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## Hispanic ethnicity and the rs4880 variant in *SOD2* are associated with elevated liver enzymes and bilirubin levels in children receiving asparaginase-containing chemotherapy for acute lymphoblastic leukemia

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### Abstract

Asparaginase is an integral component of acute lymphoblastic leukemia (ALL)<sup>3</sup> treatment. Hepatotoxicity related to asparaginase is one of the most common treatment-related toxicities in ALL therapy. Hispanic children are at higher risk of developing ALL, and toxicities from ALL therapy. The rs4880 variant in the superoxide dismutase 2 (*SOD2*)<sup>4</sup> gene, a critical mitochondrial enzyme that protects cells against oxidative stress, was found to be associated with increased incidence of asparaginase-related hepatotoxicity in adult cohort of largely White non-Hispanics patients with ALL. The risk genotype (rs4880-CC) is more frequent among adult Hispanic patients with ALL. To assess the prevalence of hepatotoxicity and risk genotype among pediatric patients with ALL, particularly of Hispanic ethnicity, we conducted a prospective study of 143 pediatric patients with ALL (62.2% Hispanic). Bilirubin and hepatic transaminase levels were collected at

<sup>3</sup>Acute Lymphoblastic Leukemia ALL

<sup>4</sup>Superoxide Dismutase 2 SOD2

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**Sharon Wu:** Methodology, Writing – original draft. **Mengxi Wang:** Methodology, Formal analysis, Visualization, Writing – original draft. **Amani Alqahtani:** Methodology, Formal analysis, Writing – original draft. **Mimi Lou:** Methodology, Formal analysis, Visualization, Supervision, Writing – original draft. **Wendy Stock:** Writing – review & editing. **Deepa Bhojwani:** Conceptualization, Data curation, Resources, Writing – review & editing. **Houda Alachkar:** Conceptualization, Writing – original draft, Visualization, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Conflict of Interest statement

The authors have no conflicts of interest to declare.

different times during multiagent therapy including asparaginase treatment. Germline DNA blood samples were genotyped for the *SOD2* rs4880. We found that the frequency of hepatotoxicity and the rs4880-CC risk genotype are higher in Hispanic patients than non-Hispanic. Patients with the CC genotype exhibit higher bilirubin and hepatic transaminase levels compared with patients with the TT and CT genotypes. In a multivariate Cox analysis, Hispanic ethnicity was identified as a strong predictor of hepatotoxicity (hazard ratio [HR] = 1.9, 95% confidence interval [95% CI] 1.0–3.5,  $p = 0.05$ ). Altogether, these findings demonstrate that hepatotoxicity is highly prevalent among Hispanic pediatric patients with ALL, and those with rs4880-CC genotype.

## Keywords

ALL; Asparaginase; Hepatotoxicity Pediatric; Hispanic; *SOD2* gene; Rs4880 polymorphism

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## 1. Introduction

Acute Lymphoblastic Leukemia (ALL) is a malignancy of the lymphoid lineage that occurs due to the abnormal proliferation and a block in differentiation of lymphoid progenitor cells [1]. ALL occurs more frequently in children; more than 50% of patients diagnosed with ALL are younger than 20 years [1]. Pediatric patients have an overall survival (OS) rate of more than 90%, [2,3] whereas adult patients have a 5-year OS of approximately 44–59% [4,5]. Several factors have contributed to the improved OS in pediatric patients with ALL, including intensification of multiagent chemotherapy and incorporation of optimal risk stratification based on leukemia subtype and early response to therapy [3,6,7].

The intensive use of Asparaginase is an essential component in treating pediatric ALL patients and has contributed to the increased survival in this population [8–10]. However, treatment-related toxicity (TRT) remains a challenge for ALL treatment in both pediatric and adult patients. TRT can often restrict delivery of optimal doses of drugs and increases risk of morbidity [6]. Hepatotoxicity is one of the most common dose-limiting TRT in ALL therapy, particularly in asparaginase-containing regimens [6]. It has been reported that the incidence of grade 3 or 4 elevation in liver enzymes and hyperbilirubinemia is 20% and 3% in pediatric ALL patients, respectively [11]. In a retrospective cohort study of 262 children and adolescents with ALL, it was reported that 27% of patients developed hepatotoxicity [6]. Asparaginase related hepatic dysfunction, including abnormalities in liver function and elevations in hepatic transaminases, bilirubin and alkaline phosphatase, often result in significant delays in treatment, and that may have a negative impact on the overall clinical outcome of patients [12]. On the other hand, inability to deliver optimal asparaginase therapy may negatively impact survival,[13] therefore it would be beneficial to predict which patients are at higher risk of toxicity to enable enhanced monitoring and hepatoprotective measures [14].

Children, adolescent and young adults (AYA) of Hispanic ethnicity have the highest incidence of ALL, and they also suffer inferior outcomes likely due to increased incidence of high-risk genetic subtypes and other factors [15,16]. Hispanic patients developed hepatotoxicity at an increased rate compared with other ethnicities, with a trend towards

increased severity of toxicity and death among Hispanic patients with a body mass index (BMI)  $\geq 25$  [17,18]. Obese and/or older children are particularly at risk for hepatotoxicity [6].

L-asparaginase, a bacterial enzyme derived from *E. coli*, depletes circulating asparagine and glutamine in the blood [12]. Asparaginase converts asparagine to aspartate and glutamine to glutamate, thereby starving leukemic cells that do not express asparagine synthetase which converts aspartate to asparagine for use in cellular activities [12]. The depleting of asparagine and glutamine activates amino acid stress response [19,20]. Oxidative stress, mediated by excessive reactive oxygen species (ROS), occurs due to anticancer treatment, leading to increase mitochondrial permeabilization and consecutive cell apoptosis [20,21]. Oxidative stress is the most common mechanism of drug-induced hepatotoxicity [19]. Several antioxidant enzymes are involved in neutralizing free radicals and protecting cells against oxidative stress related cell damage [21]. SOD2 is a critical mitochondrial enzyme that protects cells against oxidative stress by catalyzing the dismutation of free  $O_2^-$  to  $H_2O_2$  and  $O_2$  [20]. The superoxide dismutase (SOD) family includes three enzymes—SOD1, SOD2, and SOD3. Of these three enzymes, SOD2 is localized in the mitochondria and is the most important one for aerobic survival [22,23]. A SNP, rs4880 was identified in the mitochondrial targeting sequence (MTS) of SOD2 to cause either a valine (C allele) or alanine (T allele) substitution at the 16th amino acid residue of the MTS [22]. This rs4880 polymorphism has been found to be significantly associated with drug-induced liver injury (DILI) [24,25]. We previously found that adult patients with ALL carrying the CC genotype of *SOD2* had significantly higher incidence of asparaginase-induced hepatotoxicity [19]. The study was conducted in patients of European ancestry that included very few Hispanic patients [19]. Hispanics not only have a greater risk of development of ALL compared with other ethnicities [26–29], but also have a higher frequency of the CC genotype of *SOD2* and higher incidence of developing hepatotoxicity after asparaginase treatment.

Herein, we report the results of a prospective study to evaluate the prevalence of asparaginase induced hepatotoxicity and investigate the genetic contribution of the *SOD2* rs4880 SNP in a primarily Hispanic pediatric patient cohort.

## 2. Methods

### 2.1. Patient data and samples

Blood samples, demographic and clinical data were obtained from 143 pediatric patients who were treated for ALL between January 2015 and August 2019 at Children's Hospital Los Angeles. Ethnicity was self-reported. The demographic data on the Hispanic patients were available for 76 (85.4%) of 89, where 43 (56.6.0%) of them were Mexican, 5 (6.6.%) were from Guatemala, 3 (3.9%) were from El Salvador, 3 (3.9%) were from the USA, 1 (1.3%) was from Guam, 1 (1.3%) was from Nicaragua, 2 (2.6%) were multiethnic, and 18 (23.7.0%) were mixed of different Hispanic origin. Subjects were all treated following pediatric ALL multiagent chemotherapy regimens, which include asparaginase. Consent for use of blood samples and clinical data for this study was obtained from patients or guardians and assent obtained when appropriate. The use of human materials was approved by the

University of Southern California Health Sciences Campus Institutional Review Board in accordance with the Helsinki Declaration.

## 2.2. Hepatotoxicity assessment

Data were collected from 143 pediatric patients with ALL for aspartate aminotransferase (AST), alanine aminotransferase (ALT), and bilirubin levels. These data were collected for each subject during multiple visits before and until 4 months after asparaginase-based treatment. Hepatotoxicity was graded per the Common Terminology Criteria for Adverse Events (CTCAE) version 5 as follows: Grade 3 transaminitis when AST or ALT  $> 5\text{--}20 \times$  upper limit of normal (ULN) where the institutional ULN is 46 Units/L for AST and 42 Units/L for ALT. Grade 4 transaminitis when AST and ALT  $> 20 \times$  ULN, Grade 3 hyperbilirubinemia when total bilirubin  $> 3\text{--}10 \times$  ULN where the ULN level of bilirubin is 1.30, Grade 4 hyperbilirubinemia when bilirubin  $> 10 \times$  ULN. Hepatotoxicity was defined as (a) elevation of both AST and ALT levels at grade 3, or (b) either AST or ALT grade 4, or (c) bilirubin grade 3 or 4.

## 2.3. DNA extraction and genotyping

Blood samples were collected from patients after remission was achieved, and cell pellets were stored at  $-80\text{ C}$ . DNA was isolated using the Taqman™ Sample-to-SNP™ kit and DNeasy Blood & Tissue kit. Genotyping was performed using the TaqMan Allelic Discrimination Assay for rs4880 of *SOD2* gene using the TaqMan™ Genotyping Master Mix (Thermo Fisher, Waltham, MA) and the TaqMan™ GTXpress™ Master Mix (Thermo Fisher, Waltham, MA). The PCR and allelic discrimination were carried out using the QuantStudio 12 K Flex Real-Time PCR System (Applied Biosystems, Foster City, CA).

## 2.4. Statistical analysis

Patient characteristics were reported as mean  $\pm$  standard deviation (SD). Deviations from Hardy–Weinberg equilibrium were considered using Chi-squared tests. For continuous variables, two independent samples t-test or Wilcoxon-Mann Whitney test (non-parametric) was used to determine difference between two groups, and one-way ANOVA test or Kruskal–Wallis test (non-parametric) was used to compare difference among more than two groups. For categorical variables, comparisons between groups were performed by Chi-square or Fisher's exact test. Cox Proportional Hazards models were used to evaluate the predictive factors of hepatotoxicity as defined above overtime. BMI of children from 2 to 20 years old was calculated based on 2000 CDC Growth Charts, children  $< 2$  years ( $N = 4$ ) were not included in BMI analysis, since BMI is not a good measurement for this age group [30]. Obesity was defined by the BMI being  $\geq 95$ th of the Centers for Disease Control and Prevention (CDC) normative BMI percentiles for children or adolescents from age 2–20 years. Whereas children  $< 2$  years of age were defined as obese if their sex-specific weight for recumbent length is  $\geq 97.7$ th percentile on the World Health Organization (WHO) charts. A p value of  $< 0.05$  denotes statistical significance. All statistical analysis was performed using SAS version 9.4 (Cary, NC).

## 2.5. Health Facts dataset analysis

We searched the Health Facts database from 2007 to 2016 to find ALL (both children and adults) patients with ALL as primary diagnosis. Data in Health Facts is extracted directly from the electronic medical record (EMR) from hospitals in which Cerner has a data use agreement. Encounters may include pharmacy, clinical and microbiology laboratory, admission, and billing information from affiliated patient care locations. All admissions, medication orders and dispensing, laboratory orders and specimens are date and time stamped, providing a temporal relationship between treatment patterns and clinical information. Cerner Corporation has established Health Insurance Portability and Accountability Act-compliant operating policies to establish de-identification for Health Facts.

The patients were grouped into asparaginase group (patients who received at least one time asparaginase after diagnosis) and No- asparaginase group (patients who received other chemo, but no asparaginase). Bilirubin, ALT or AST levels were obtained on encounters in which medication start time was earlier than the lab tests. If any of bilirubin, ALT or AST was higher than normal range, then the test result was counted as abnormal. Normal range of each test were provided by Health fact. Patients with at least one abnormal result were counted as abnormal.

## 3. Results

### 3.1. Demographics of study population

One hundred forty-three pediatric patients with ALL were recruited to this study. The demographics and presenting features of patients are summarized in Table 1. Approximately, 62.2% of the cohort were Hispanic (n = 89). The age of patients ranged from < 1–20 years. The average body mass index (BMI) was 20.4(± 6.0). Among the 143 patients, 37.1% were female. Patients were treated with contemporary ALL regimens based on NCI risk, immunophenotype, and minimal residual disease response at day 29 of therapy: 67 patients (46.9%) were treated with chemotherapy regimens for standard-risk B-ALL, 60 (41.9%) for high-risk B-ALL and 8 (5.6%) for T-ALL. Eight patients (5.6%) received tyrosine kinase inhibitors in combination with chemotherapy for Philadelphia (Ph) chromosome-positive, or Ph-like ALL.

### 3.2. Assessment of hepatotoxicity in pediatric patients with ALL

Asparaginase related hepatotoxicity was estimated by assessing the following clinical laboratory biomarkers: bilirubin levels, aspartate transaminase (AST) and alanine transaminase (ALT) in patients before starting asparaginase and at a different time point during asparaginase therapy. The mean levels of bilirubin, AST and ALT before starting asparaginase for the study cohort were 1.34 mg/dl, 61.85 units/liter, and 104.55 units/liter, respectively. Among the 143 patients, 13 (9.1%) patients developed grade 3 elevated bilirubin levels, and 3 (2.1%) and 55 (38.5%) patients had grade 4 and 3 elevated AST levels respectively. Nineteen (13.3%) and 109 (76.2%) developed grade 4 and 3 elevated ALT levels respectively. Hepatotoxicity defined as grade 3 of both AST and ALT, grade 4 of either AST or ALT, or grade 3 of bilirubin elevation was observed in 60 (41.9%) patients.

### 3.3. Hispanic patients are at higher risk of developing hepatotoxicity

Patient's characteristics according to their ethnicity, Hispanic and non-Hispanic, are summarized and compared in Table 2. There was a statistically significant difference in BMI between Hispanic and non-Hispanic patients ( $p = 0.002$ ). Accordingly, obesity was more frequent among Hispanic patients compared with non-Hispanic patients ( $p = 0.02$ ). Among the 89 Hispanic patients, 11 (12.4%) had grade 3 or 4 elevated bilirubin, while only 2 of 54 (3.7%) non-Hispanic patients had grade 3 or 4 elevated bilirubin during asparaginase treatment. There was a statistically significant difference in both grade 4 and grade 3 or higher elevated ALT levels between Hispanic and non-Hispanic patients ( $p = 0.01$ ), with grade 4 observed in 17 (19.1%) and 2 (3.7%) and grade 3 in 74 (83.2%) and 35 (64.8%) in Hispanic and non-Hispanic, respectively (Table 2). Additionally, 38 (42.7%) of Hispanic patients showed an elevation in the AST level at grade 3, whereas only 17 (31.5%) of non-Hispanic had this level of elevation. Consequently, hepatotoxicity was more frequent among Hispanic patients than non-Hispanic, 42 (47.2%) and 18 (33.3%), respectively ( $p = 0.10$ ).

There was no significant difference in the mean baseline level of bilirubin and liver enzymes between Hispanic and non-Hispanic ALL patients (bilirubin: 1.1 vs. 1.8 mg/dl,  $p = 0.58$ ; AST: 65.7 vs. 55.6 units/liter,  $p = 0.61$ ; ALT: 113.1 vs. 86.7 units/liter,  $p = 0.49$  Table 3); however, the significant difference was identified during chemotherapy treatment. We compared the mean enzyme levels of bilirubin, AST and ALT between Hispanic and non-Hispanic. We found that Hispanics exhibited significant higher levels of AST and ALT compared with non-Hispanic patients. Bilirubin mean levels were slightly higher in non-Hispanics but likely driven by one outlier value (bilirubin: 1.0 mg/dl vs. 1.1 mg/dl,  $p = 0.04$ ; AST: 101.8 vs. 77.9 units/liter,  $p = 0.004$ ; ALT: 171.7 vs. 112.9 units/liter,  $p = 0.004$ , Fig. 1).

### 3.4. The CC genotype of the rs4880 SNP is more frequent in Hispanic patients than non-Hispanic patients with ALL

The CC, CT, and TT genotypes were found in 46 (32.2%), 70 (49.0%), and 27 (18.9%) of the study cohort, respectively. These results did not deviate from the Hardy-Weinberg equilibrium (Chi-square ( $\chi^2$ ) test  $P = 0.96$  with 1 degree of freedom). We compared the frequencies of the variant genotype, CC, between Hispanic and non-Hispanic patients. The frequency of the CC genotype is higher in Hispanic patients compared with non-Hispanic patients (42.7% vs 14.8%). We found that the CT genotype was slightly lower in Hispanic patients compared with non-Hispanic patients (44.9% vs 55.6%). The TT genotype was significantly lower in Hispanic patients compared with non-Hispanic pediatric patients (12.4% vs 29.6%). The data is consistent with previously reported frequency of the CC genotype being more frequent among Hispanics than non-Hispanics (HSP-GENO-panel cohort;  $N = 108$  and Hispanic patients with ALL;  $N = 16$ ) [19].

### 3.5. The rs4880 SNP CC genotype is associated with high BMI and elevated liver enzymes in pediatric patients with ALL

Patient clinical characteristics, including hepatotoxicity parameters according to their rs4880 genotypes, CC, CT or TT, are summarized in Table 4. We tested the two possible

genetic models, including recessive and additive. The recessive inheritance model was most significant and thus was further implemented in the multivariate analysis. This was consistent with our previous study in adult patients [19]. Patients with CC genotype had higher BMI levels than patients with the CT or TT genotypes (22.1 ( $\pm$  6.9) vs. 19.6 ( $\pm$  5.4),  $P = 0.03$ ). The CC genotype was found more frequently in patients who experienced either elevated AST, ALT, or hepatotoxicity, as defined previously. Grade 3 bilirubin was found more frequently in patients with the *SOD2*rs4880 CC genotype compared with those with the CT or TT genotypes (7 (15.2%) vs. 6 (6.2%) patients). Additionally, grade 4 and grade 3 of elevated AST was reported in 3 (6.5%) and 23 (50.0%) patients with CC genotype compared with 0 (0.0%) and 32 (33.0%) patients with CT and TT genotype, respectively (CC vs. CT or TT; Grade 4:  $p = 0.03$ ; Grade 3:  $p = 0.05$ ). We also found that hepatotoxicity as defined above occurred in 24 (52.2%) patients with CC genotype, and in 36 (37.1%) patients with CT and TT genotype, further showing a higher frequency of CC genotype in individuals who developed hepatotoxicity during chemotherapy.

Mean bilirubin and liver enzyme levels (AST and ALT) during chemotherapy were compared among the genotype groups using recessive model (CC vs. CT or TT), Fig. 2. There was a statistically significant difference in bilirubin, AST and ALT mean levels between patients with CC genotype and those with CT or TT genotype (bilirubin: 1.1 vs. 1.0 mg/dl,  $p < 0.001$ ; AST: 105.0 vs. 68.7 units/liter,  $p = 0.02$ ; ALT: 169.1 vs. 139.4 units/liter,  $p = 0.02$ ). We also analyzed the enzyme levels by genotype using additive model CC vs. CT vs. TT (Fig. 2). There was a statistically significant difference between individuals with CC, CT, and TT genotype in the mean level of bilirubin (bilirubin: 1.1 vs. 1.0 vs. 0.9 mg/dl,  $p = 0.002$ ). There was not a statistically significant difference in the mean of AST and ALT level between those three groups (AST: 105.0 vs. 86.8 vs. 86.6 units/liter,  $p = 0.06$ ; ALT 169.1 vs. 139.7 vs. 138.7 units/liter,  $p = 0.06$ ).

### 3.6. Hispanic ethnicity is strong predictor of hepatotoxicity in pediatric patients with ALL

We tested whether the risk of hepatotoxicity secondary to asparaginase treatment is attributable to several factors, including ethnicity (Hispanic), the risk genotype of rs4880 variant (CC), age, BMI, obesity, and gender (female), by the univariate analyses (Table 5). We performed a multivariable Cox analysis for predicting incidence of hepatotoxicity with respect to these factors. We implemented two multivariate models one included BMI for 139 patients excluding those  $< 2$  years (Table 6) and other included obesity for all patients including those  $< 2$  years (Table 7). We found that patients' ethnicity (hazard ratio [HR] Hispanic=1.9, 95% CI 1.0–3.5,  $P = 0.05$ ; 1.8, 95% CI 1.0–3.3,  $P = 0.04$ , respectively) was associated significantly with incidence of hepatotoxicity over time in two models, after controlling other possible confounding factors such as genotype, age, gender, and either BMI or obesity in the model (Tables 6 and 7). These results suggest that Hispanic ethnicity is a strong predictor of hepatotoxicity in pediatrics with ALL.

### 3.7. Elevations in liver enzymes and bilirubin occur more frequently post asparaginase treatment compared with other chemotherapies

We identified 1532 encounters in total corresponding to 586 adult and pediatric patients with ALL in the Health Facts database between 2007 and 2016. 78 patients were missing

laboratory test information. When comparing the frequency of abnormal lab tests (bilirubin, ALT or AST) between patients who received asparaginase with patients received other chemotherapies that did not include asparaginase, we found that 83.7% of patients who received asparaginase had abnormal (bilirubin, ALT or AST) lab tests compared with 68.1% of patients who received other chemotherapies ( $P = 0.002$ ).

#### 4. Discussion

In the current study, the prevalence of hepatotoxicity and risk genotype of rs4880 (CC) was assessed prospectively in a cohort of pediatric patients with ALL, to seek potential association between this variant and incidence of asparaginase related hepatotoxicity. We identified the frequency of hepatotoxicity and elevated liver enzymes of pediatric patients with ALL during chemotherapy and stratified these parameters by ethnicity (Hispanic and non-Hispanic) and *SOD2* SNP rs4880 genotypes (CC and CT or TT). We found that Hispanic patients with ALL are more susceptible to developing hepatotoxicity than non-Hispanic during chemotherapy. We found a significant difference in the frequency of high grades elevated liver enzymes, particularly in ALT during asparaginase treatment in Hispanics compared with non-Hispanics. This was also consistent with higher mean liver enzyme levels observed in Hispanic than non-Hispanic pediatric patients with ALL. Our findings are in line with previous reports showing that the Hispanic pediatric patients with ALL were more likely to experience elevated liver enzyme (ALT) post induction chemotherapy than other ethnicities [31]. Although several factors may influence the risk of developing hepatotoxicity, our study shows that in the multivariate cox analysis, the Hispanic ethnicity is the strongest predictor of hepatotoxicity. Altogether, our study further confirms that being Hispanic contributes to an increased risk of developing hepatotoxicity secondary to asparaginase. A limitation of the study is the use of self-identified ethnicity rather than a genomic approach. The Hispanic ethnicity includes different nationalities with various genetic admixtures. Hispanic patients in our cohort were mainly from Mexico. Mexicans have a heterogeneous genetic admixture by their region and evaluating genetic admixtures would improve our study [32,33].

Importantly, in the present study, we found that the CC genotype of rs4880 was more frequent in Hispanic pediatric patients with ALL than non-Hispanic. This is consistent with our previous study which included very few Hispanic patients, yet showed that 50% of the Hispanic adult patients with ALL carry the CC genotype [19]. Additionally, our study showed that patients with the CC genotype had higher liver enzyme and bilirubin levels compared with patients carrying the CT and TT genotypes. Also, the frequency of the elevated liver enzymes' especially in AST was higher in patients with CC genotype compared with patients with CT or TT genotype. This finding is consistent with our previous study that reported the association between the CC genotype of rs4880 and increased hepatotoxicity after asparaginase-based treatment [19]. Additionally, a recent cross-sectional study that was conducted in pediatric patients with ALL reported a recessive model of CC genotype being significantly associated with hepatotoxicity following asparaginase based treatment (CC vs CT and TT genotype; OR = 7.82, 95% CI=3.86–15.85,  $P < 0.05$ ) [34]. While the frequency of hepatotoxicity among the CC genotype was higher than that among the CT and TT genotypes, the association between the *SOD2* rs4880 genotypes and



hepatotoxicity did not reach statistical significance in univariate or multivariate analysis. This is likely due to the small sample size and the correlation between the risk genotype and ethnicity.

Other study has shown that the polymorphism rs4880, particularly C allele, was strongly associated with hepatotoxicity development secondary to anti-tuberculosis drugs (OR = 2.4, P = 0.02), [24] showing that this variant may contribute to the incidence of hepatotoxicity induced by different treatment modalities. Similarly, a recent prospective study on a Chinese cohort of 1060 subjects with tuberculosis (TB) found a potential association between the *SOD2* rs4880 and the susceptibility to anti-tuberculosis drug-induced liver injury (OR = 1.5, P = 0.155) [35]. Furthermore, another study conducted to investigate the potential association between the *SOD2* rs4880C allele and the risk of developing idiosyncratic hepatotoxicity in the Hispanic population reported that the CC genotype was associated with a significant risk of developing various types of liver injury (OR = 2.3, 95% CI = 1.4–3.8, P = 0.0058) [25].

Oxidative stress is developed due to an imbalance between the generation and accumulation of ROS in cells and tissues and the ability of various antioxidant enzymes to eradicate these reactive products [36]. Drug-induced hepatotoxicity is commonly associated with oxidative stress. Antioxidant enzymes, including SOD, catalase (CAT), and glutathione peroxidase (GPX), play an essential role in protecting cells and tissues from oxidative stress-related damage [21,36]. Therefore, an elevated ROS level resulting from variability in the function or expression of antioxidant enzymes may affect treatment outcome and toxicity [21]. For instance, a SNP, rs1050450, was identified in the human GPX1 gene causes either a proline (C allele) or a leucine (T allele) substitution at the 198 amino acid residues. The TT genotype of this variant has been shown to reduce the enzyme activity by 40% [25]. That may explain the significant association between the TT genotype of rs1050450 and cholestatic injury, a type of drug-induced liver injury (OR = 5.1, 95% CI = 1.6–16.0, P = 0.01) [25].

Our study also confirmed a significant difference in the BMI between Hispanic and non-Hispanic pediatric patients with ALL. The average BMI of Hispanic pediatric patients were much higher compared with non-Hispanic. This result is consistent with that of Butturini et al., [37] who also found that obese pediatric patients with ALL were more likely to be Hispanic (P = 0.001). Furthermore, it has been reported that obese pediatric patients with ALL are at high risk for developing hepatotoxicity or pancreatitis following chemotherapy [6]. Our study also found that patients with the CC genotype have significantly higher BMI than patients with the CT or TT genotypes. Obesity likely contributes to the risk for developing asparaginase-induced hepatotoxicity along with ethnicity and genetic variation in the *SOD2* gene.

In summary, we have identified that liver enzymes during chemotherapy were more elevated in the Hispanic pediatric patients with ALL than non-Hispanic, and we confirmed that the *SOD2* rs4880 risk genotype CC is more prevalent in Hispanics and is associated with elevated liver enzymes in patients treated with asparaginase. Further functional and mechanistic studies are needed to investigate this genetic contribution in Hispanic patients

with ALL to improve their outcomes and develop therapeutic approaches to mitigate asparaginase-related hepatotoxicity.

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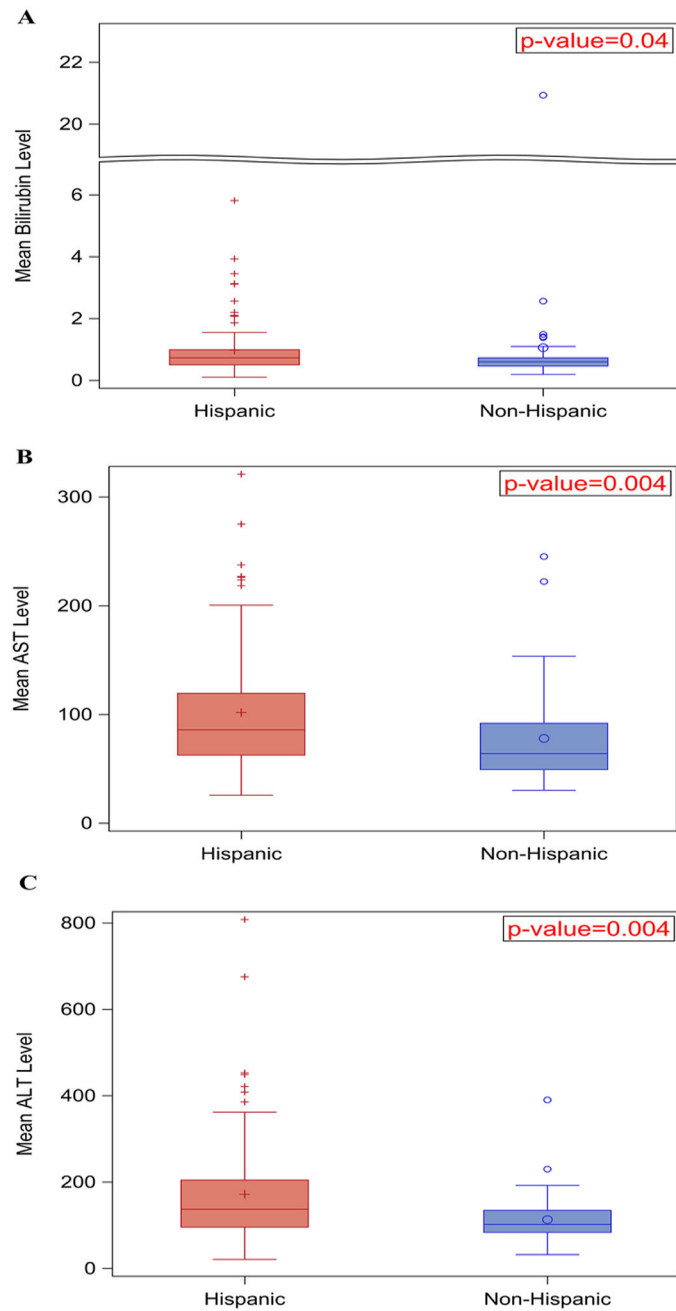
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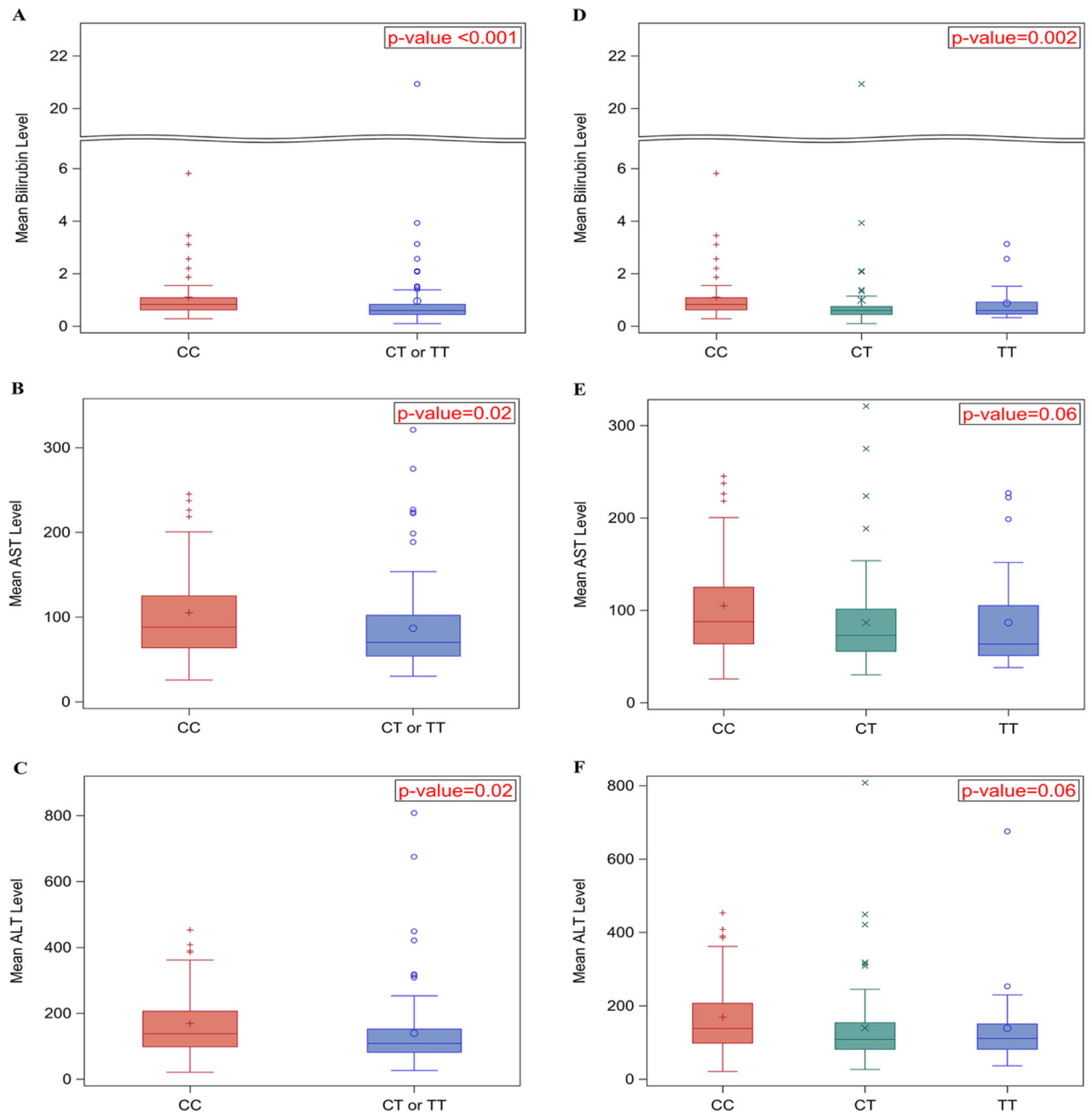
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**Fig. 1.** The mean levels of bilirubin, AST, and ALT by ethnic groups during asparaginase treatment. (A) The mean level of bilirubin in Hispanic and non-Hispanic pediatric patients with ALL. (B) The mean level of AST among Hispanic and non-Hispanic patients. (C) The mean level of ALT in Hispanic patients compared with non-Hispanic patients.



**Fig. 2.**

The mean levels of bilirubin, AST, and ALT by rs4880 genotype. (A) The mean level of bilirubin of patients with CC vs. those with CT or TT (B) The mean level of AST in patients with CC vs. CT or TT. (C) The mean level of ALT of patients with CC vs. those with CT or TT genotype. (D) The mean bilirubin level of a pediatric patient with CC, CT, and TT genotypes during treatment. (E) The mean AST level of pediatric patients with CC, CT and TT genotype. (F) The mean ALT level of patients with CC, CT and TT genotypes.

**Table 1**

Baseline Demographics (N = 143).

Patient characteristics	Total (N = 143)
Age- no. (%)	
< 1 year	1 (0.7%)
1–4 years	66 (46.2%)
5–9 years	40 (28.0%)
10–14 years	22 (15.4%)
15–19 years	10 (7.0%)
19–20 years	4 (2.8%)
Gender- no. (%)	
Male	90 (62.9%)
Female	53 (37.1%)
Ethnicity -no. (%)	
Hispanic	89 (62.2%)
Non-Hispanic	54 (37.8%)
BMI mean ( $\pm$ SD) (n = 139)	20.4 ( $\pm$ 6.0)
Obesity -no. (%) (n = 143)	52 (36.4%)

<sup>^</sup>Data are presented as number (percentage) or mean ( $\pm$  SD) BMI calculation excluding patients < 2 years

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**Table 2**

Patient Characteristics stratified by Ethnicity.

Patient characteristics	Hispanic (n = 89)	Non-Hispanic (n = 54)	p-value
Age- no. (%)			0.85
< 1 year	1 (1.1%)	0 (0%)	–
1–4 years	40 (44.9%)	26 (48.2%)	–
5–9 years	24 (27.0%)	16 (29.6%)	–
10–14 years	14 (15.7%)	8 (14.8%)	–
15–19 years	8 (9.0%)	2 (3.7%)	–
19–20 years	2 (2.2%)	2 (3.7%)	–
Gender- no. (%)			0.28
Male	53 (59.6%)	37 (68.5%)	–
Female	36 (40.5%)	17 (31.5%)	–
BMI mean ( $\pm$ SD)	21.6 ( $\pm$ 6.9)	18.6 ( $\pm$ 3.7)	0.002 *
Obesity -no. (%)	39 (43.8%)	13 (24.1%)	0.02
Obese + Overweight- no. (%)	47 (52.8%)	24 (44.4%)	0.33
Outcome			
Bilirubin (grade 3 or 4)	11 (12.4%)	2 (3.7%)	0.13
AST (grade 4)	2 (2.3%)	1 (1.9%)	1.00
AST (grade 3)	38 (42.7%)	17 (31.5%)	0.18
ALT (grade 4)	17 (19.1%)	2 (3.7%)	0.01 *
ALT (grade 3)	74 (83.2%)	35 (64.8%)	0.01 *
Hepatotoxicity	42 (47.2%)	18 (33.3%)	0.10

<sup>^</sup>Data are presented as number (percentage) or mean ( $\pm$  SD); P-values were calculated from Chi-square (or Fisher's exact) tests for categorical variables, and independent two-sample T-tests or Wilcoxon-Mann-Whitney tests for continuous variables.

\* Denotes p value  $\leq$  0.01.

BMI calculation excluding patients < 2 years



**Table 3**

Baseline Levels of Bilirubin and Liver Enzymes by Ethnicity.

Variables	Hispanic (n = 89) Mean ( $\pm$ SD)	Non-Hispanic (n = 54) Mean ( $\pm$ SD)	P-value
Bilirubin	1.1 ( $\pm$ 1.2)	1.8 ( $\pm$ 7.4)	0.58
AST	65.7 ( $\pm$ 56.2)	55.6 ( $\pm$ 60.2)	0.61
ALT	113.1 ( $\pm$ 116.6)	86.7 ( $\pm$ 85.4)	0.49

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**Table 4**

Patients Demographic and Clinical Characteristics Stratified by Genotype.

Patient characteristics	CC (n = 46)	CT or TT (n = 97)	p-value
Age- no. (%)			0.07
< 1 year	1 (2.2%)	0 (0.0%)	–
1–4 years	16 (34.8%)	50 (51.6%)	–
5–9 years	12 (26.1%)	28 (28.9%)	–
10–14 years	12 (26.1%)	10 (10.3%)	–
15–19 years	4 (8.7%)	6 (6.2%)	–
19–20 years	1 (2.2%)	3 (3.1%)	–
Gender- no. (%)			0.45
Male	31 (67.4%)	59 (60.8%)	–
Female	15 (32.6%)	38 (39.2%)	–
Ethnicity (Hispanic)	38 (82.6%)	51 (52.6%)	< 0.001 * *
BMI mean ( $\pm$ SD)	22.1 ( $\pm$ 6.9)	19.6 ( $\pm$ 5.4)	0.03 *
Obesity -no. (%)	19 (41.3%)	33 (34.0%)	0.40
Outcome			
Bilirubin (grade 3)	7 (15.2%)	6 (6.2%)	0.12
AST (grade 4)	3 (6.5%)	0 (0.0%)	0.03 *
AST (grade 3)	23 (50.0%)	32 (33.0%)	0.05
ALT (grade 4)	9 (19.6%)	10 (10.3%)	0.13
ALT (grade 3)	39 (84.8%)	70 (72.2%)	0.10
Hepatotoxicity	24 (52.2%)	36 (37.1%)	0.09

<sup>^</sup>Data are presented as number (percentage) or mean ( $\pm$  SD); P-values were calculated from Chi-square (or Fisher's exact) tests for categorical variables, and independent two-sample T-tests or Wilcoxon-Mann-Whitney tests for continuous variables.

\* Denotes p-value < 0.05;

\* \* denotes p-value < 0.001.

BMI calculation excluding patients < 2 years

**Table 5**

Univariate Cox Analysis for Predicting Asparaginase Induced Hepatotoxicity.

Variables	Hazard Ratio	95% CI	P-value
Hispanic	2.0	1.2 3.6	0.01 <sup>a</sup>
Genotype (CC) vs. CT or TT	1.5	0.9 2.6	0.11
Age	1.0	1.0 1.1	0.33
BMI	1.0	1.0 1.1	0.03
Obese	0.8	0.5 1.3	0.38
Female	1.1	0.7 1.9	0.70

BMI calculation excluding patients &lt; 2 years

<sup>a</sup>Denotes p-value < 0.01

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**Table 6**

Multivariate Cox Analysis for Predicting Asparaginase Induced Hepatotoxicity.

Variables	Hazard Ratio	95% CI	P-value	
Hispanic	1.9	1.0	3.5	0.05 <sup>a</sup>
Genotype (CC) vs. CT or TT	1.2	0.7	2.1	0.54
Age	1.0	0.9	1.1	0.65
BMI	1.0	1.0	1.1	0.13
Female	1.2	0.7	2.0	0.61

BMI calculation excluding patients &lt; 2 years

<sup>a</sup>Denotes p-value < 0.05

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**Table 7**

Multivariate Cox Analysis for Predicting Asparaginase Induced Hepatotoxicity.

Variables	Hazard Ratio	95% CI	P-value
Hispanic	1.8	1.0 3.3	0.04 <sup>a</sup>
Genotype (CC) vs. CT or TT	1.2	0.7 2.2	0.42
Age	1.0	0.9 1.1	0.48
Obese	1.1	0.6 1.9	0.62
Female	1.1	0.6 1.9	0.63

<sup>a</sup>Denotes p-value < 0.05

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