# NQO1 ALLELE FREQUENCIES IN AN AMERICAN INDIAN POPULATION AND CANCER SUSCEPTIBILITY

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## ABSTRACT

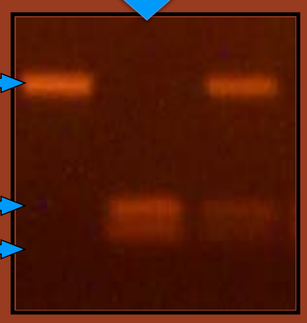
NQO1 is involved in the reductive bioactivation of cytotoxic antitumor quinone compounds, but also plays a protective role against the carcinogenicity and mutagenicity of quinones, their precursors and metabolites. One allele that predicts the function of this enzyme, NQO1*2,has been identified. Three forms of this allele exist, a functional allele, a nonfunctional allele, and an allele associated with diminished activity. The purpose of this study was to characterize interethnic variability in the frequency of the NQO1*2 nonfunctional allele in the Confederated Salish & Kootenai (CSK) population. One-hundred and twenty-five blood samples were tested for the allele by isolating the DNA from peripheral blood, performing PCR, digesting with restriction enzymes, and then analyzing the DNA fragments on a 1.5% agarose electrophoresis gel. The data analysis showed an elevated frequency of the nonfunctional allele in Native Americans when compared to Caucasian populations. The frequency of the variant in NQO1*2 is 16% in Caucasians as established in previous studies. As the blood quantum of the CSK blood samples approached 100% the NQO1*2 allele frequency approached 36%. Our data suggests that CSK population is at an increased risk of cancer due to a higher frequency of defective NQO1 enzyme. The higher allele frequency present in CSK members would also make genetic testing for the variant allele necessary before treatment with quinone based cancer therapies.

## EXPERIMENTAL APPROACH AND METHODS

DNA was isolated from the blood samples using a Qiagen kit and diluted to 10ng/µL solutions. The polymorphic alleles of the human NQO2 promoter samples were amplified from the DNA samples using polymerase chain reaction (PCR) as in Gaedigk et al. Twenty nanograms of genomic DNA were used as the template.

The PCR products were digested with Hinf I and incubated for three hours at 37°C before being separated in the gel.

PCR products were resolved on a 1.5% agarose-ethidium bromide gel in 0.5 X Tris-borate-EDTA buffer and photographed under UV light. Electrophoresis was carried out at 100V for two hours at room temperature.




The length of the uncut PCR amplicon from the alleles was 283 base pairs. The lengths of the polymorphic allele pieces post digestion by Hinf1 were 151 and 132 basepairs long.

Wild Type Variant Heterozygous

## INTRODUCTION

Pharmacogenomics is the study of the relationship between genetic variations and drug response. Adverse drug reactions (ADRs) occur over 2 million times a year causing an estimated 137,000 deaths according to a metanalysis done by Lazarou et al (2) in 1998 at the University of Toronto. Many of these ADRs are caused by single nucleotide polymorphisms (SNPs) in genes encoding enzymes. Variations of these genes lead to different efficiencies; either increased or decreased, in the way the enzymes process toxins and drugs. Increased metabolism leads to ineffective drugs and decreased metabolism leads to toxicity. The development of personalized medicine would allow patients to receive a drug or dosage resulting in the least side effects for their genetic makeup.

NQO1 is a two-electron quinone oxidoreductase that prevents the one electron reduction of quinones which results in the production of radical species. In essence this phase II enzyme catalyzes detoxification reactions. A single nucleotide polymorphism has been characterized on the gene, NQO1*2, having significant phenotypic consequences. A “C” to “T” basepair change at position 609 produces a mutant NQO1 protein quickly degenerated. If an individual is heterozygous for the polymorphism metabolism is slowed, but if they are homozygous for the variant allele a null phenotype results.

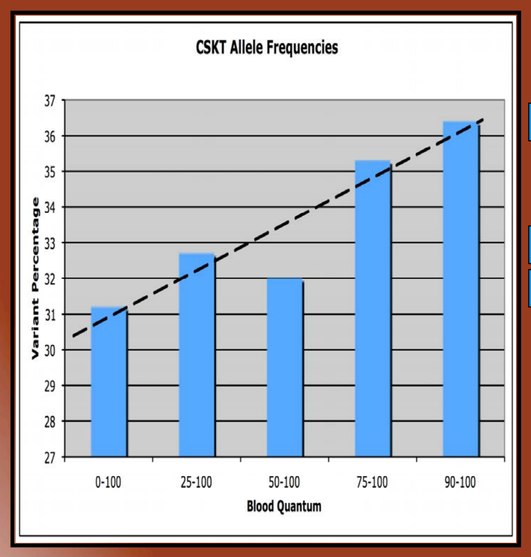
NQO1’s high expression levels in human solid tumors makes it a target for cancer therapy due to its potential for selective toxicity. With the advent of NQO1 targeting drugs, a patient’s NQO1 protein expression rate becomes important if ADRs and/or failed drug response are to be avoided. In addition, low NQO1 activity has also been linked to increased cancer susceptibility.

The allele frequency of NQO1 has been characterized in most ethnic populations. These frequencies are essential for the prescription of quinone based drugs and are useful in the early detection of cancer. The aim of this study is to characterize the allele frequency for CSK tribal members. If the frequency of a specific ethnicity is high genetic tests should be performed before the patient is given NQO1 targeted drugs or alternative drugs prescribed. By characterizing the allele frequency of the CSK tribal members we are providing them with the opportunity to receive the same level of healthcare available to patients of other ethnicities.

## RESULTS & CONCLUSION

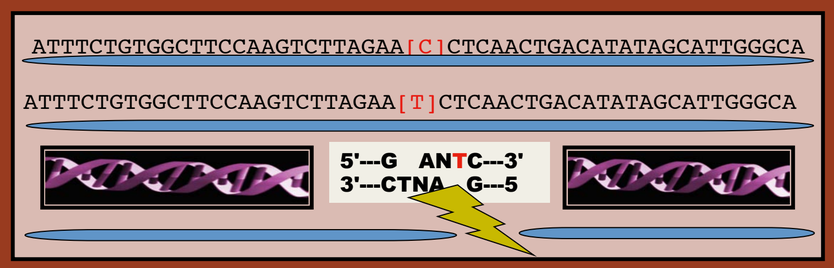
- As the blood quantum samples approached 100% the variant allele frequency approached 36%. This number is more than twice as large as the Caucasian NQO1*2 frequency.
- This allele frequency is crucial for helping doctors correctly prescribe medication to CSK patients, avoiding unnecessary and sometimes fatal ADRs.
- This data could help explain the elevated rate of certain cancers in American Indian populations.

## ALLELE FREQUENCY VS BLOOD QUANTA




| Blood Quanta | Sum of T | Sum of C | Allele Frequency | n |
|---|---|---|---|---|
| 0-100 | 93 | 205 | 31.2 | 149 |
| 25-100 | 87 | 179 | 32.7 | 133 |
| 50-100 | 48 | 102 | 32.0 | 75 |
| 75-100 | 24 | 44 | 35.3 | 34 |
| 90-100 | 8 | 14 | 36.4 | 11 |

## Hinf1 Cutting Mechanism




Cancer Rates in Native Americans vs Non-Hispanic Whites in the Northern Plains Region (3)

| Cancer Type | Ratio(NA/NHW) |
|---|---|
| All | 1.17 |
| Gallbladder | 3.97 |
| Liver | 2.98 |
| Penis | 2.9 |
| Stomach | 2.21 |

NQO1*2 Allele Frequencies in Selected Ethnicities (1)

| Ethnicity | Wild Type | Variant |
|---|---|---|
| Caucasian | 84% | 16% |
| **CSKT** | **64%** | **36%** |
| First Nation | 60% | 40% |
| Inuit | 54% | 46% |
| Chinese | 51% | 49% |

## REFERENCES

(1) Gaedigk, Andrea et al. NAD(P)H: quinone oxidoreductase: polymorphisms and allele frequencies in Caucasian, Chinese and Canadian Native Indian and Inuit populations. Lippincott-Raven Publishers. 1998. 25 November 1997
(2) Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. *JAMA.* 1998;279(15): 1200-1205.
(3)Wiggens, Charles L. An Update on Cancer in American Indians and Alaska Natives, 1999-2004. American Cancer Society. 2008

## ACKNOWLEDGMENTS

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