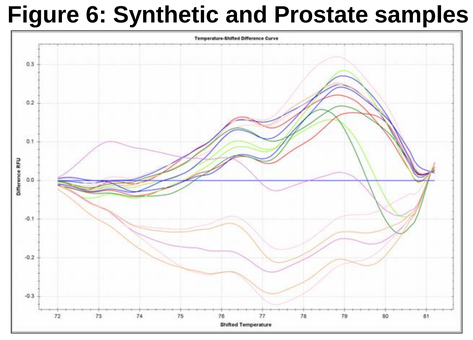
=Sample 150
=Sample 109
=Sample 124
=Sample 147
=Sample 148
=Sample 149
=Control 165
=Control 45
=Control 464
=Control 132
=Control 152
=Control 1314

Figure 5: Control and Synthetic Samples



=Synthetic Wild Type
=Synthetic Mutation G640T
=Control 165
=Control 45
=Control 464
=Control 132
=Control 152
=Control 1314

Figure 6: Synthetic and Prostate samples



=Synthetic Wild Type
=Synthetic Mutation G640T
=Sample 124
=Sample 149
=Sample 150
=Sample 148
=Sample 147
=Sample 109

Figure 3 shows the standard curve of 5 serial dilutions of a prostate sample, a control sample with the SNP and a control sample without the SNP. This can be used to observe the level of gene expression and the behaviour of the samples in these conditions. **Figure 4** compares unknown prostate samples with controls positive for G640T. It was observed that samples 147, 150 and 149 produced melt curves very similar to those of the controls. Also 2 controls were found to be inconsistent with the other controls. **Figure 5** compares controls with a sample designed to have the G640T SNP and a synthetic wild type sample. While the shapes of the melt curves of the control and the synthetic SNP sample are similar, the separation is still too great to confirm concordance. Also, the wild type and synthetic SNP sample display a greater resemblance than expected. **Figure 6** shows prostate and the synthetic samples. Again, despite similar shapes, there is little agreement between the samples and more analysis is needed to confirm this.

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.

I would like to express gratitude to Dr. James Gomes for giving me the opportunity to work in his lab and for guiding me in throughout the research. I would also like to thank my colleagues , and a special thanks to Paul Riley for his expert advice and assistance. Lastly I would like to thank UROP for giving me this amazing opportunity to learn so much from.

References

Diagrams:

[1]*The Cregan Lab Research Projects: Discovery of Single Nucleotide Polymorphisms (SNPs) in the Soybean Genome.* http://bldg6.arsusda.gov/pberkum/Public/sarl/cregan/snps.htm.

[2] *NAT1 (N-acetyltransferase 1(arylamine N-acetyltransferase)).* http://atlasgeneticsoncology.org/Genes/NAT1ID41497ch8p22.html (2009).

[3] Blood Genomic DNA Miniprep Kit. *Sigma Alrich*. http://www.sigmaaldrich.com/life-science/molecular-biology/dna-and-rna-purification/blood-genomic-miniprep-kit.html.

[4] The Thermo Scientific NanoDrop 2000/c and NanoDrop Lite. http://www.nanodrop.com/NDlite/web_ft.html

[5] DNA Amplification and PCR. *New England Biolabs.* https://www.neb.com/applications/dna-amplification-and-pcr.

[6] Principle of PCR. http://users.ugent.be/~avierstr/principles/pcr.html. (1999)

Data:

[7] Twyman, Richard M. "Single Nucleotide Polymorphism (SNP) Genotyping Techniques—An Overview." *Encyclopedia of Diagnostic Genomics and Proteomics.* Ed. Marcel Dekker. University of York, 2005.

[8] Yang M et al. "Relationship between NAT1 genotype and phenotype in a Japanese population." *Pharmacogenetic* (2000): 225-32.