Ethnic and Gender Differences in CYP2A6 Expression and Progression of Hepatocellular Carcinoma

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Received May 28, 2024
Accepted November 21, 2024
Electronic access November 15, 2024

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer. This paper studies the role of the gene CYP2A6 (Cytochrome P450 2A6) and its differential expression in hepatocellular carcinoma (HCC). The expression of CYP2A6 has been shown to have significant impact on nicotine-related disease outcomes and very few studies have studied its impact on HCC, despite the fact that CYP2A6 is maximally expressed in the liver. This study uses tissue analysis and Kaplan-Meier survival analysis to explore CYP2A6 expression's impact on HCC survival outcomes for Asian versus non-Asian population groups and for male versus female population groups. Results indicate a significant link between CYP2A6 expression and patient prognosis, with strong variations in outcomes based on ethnicity and gender. The median survival time differences between groups with high and low CYP2A6 expression are significantly different between Asian ($\sim$9-fold) vs non-Asian populations ($\sim$2-fold). These findings are significant in the context of personalized medicine, suggesting future research directions to further understand the role of CYP2A6 in disease progression and treatment.

Introduction

The liver is a central organ in the metabolism of both internal and external compounds, including drugs and carcinogens. As part of the cytochrome P450 superfamily, CYP2A6 is involved in these metabolic processes, particularly in the conversion of procarcinogens into carcinogens. This process plays a key role in cancer progression, as the activation of procarcinogens can lead to DNA damage, mutations, and subsequent tumor development[1,2]. It has been found that CYP2A6 is expressed in multiple organs, but is predominantly expressed in the liver (Figure 1)[3], where its influence on liver diseases has been observed. It has also been shown that this gene shows notable variation in its activity across different populations, especially impacting nicotine-related disease outcomes in Asians[1,2]. The polymorphic nature, meaning the existence of multiple forms of a gene in a population due to genetic variation, of CYP2A6 and its effect on expression underscores the complexity of studying Cytochrome P450 2A6.

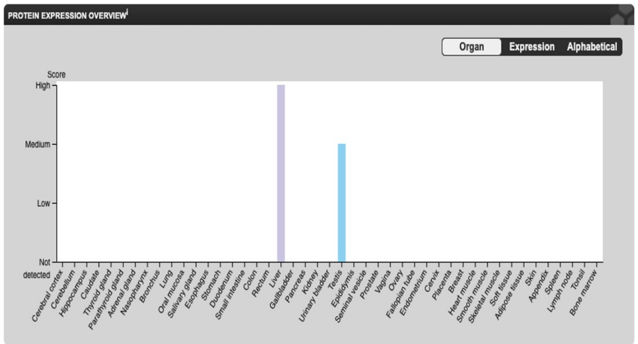


Fig. 1 CYP2A6 is maximally expressed in the liver

Prior Work

Multiple studies have examined the role of the cytochrome P450 (CYP) family in cancer development and progression, with a particular focus on hepatocellular carcinoma (HCC) due to the liver's central role in drug metabolism. One significant finding is that CYP enzymes, including CYP2A6, are crucial in the bioactivation of procarcinogens, substances that must be metabolized into carcinogenic compounds before they can cause cellular damage[1,2]. For example, Ren et al. (2018) demonstrated that downregulation of both CYP2A6 and CYP2C8 is linked to poorer overall survival and recurrence-free survival in HCC patients[4]. Similarly, Jiang et al. (2021) identified CYP2A6 as a potential biomarker for macrophage polarization in HCC tissues, proposing that its expression is associated with tumor microenvironment modulation and patient prognosis[5]. Other studies have highlighted the genetic variability of CYP2A6 activity among different ethnic groups, particularly in relation to nicotine metabolism and related cancers. Tanner et. al (2017) explored how genetic polymorphisms in CYP2A6 affect drug metabolism, identifying and noting that individuals of Asian descent tend to exhibit lower enzymatic activity, which can impact their response to cancer therapies[2]. This variability highlights the importance of considering both ethnic and genetic factors in personalized medicine approaches for HCC treatment. To further build on this foundational understanding, research has also explored how environmental and lifestyle factors can influence CYP2A6 activity, particularly in relation to HCC progression. For instance, Topcu et al. (2001) showed that the deletion of the CYP2A6 gene significantly reduces the risk of oral cancer among betel quid chewers, a common practice in certain Asian populations, linking CYP2A6 activity with carcinogen metabolism in culturally specific contexts[6]. Furthermore, studies have suggested that hormonal differences between males and females may modulate CYP2A6 expression, influencing cancer progression and patient outcomes. Estrogen, for example, has been shown to downregulate certain CYP enzymes, potentially affecting how CYP2A6 interacts with carcinogens and modulates HCC progression in female patients[7].

Focus of this work

The findings above show the complex interplay between genetic, environmental, and hormonal factors in determining the role of CYP2A6 in HCC, and highlight the need for a deeper exploration within this work. There is also a gap in the understanding of how CYP2A6 expression levels specifically influence survival outcomes in diverse ethnic and gender groups. This study aims to address that gap, building on the existing body of research by focusing on CYP2A6 expression's prognostic significance in HCC across different populations. Specifically, I study the correlation of CYP2A6 expression levels on HCC outcomes in Asian vs non-Asian populations and in male vs female patients. My hypothesis is that there is a significant link between CYP2A6 expression and patient prognosis, with strong variations in outcomes based on different ethnicities and genders.

Results

Kaplan-Meier analysis is a statistical method used to estimate the survival function from lifetime data. It provides a way to model the time until an event of interest occurs—typically, this event is death, disease recurrence, or some other significant endpoint in a clinical study. The Kaplan-Meier estimator generates a survival curve, which shows the proportion of subjects surviving (or free of an event) as a function of time. Utilizing Kaplan-Meier analysis[8,9] from a cancer database, GEO[3], the study examined the relationship between CYP2A6 expression and survival in hepatocellular carcinoma. Patients are classified into 'high' or 'low' CYP2A6 expression groups based on the relative levels of enzyme activity in their bodies. 'High expression' refers to elevated levels of CYP2A6. In contrast, 'low expression' indicates reduced enzyme activity. These groups were split along the median.

The results are based on survival outcomes, defined as the duration patients live after the diagnosis of HCC. The p-values are calculated using the log-rank test, which compares the survival distributions of the groups. A p-value of less than 0.05 is considered statistically significant, indicating a significant difference in survival between the groups. HR (Hazard ratio quantifying magnitude of survival probability) values are recorded.

As seen in Figure 2 that plots the probability of survival over a span of 10 years for cohorts with low (black) and high (red) expression, higher expression levels of CYP2A6 correlated with markedly better survival outcomes, with a p-value of 7e-4, underscoring its statistical significance. Notably, as seen in the tabulated data, there was a median difference of 37 months in survival rates favoring high CYP2A6 expression. This analysis robustly confirms the strong predictive capacity of CYP2A6 expression levels for survival rates in patients with hepatocellular carcinoma.

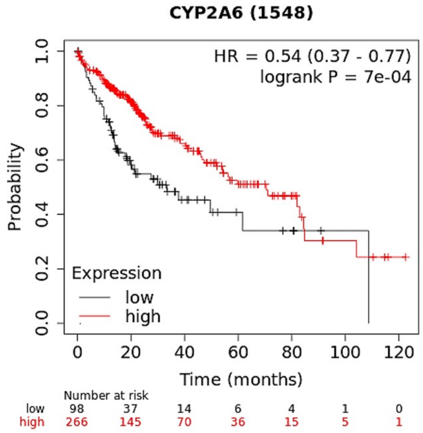


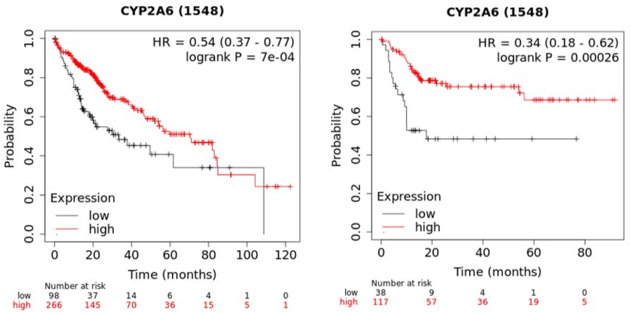
Fig. 2 CYP2A6 is a strong Hepatocellular Carcinoma Prognosticator

	Low Expression (months)	High Expression (months)
Probability of Survival	33.5	70.5

Table 1 Probability of survival over time based on expression levels.

Next, I study the impact of higher CYP2A6 expression and improved overall survival outcomes in hepatocellular carcinoma on Asian vs non-Asian populations. This is shown in Figure 3, which plots the probability of survival over time for cohorts with low (black) and high (red) expression for Asian and non-Asian populations.

The association, as shown in the Table below, is particularly strong in Asian populations, where the median survival time is significantly longer, with 9.5x median survival time differences compared to non-Asian populations (as available from the GEO datasets), with 2x median survival time difference. The very low p-values obtained in the analysis underscore the robust statistical significance of this data, reinforcing the crucial role of CYP2A6 expression in determining survival outcomes in hepatocellular carcinoma, especially among different ethnic groups.



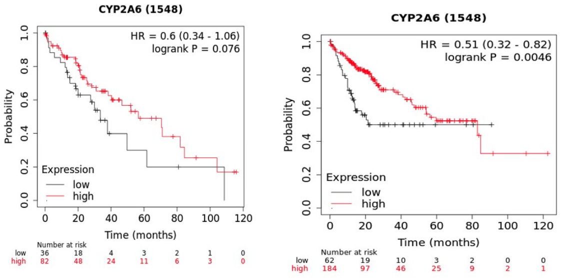
Asian survival *Non-Asian survival*

Fig. 3 CYP2A6 expression has a stronger impact on survival outcomes for Asians

Ethnicity	**Low expression survival (months)**	**High expression survival (months)**
Asian	5.7	54.1
Non-Asian	33.5	70.5

Table 2 Comparison of survival months based on ethnicity and expression levels

Next, I proceed to study the impact of CYP2A6 expression on survival outcomes in hepatocellular carcinoma for both genders. As shown in Figure 4, it is observed that males generally have worse outcomes compared to females. The strong p-values observed in the male population support the correlation between CYP2A6 expression and survival outcomes.



Female survival *Male survival*

Fig. 4 Males have worse outcomes than females in general

Gender	**Low expression survival (months)**	**High expression survival (months)**
Female	33.5	56.5
Male	10	25.6

Table 3 Comparison of survival months based on gender and expression levels

Discussion

Previous research[4,5] has studied the links between CYP450 family genes, including CYP2A6, in tumor tissues and hepatocellular carcinoma (HCC) outcomes. However, it was not understood if CYP2A6 expression levels can serve as an effective predictor of survival rates in HCC across ethnic and gender groups. Based on the results in this study, we have shown that CYP2A6's predictive strength is more pronounced in Asian ethnic groups and male populations. This highlights the need for future research, including larger datasets to validate these findings, investigation into gene clusters interacting with CYP2A6, and a focused study on CYP2A6 polymorphisms in the Asian population, akin to prior CYP2A6-nicotine research. Such studies could unravel the reasons behind observed ethnic disparities and pave the way for personalized treatment strategies for HCC in Asian populations. Additionally, while CYP2A6 demonstrates strong predictive power in males, outcomes for females appear less significant. The weaker correlation between CYP2A6 expression and survival in females could be due to hormonal influences, particularly the role of estrogen in regulating the activity of CYP enzymes. Previous research has indicated that estrogen can downregulate certain CYP enzymes, potentially impacting how CYP2A6 modulates cancer progression in female patients[7]. There may also be other gender-specific factors contributing, such as hormonal balance, liver physiology, or immune responses may play a role in improving outcomes in female HCC patients.

It is also important to recognize the potential limitations of the study. First, the sample size may have affected the ability to generalize findings across broader populations, as small cohorts can introduce variability within the results and reduce statistical power of those results. Additionally, the datasets used, although robust, could contain inherent limitations or biases.

Conclusion

Overall, this study demonstrates that CYP2A6 expression levels have a significant impact on survival outcomes for hepatocellular carcinoma patients, with pronounced variations observed across different ethnic and gender groups. High expression of CYP2A6 is strongly correlated with better prognosis, particularly in Asian populations and males. These findings underscore the potential of CYP2A6 as a biomarker for predicting patient outcomes and tailoring therapeutic interventions in HCC. The results support further exploration into the role of CYP2A6 in cancer treatment, particularly in the context of personalized medicine. Future research should continue to investigate the genetic and environmental factors that influence CYP2A6 activity, with a focus on developing targeted therapies for patients with high CYP2A6 expression.

Limitations and Future Recommendations

This study does have certain limitations that should be addressed in future research. First, the sample size, while it provides adequate for initial analysis, limits the generalizability of the findings across larger and broader populations. Larger cohorts would provide more robust data and increase the statistical power of the analysis. Additionally, the datasets used in this study, though well-validated, may contain inherent biases related to patient selection, clinical information, or geographic location, which could impact the results.

Another limitation involves the focus on CYP2A6 expression alone. While this gene looks to have a significant impact on HCC survival outcomes, other factors, such as genetic polymorphisms, co-regulatory genes, and environmental exposures, may also play a role in cancer progression. Future research should examine these additional variables to gain a more comprehensive understanding of how CYP2A6 interacts with other biological pathways.

Given its central role in carcinogen metabolism and its apparent impact on HCC, CYP2A6 represents a promising target for drug development. One potential approach could involve CYP2A6 inhibitors that reduce the enzyme's activity and thus decrease the bioactivation of carcinogens. Inhibiting CYP2A6, with compounds such as tranylcypromine, could theoretically lower the risk of cancer progression in patients with high exposure to environmental carcinogens, particularly those who smoke or are exposed to other environmental toxins. Another avenue for therapeutic intervention lies in gene-editing technologies, such as CRISPR, which could be used to target and modify CYP2A6 expression in the liver. While gene editing is still in its infancy as a clinical tool, the ability to downregulate or correct polymorphisms in CYP2A6 could provide a personalized approach to managing HCC risk, particularly in patients with high enzyme activity. Furthermore, the development of drugs that are less dependent on CYP2A6 for metabolism could offer another strategy for treating HCC patients. By identifying alternative metabolic pathways for drug breakdown, clinicians could tailor treatments to the patient's specific CYP2A6 expression profile, thus optimizing therapeutic outcomes and minimizing side effects.

Methods

Kaplan-Meier analysis is a statistical method used to estimate the survival function from lifetime data. It provides a way to model the time until an event of interest occurs—typically, this event is death, disease recurrence, or some other significant endpoint in a clinical study. The Kaplan-Meier estimator generates a survival curve, which shows the proportion of subjects surviving (or free of an event) as a function of time. Utilizing Kaplan-Meier analysis[8,9] from a cancer database, GEO[3], the study examined the relationship between CYP2A6 expression and survival in hepatocellular carcinoma. The specific criteria for dataset collection were based on: the availability of detailed demographic information, including ethnicity and gender, as well as complete data for clinical and survival for at least 100 patients. The resultant datasets were: GSE14520 and additional analysis was also done using datasets preloaded into the site kmplot.com[10].

The following experimental methodology is used in this study.

1. Find GEO dataset for hepatocellular diseases that have gene analysis for tissues with and without tumors.
2. Create R scripts to analyze CYP2A6 expression correlation on tissues with and without tumor.
3. Perform Kaplan Meier analysis and compare Asian versus non-Asian population. Analyze data based on strongest (=small) p-values for statistical significance
4. Perform Kaplan Meier analysis and compare Male versus Female population. Analyze data based on strongest (=small) p-values for statistical significance.

Acknowledgments

I would like to acknowledge Katie Sie at UC Berkeley CRISPR camp and Dr. Vamsi Boppana, whose mentorship provided guidance and encouragement for me to write this research paper.

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9 `https://www.r-project.org/`, R programming language:.

10 `https://www.proteinatlas.org/`, Protein Atlas:.