# Understanding the Association of CYP2A6 Gene Variations with Drug Resistance in Prostate Cancer

Katherine G. Marlow<sup>1,\*</sup>, Abigail Weir<sup>2</sup>, Razan Waliagha<sup>3,4</sup>, and Amit Kumar Mitra<sup>3</sup>

<sup>1</sup> Department of Biological Sciences, Auburn University
<sup>2</sup> Department of Chemistry and Biochemistry, Auburn University
<sup>3</sup> Harrison College of Pharmacy, Auburn University
<sup>4</sup> Center for Pharmacogenomics and Single-Cell Omics initiative (AUPharmGx), Auburn University

Pharmacogenomics is an emerging field of study that focuses on investigating the genetic basis of inter-individual variations in drug response, specifically linked to Single Nucleotide Polymorphisms (SNPs). SNPs are genetic variations/mutations at a single base position in a DNA sequence and are the most common type of genetic variation. Certain SNPs within drug metabolism enzymes (DMEs) could lead to the development of adverse drug reactions or toxicity following drug treatment, which we aimed to investigate [1]. Prostate cancer (PCa) is the second leading cause of cancer-related deaths among men in the US [2]. Treatment options for mCRPC patients are docetaxel or cabazitaxel (types of chemotherapy drugs) alone or in combination with other drugs or immunotherapy. However, it is typical that these chemotherapy drugs only slightly improve survival by 3-4 months in patients, often resulting in more aggressive variants of aggressive or lethal PCa [3]. Certain mutations in drug metabolizing enzymes (DMEs) could lead to the development of adverse drug reactions, caused by the change in efficacy or toxicity of the drug. Having this genetic information will aid in prescribing the best treatment for a beneficial outcome while also avoiding unwanted effects in a particular patient in a timely manner [4]. CYP2A6 enzyme is responsible for metabolizing a wide variety of drugs, including anti-cancer agents (Table 1). Discerning genetic diversity within the CYP2A6 gene may provide a unique resource in understanding the population-specific role of SNPs in drug resistance within prostate cancer, including aggressive PCa forms metastatic castration resistance PCa (mCRPC). Previously, we used DNA samples from ~2000 healthy individuals from >50

\* Corresponding author: gm0045@auburn.edu

sub-populations across the world and performed targeted exon sequencing followed by multiple sequence alignment and bioinformatics analysis and identified several potentially relevant SNPs within CYP2A6.

Drug	Role
Tegafur	Prodrug component in combination therapies
Letrozole	Aromatase inhibitor for postmenopausal hormone receptor-positive breast cancer
Valproic acid	Antiepileptic with potential anticancer effects via HDAC inhibition
Fadrozole	Aromatase inhibitor for hormone receptor- positive breast cancer
Erlotinib	EGFR inhibitor for non-small cell lung and pancreatic cancer
Ifosfamide	Alkylating agent for various cancers, including prostate cancer
Lapatinib	Dual tyrosine kinase inhibitor for HER2- positive breast cancer

**Table 1.** Anti-cancer drugs potentially metabolized byCYP2A6.

Our overarching goal is to identify prostate cancer cell lines displaying variable responses to primary anti-cancer drugs. This critical step lays the foundation for understanding the interplay between genetic variations and drug sensitivity, paving the way for personalized therapeutic approaches. A key aspect of our study is to discern the association between cytotoxicity and the presence of functionally important CYP2A6 SNPs. By honing in on this specific gene, we aim to unravel its role in mediating drug response and potentially identify novel targets for therapeutic interventions.

Commencing our study, a thorough bioinformatic analysis was executed to discern patterns and potential functional ramifications of previously identified SNPs. This initial computational analysis laid the foundation for subsequent investigations, providing a comprehensive map of genetic variations within the prostate cancer context. Expanding our analytical scope, SNPs were selected based on their predicted impact on protein function. Additional SNPs were included based on their predicted effect on protein function using SIF and PolyPhen. So far, we have identified nine SNPs within CYP2A6 in several world subpopulations. Bioinformatics analysis revealed that several of these SNP may be deleterious in effect, including rs4997557 and rs8192730.

Next, we used a panel of prostate cancer cell lines, spanning a spectrum of aggressiveness, which were cultured and subjected to cytotoxicity assays using standard-ofcare drugs (Fig. 1). To quantify the cell viability and response to standard-of-care drugs from the in vitro cytotoxicity studies, we utilized the MTT assay. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is a widely used colorimetric assay employed in cellular and molecular biology to assess cell viability and proliferation. This assay is based on the reduction of the yellow tetrazolium salt MTT by mitochondrial dehydrogenases in metabolically active cells, producing a purple formazan product. The intensity of the color is directly proportional to the number of viable cells [5].

In our upcoming phases, we plan to employ quantitative PCR (qPCR) with TaqMan gene expression assays to correlate CYP2A6 gene expression with drug response in wild type and mutant prostate cancer cell lines.

TaqMan assays utilize a fluorogenic 5' nuclease probe that is specific to the target gene of interest. These probes are designed to anneal to the target sequence during the PCR amplification process. The 5' nuclease activity of Taq DNA polymerase cleaves the probe, releasing a fluorescent signal that is proportional to the amount of amplified DNA [6]. This real-time quantitative PCR (qPCR) technology allows for the accurate quantification of gene expression levels, enabling researchers to analyze changes in gene expression under different experimental conditions or in response to various treatments This advanced molecular technique will allow us to delve deeper into the mechanistic underpinnings of drug sensitivity, providing valuable information for the development of targeted therapies.



**Dose-response curve DTX** 

**Figure 1.** Dose-response curve illustrating the pharmacological effect of Docetaxel across a range of concentrations in DU145 PCa cell line.

As our study progresses, the integration of diverse methodologies and comprehensive analyses promises to unravel the complexities of prostate cancer genetics, ultimately contributing to the advancement of personalized medicine in the field of oncology.

### **Statement of Research Advisor**

Katie's research involves the functional analysis of key drug metabolizing gene mutations using a combination of several dry-lab and wet-lab approaches: Bioinformatic analysis of high-risk variations, drug sensitivity studies and molecular biology-based validation of top SNP biomarkers. We are planning to further this work with a particular focus on world populations and under-served subpopulations.

- Amit K. Mitra, Harrison College of Pharmacy

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## **Authors Biography**



Katherine G. Marlow is a senior-year student pursuing a B.S. degree in Genetics at Auburn University. She has played key research roles in understanding pharmacogenomic biomarkers in drug metabolism genes and DNA repair gene



Abigail Weir is a senior-year undergraduate pursuing a B.S. degree in Biochemistry at Auburn University. She has performed mutational analysis of mutations in DNA repair genes such as LIG1 and MSH2.



Razan Waliagha is a current third year dual degree PharmD./PhD. student at Auburn University Harrison College of Pharmacy. Her current work involves understanding the novel mechanisms of resistance to anti-cancer therapis in solid tumors.



Dr. Amit K. Mitra is an Assistant Professor at the Harrison College of Pharmacy at Auburn University. He is also the founding Director of the Center for Pharmacogenomics and Single-Cell Omics (AU-PharmGx). Dr. Mitra's current research involves integrating single-cell multi-omics with in vitro drug

response modeling, and functional genomics with translational bioinformatics to investigate inter-tumor and intra-tumor (subclonal) heterogeneity to drug response in human cancers.