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Expression and polymorphism (rs4880) of mitochondrial superoxide dismutase (SOD2) and asparaginase induced hepatotoxicity in adult patients with acute lymphoblastic leukemia

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Abstract

Asparaginase, which depletes asparagine and glutamine, activates amino acid stress response. Oxidative stress mediated by excessive reactive oxygen species (ROS) causes enhanced mitochondrial permeabilization and subsequent cell apoptosis and is considered a plausible mechanism for drug-induced hepatotoxicity, a common toxicity of asparaginase in adults with acute lymphoblastic leukemia (ALL). Studies investigating the pharmacogenetics of asparaginase in ALL are limited and focused on asparaginase-induced allergic reaction common in pediatric patients. Here, we sought to determine a potential association between the variant rs4880 in *SOD2* gene, a key mitochondrial enzyme that protects cells against ROS, and hepatotoxicity during asparaginase-based therapy in 224 patients enrolled on CALGB-10102, a treatment trial for adults with ALL. We report that the CC genotype of rs4880 is associated with increased hepatotoxicity following asparaginase-based treatment. Thus, rs4880 likely contributes to asparaginase-induced hepatotoxicity, and functional studies investigating this SNP are needed to develop therapeutic approaches that mitigate this toxicity.

Keywords

Acute Lymphoblastic Leukemia; Asparaginase; SOD2; rs4880; polymorphism; hepatotoxicity

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Conflict of interest

Authors do not have any conflict of interest to declare.

Introduction

The intensive use of asparaginase is an essential component of pediatric regimens for acute lymphoblastic leukemia (ALL) and has been associated with significant improvements in survival. Accordingly, many attempts have incorporated asparaginase in treatment regimens for adults with ALL; yet, the higher rate of asparaginase-related toxicities in adults with ALL have limited its widespread use and new insights are needed. (1) Studies investigating the pharmacogenetics of asparaginase in ALL are limited and mostly focused on pediatric patients with ALL. A recent study investigated more than 500,000 single nucleotide polymorphisms (SNPs) in 485 children with ALL and found an association of five SNPs in the *GRIA1* gene with hypersensitivity to the drug.(2) The same group also tested more than 2 million SNPs using the HapMap lymphoblastoid cell lines and identified the aspartate metabolic routes as the most likely candidate pathway for asparaginase sensitivity.(3) In addition, polymorphisms in genes that mediate the antileukemic effect of asparaginase, such as the asparaginase synthetase gene, the basic region leucine zipper activating transcription factor 5, and the argininosuccinate synthase 1 gene, were found to be associated with reduced event-free survival of childhood patients with ALL, but not with toxicity.(4)

While asparaginase allergy is the main toxicity observed in children, hepatotoxicity is one of the most common toxicities of this drug in adults with ALL and often limits the use of this effective drug in this age group.(1, 5) The incidence rate of elevated liver enzymes and hyperbilirubinemia (grade 3 or 4) was reported to be 36% and 14%, respectively, in adults compared to 20% and 3% in pediatric patients.(1) Studies that focused on exploring these toxicities in association with polymorphisms in adult ALL are still limited.

Superoxide dismutase (SOD), an enzyme that catalyzes the dismutation of superoxide ($O2^{-}$) into oxygen and hydrogen peroxide, is a crucial antioxidant that protects cells against oxidative stress. Three forms of SOD enzymes are present in mammalian cells; cytoplasmic superoxide dismutase (SOD1), mitochondrial superoxide dismutase (SOD2), and extracellular superoxide dismutase (SOD3).(6) Two previous studies have reported that the SOD2 polymorphism causing a V16A amino-acid substitution (rs4880) is significantly associated with drug induced liver injury (DILI).(7, 8) Recently, the same polymorphism was found to be significantly correlated with breast cancer survival after cyclophosphamide-containing chemotherapy.(9)

Here we studied 224 patients enrolled on CALGB 10102, a treatment trial for adults with previously untreated ALL who received L-asparaginase as part of their chemotherapy regimen. The aim of the study is to investigate potential associations between the *SOD2* rs4880 polymorphism and asparaginase-related hepatotoxicity in adult patients with ALL. Secondary objectives of this study are to assess a possible correlation between this polymorphism and ALL susceptibility in adults, and to determine whether this polymorphism is associated with *SOD2* transcript levels as a possible mechanism for this functional variant. Here we also genotyped rs4958351 in the *GRIA1* gene, one of the SNPs that was previously identified to be associated with hypersensitivity to asparaginase in children with ALL. (2)

Materials and Methods

Patient population

We studied samples obtained from 224 patients with previously untreated ALL, enrolled on a national clinical trial for adults with ALL {Cancer and Leukemia Group B [CALGB] trial 10102}. Informed consent to use the tissue for investigational studies was obtained from each patient enrolled on the trial and according to institutional guidelines. Complete clinical data was available for 221 of 224 patients. Samples at remission (after Cycle III of treatment) were available from 196 patients for DNA extraction and genotyping. Paired samples of pretreatment and post remission peripheral blood (bone marrow paired samples were obtained from two patients) samples from 30 patients were available for RNA analysis. Remission samples (after Cycle III of treatment) obtained from 86 patients were used for RNA analysis and correlation with hepatotoxicity and genotypes (Table S1).

By Cycle III of the treatment regimen on the CALGB 10102 protocol, patients would have received the following chemotherapy: Cyclophosphamide, Daunorubicin, Vincristine, L-asparaginase, Cytarabine, Methotrexate, and 6-Mercaptopurine. L-asparaginase was given as 6000 U/m^2 SC or IM twice a week for six doses beginning on day 5 during the first month of treatment and on days 15, 18 and 22 during the 2^{nd} and 4^{th} month of treatment. Common Terminology Criteria for Adverse Events v4.0 (*CTCAE*) was used to grade hepatotoxicity.

RNA extraction and Real-time quantitative PCR

Total RNA was isolated from bone marrow and peripheral blood samples using Trizol reagent (Life Technologies Carlsbad, CA, USA). cDNA was synthesized using SuperScript III reagents (Life technologies) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using commercially available TaqMan Gene Expression Assay primers and probes for *SOD2* and *B2M* and the LightCycler 480II Real-Time PCR System (Roche, Basel, Switzerland). The expression levels were normalized to *B2M* gene expression.

DNA extraction and genotyping

Genotyping was performed using the TaqMan Allelic Discrimination Assay for rs4880 of *SOD2* gene and rs4958351 in the *GRIA1* gene (Life Technologies). The PCR was carried out using The LightCycler® 480II System (Roche). After PCR, fluorescence from reaction products was measured and analyzed using the System LightCycler® 480 Software.

Statistical analysis

We tested agreement with the Hardy-Weinberg Equilibrium using a χ^2 goodness-of-fit test for rs4880. Because 84% of patients with ALL in our study reported to be white, we used genotyping data obtained in European cohorts, after excluding patients of Hispanics origin (N=16). We then compared genotype frequencies of the polymorphisms between cases with ALL and reported frequencies obtained from the NCBI European cohort data sets (ESP-cohort, Hap-Map-CEU and CEU-GENO). We also compared genotype frequencies in Hispanic patients with ALL and Hispanic/Latino/Mexican-American cohort data set (HSP-geno-panel). We used the Fisher Exact two-tail test, and calculated odds ratios (ORs) with

95 percent confidence intervals (95% CI). In the association study, P values of less than 0.05 were considered to be statistically significant. Expression levels of *SOD2* transcripts between three or two groups were tested with the Kruskal-Wallis test or an unpaired Mann-Whitney test, respectively. The Chi-square test was then used to compare differences in allele frequencies and genotype distribution of the polymorphism between patients with hepatotoxicity and those without. The Fisher Exact test was used to test the recessive model.

Results

Patient population and hepatotoxicity data

We studied samples obtained from 224 patients with ALL. Demographic and clinical data were available for 221 patients (age range, 17–80 years; median age, 43.9 years; 85 female and 136 male), enrolled on treatment trial (CALGB protocol 10102) for adults with ALL. Asparaginase hepatotoxicity was estimated by assessing the following clinical laboratory markers: aspartate transaminase (AST), alanine transaminase (ALT), albumin, alkaline phosphatase, and bilirubin levels in patients following induction and two cycles of post-remission therapy. Among the 221 patients, 51 (23%) patients had grade 3 or 4 elevated AST levels, and 82 (37%) patients had grade 3 or 4 elevated ALT levels, 53 (24%) patients had grade 3 or 4 elevated bilirubin levels. AST and ALT levels may be increased several folds above normal in hepatocyte injury. On the other hand, elevations of alkaline phosphatase and bilirubin levels predominate in cholestatic syndromes (10). Therefore, we defined hepatotoxicity as grade 3/4 of both AST and ALT, grade 4 of either AST or ALT, or grade 3/4 of bilirubin elevation. Using this classification, we identified 73 patients (33.03%) meeting these criteria (Table 1).

Genotyping analysis for *SOD2* rs4880 in patients with ALL

DNA samples from 196 patients with ALL were genotyped for *SOD2* rs4880 and GRIA1 rs4958351. Among these, four samples were excluded from the *SOD2* rs4880 analysis due to poor discrimination/quality of genotyping results. The genotypes of the remaining 192 samples were used for further analysis. Our genotyping results showed a minor allelic frequency of 0.52 (for the C allele). Among the 192 patients, 48 patients had a TT genotype (25%), 55 patients had a CC genotype (28.6%), and 89 patients had a CT genotype (46%). These results did not deviate from the Hardy-Weinberg equilibrium (Chi-square (χ^2) test P=0.32 with 1 degree of freedom).

We compared genotype frequencies of *SOD2* rs4880 obtained from our data set of patients with ALL, with publicly available databases. Because 84% of the patients enrolled on CALGB 10102 were Caucasian, we compared our results of *SOD2* rs4880 genotype frequencies obtained from 192 patients with data from public data bases of European cohorts. We found that the frequency of the CC genotype (28.6%) in our data set was significantly higher than that (14.8%) of the CEUGENO; (two tailed Fisher's Exact test; P=0.007, Table 2). Higher frequency of the CC genotype was also found in the population of patients with ALL when compared with that in the ESP cohort (P=0.029) or the Hap-Map-CEU cohort (P=0.14; Table 2, S2). Since the rs4880 CC genotype is more frequent in the Hispanic population, we reanalyzed the samples after excluding patients of Hispanic

ethnicity (N=16). We found that the frequency of the CC genotype remained higher in patients with ALL in comparison with the CEU-GENO panel (P=0.049) and a trend for higher frequency was observed when we compared our data with that of the ESP or Hap-Map-CEU cohorts. In patients with ALL of Hispanic ethnicity, we found the rs4880 genotype CC present in 8 out of 16 (50%) patients, while the TT genotype was only present in 1 patient (6%). The frequencies of CC, CT and TT in the reported database of Hispanic ethnicity (HSP-GENO-panel cohort; N=108) are 33%, 38% and 27% respectively. Although the numbers are small, nevertheless a trend for higher frequency of the CC genotype was present in patients with ALL of Hispanics origin in comparison with that in the HSP-GENO cohort (P=0.165).

On the other hand, among the 196 patients genotyped for *GRIA1* rs4958351 there were: 21 or 10.7% AA; 86 or 43.8% AG; and 89 or 45.4% GG. The MAF was 0.33, and the genotyping results did not deviate from the Hardy-Weinberg equilibrium (Chi-square (χ^2) test P = 0.97 with 1 degree of freedom). The observed frequency distribution was similar to that of the Hap-Map-CEU population (P=0.98, Table S3).

Association of *SOD2* polymorphism rs4880 and hepatotoxicity

We analyzed 221 patients for whom hepatotoxicity data were available; among them we obtained genotyping data on 190 patients for *SOD2* rs4880, and 193 patients for *GRIA1* rs4958351. Two and three patients with genotyping data but no hepatotoxicity data were excluded from the analysis of rs4880 and rs4958351, respectively. The correlation between the rs4880 genotype and hepatotoxicity parameters is shown in Table 3. Grade 3 and 4 of bilirubinemia was found more frequently in patients with the *SOD2* rs4880 CC genotype compared with those with the CT genotype (Fisher Exact test P=0.055). In addition, the *SOD2* rs4880 CC genotype was associated with a trend towards more serious (Grade 4) hepatotoxicity compared with the rs4880 CT or TT genotypes (P=0.09). Albumin levels did not correlate significantly with rs4880 genotype.

As previously described, we defined hepatotoxicity as grade 3/4 of both AST and ALT, grade 4 of either AST or ALT, or grade 3/4 bilirubin elevation. Using this classification, we identified 61 of the 190 patients (32%) meeting these criteria. We tested the possible genetic models (dominant, recessive, or additive effect). We found that only the CC genotype was associated with increased risk of hepatotoxicity (Chi-square test P=0.018, OR=2.6, 95% CI 1.1-6.07; P=0.026 when CC vs TT were compared, OR=2.5 95% CI 1.2-5.1; P=0.01 when CC vs CT were compared) suggesting a recessive model. Therefore we implemented the recessive model for further analysis and found that patients with the *SOD2* rs4880 CC genotype had a significantly higher frequency of hepatotoxicity than those with the TT or CT genotypes (Fisher Exact test P=0.006; OR=2.53; 95% CI 1.3-4.8; Table 3 and Figure 1, A and B). *GRIA1* rs4958351 did not show a significant association with hepatotoxicity except and association with elevated ALK-Phos, (Table S4).

SOD2 mRNA expression in patients with ALL

Next we sought to examine *SOD2* mRNA levels in cells obtained from patients with ALL, assess changes in *SOD2* mRNA levels following treatment, and correlate these levels with

hepatotoxicity. *SOD2* mRNA expression was measured by RT-PCR in samples obtained from patients with ALL (N=86) that completed induction therapy and two post remission cycles on CALGB10102. *SOD2* mRNA levels were not significantly different between patients with hepatotoxicity, and those without (Figure 2A). Patients were dichotomized into *SOD2* high expressers and *SOD2* low expressers, using the median expression as the cut off. No significant correlation was observed when we assessed each toxicity parameter, or toxicity with grade 3 and higher.

In those patients, for whom we had paired samples (pretreatment and post remission, N=30) for, we analyzed changes in *SOD2* mRNA expression changes. Interestingly, *SOD2* mRNA expression was significantly lower in pretreatment samples in comparison to remission samples (Figure 2B). Although rs4880 is known to affect the mitochondrial translocation of SOD2, in order, to exclude other possible transcriptional regulatory mechanisms for this SNP, we examined the expression of *SOD2* mRNA in pretreatment (N=25) and remission (N=86) samples relative to the rs4880 genotype. We did not observe significant correlation between *SOD2* mRNA levels prior to, or during treatment and rs4880 genotypes (Figure 3A,B).

Discussion

Asparaginase, which depletes asparagine and glutamine, activates an amino acid stress response. Similar to other cytotoxic agents, the anti-cancer activity is associated with oxidative stress, which is mediated by excessive reactive oxygen species (ROS), resulting in elevated mitochondrial permeabilization and subsequent cell apoptosis.(11, 12) This process is thought to represent a common mechanism of drug-induced hepatotoxicity. High ROS levels resulting from variability in the function or expression of enzymes involved in these pathways may affect therapeutic outcomes and toxicities.(13)

SOD2 is located predominantly in the mitochondrial matrix and plays an important role in the detoxification of mitochondrial superoxide (14, 15) by converting superoxide into hydrogen peroxide and oxygen.(16) This enzyme is indispensable for cell survival; while, homozygous SOD2-deficient mice die shortly after birth, (17) heterozygous SOD2-deficient mice are viable but present with increased susceptibility to chemical-induced mitochondrial toxicity in the liver. (18-20) This suggests an important role of this gene in protecting the liver from drug-induced toxicities.

The polymorphism rs4880 in *SOD2* results in the incorporation of either alanine (C allele) or valine (T allele) in the mitochondrial targeting sequence of the protein. The alanine form of SOD2 is transported normally into the mitochondria, while the protein with valine is partially trapped in the inner mitochondrial membrane.(21) Although this suggests that the T allele would be associated with lower enzymatic efficiency, higher levels of ROS and greater risk of cancer and toxicities, most pharmacogenomic correlative studies have implicated the C allele as a risk allele for cancer.(22-24) Here we reveal that the CC genotype, but not CT or TT, is likely present at higher frequencies in patients with ALL. We also found this genotype to be more frequent in the Hispanic cohort and are known to have a higher incidence of ALL compared to other ethnicities.(25) Polymorphisms in *SOD2* have not

previously been associated with susceptibility to ALL; perhaps the lack of large pharmacogenomics studies in adult patients with ALL may have previously limited the statistical power to identify this locus.

Importantly, in this study we found that the CC genotype was associated with asparaginase related hepatotoxicity in adult patients with ALL. Patients in the study have also received methotrexate and 6-Mercaptopurine, both drugs may contribute to the elevation in transaminases and hepatotoxicity. Liver enzymes and bilirubin levels are measured regularly to monitor asparaginase-associated hepatotoxicity. Current clinical guidelines recommend holding asparaginase treatment in adult when grade 3–4 hepatotoxicity develops and then re-challenging with careful monitoring if toxicity resolves to grade 1, often resulting in prolonged delays in treatment and suboptimal dosing which may impair treatment outcomes. (1) Therefore the findings resulted from our pharmacogenomic approach may have important clinical implications.

Interestingly, *SOD2* transcript levels were significantly higher in samples obtained at remission compared to those obtained at diagnosis. This may be attributed to the different cell composition, being leukemic cells in the diagnosis samples and normal cells in the remission samples. Asparaginase induced *SOD2* mRNA upregulation, may also contribute to this finding. The transcript levels of *SOD2* did not correlate with this polymorphism, or differ between patients with and without hepatotoxicity. Previous studies have shown that this variant affected the enzymatic activity but not the transcript level of SOD2. SOD2 activity has been reported to be 33% higher in CT or TT individuals compared to CC individuals.(26) While we recognize that our study is limited by the relatively small number of patients analyzed and the lack of a control cohort, our data suggest that genetic variation in the *SOD2* gene is associated with susceptibility to ALL in this adult cohort and is associated with treatment related hepatic toxicities; thus, a larger cohort is required to validate these findings. Furthermore, functional studies that investigate this genetic association are needed in order to develop therapeutic approaches that might mitigate this toxicity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

1. Stock W, Douer D, DeAngelo DJ, Arellano M, Advani A, Damon L, et al. Prevention and management of asparaginase/pegasparaginase-associated toxicities in adults and older adolescents: recommendations of an expert panel. Leukemia & lymphoma. Dec; 2011 52(12):2237–53. PubMed PMID: 21827361. [PubMed: 21827361]
2. Chen SH, Pei D, Yang W, Cheng C, Jeha S, Cox NJ, et al. Genetic variations in GRIA1 on chromosome 5q33 related to asparaginase hypersensitivity. Clinical pharmacology and therapeutics.

Aug; 2010 88(2):191–6. PubMed PMID: 20592726. Pubmed Central PMCID: 3000799. [PubMed: 20592726]

3. Chen SH, Yang W, Fan Y, Stocco G, Crews KR, Yang JJ, et al. A genome-wide approach identifies that the aspartate metabolism pathway contributes to asparaginase sensitivity. Leukemia. Jan; 2011 25(1):66–74. PubMed PMID: 21072045. Pubmed Central PMCID: 3097057. [PubMed: 21072045]
4. Rousseau J, Gagne V, Labuda M, Beaubois C, Sinnett D, Laverdiere C, et al. ATF5 polymorphisms influence ATF function and response to treatment in children with childhood acute lymphoblastic leukemia. Blood. Nov 24; 2011 118(22):5883–90. PubMed PMID: 21972289. Pubmed Central PMCID: 3342855. [PubMed: 21972289]
5. Kearney SL, Dahlberg SE, Levy DE, Voss SD, Sallan SE, Silverman LB. Clinical course and outcome in children with acute lymphoblastic leukemia and asparaginase-associated pancreatitis. Pediatric blood & cancer. Aug; 2009 53(2):162–7. PubMed PMID: 19405141. Pubmed Central PMCID: 2721691. [PubMed: 19405141]
6. Miao L, St Clair DK. Regulation of superoxide dismutase genes: implications in disease. Free radical biology & medicine. Aug 15; 2009 47(4):344–56. PubMed PMID: 19477268. Pubmed Central PMCID: 2731574. [PubMed: 19477268]
7. Huang YS, Su WJ, Huang YH, Chen CY, Chang FY, Lin HC, et al. Genetic polymorphisms of manganese superoxide dismutase, NAD(P)H:quinone oxidoreductase, glutathione S-transferase M1 and T1, and the susceptibility to drug-induced liver injury. Journal of hepatology. Jul; 2007 47(1): 128–34. PubMed PMID: 17400324. [PubMed: 17400324]
8. Lucena MI, Garcia-Martin E, Andrade RJ, Martinez C, Stephens C, Ruiz JD, et al. Mitochondrial superoxide dismutase and glutathione peroxidase in idiosyncratic drug-induced liver injury. Hepatology. Jul; 2010 52(1):303–12. PubMed PMID: 20578157. [PubMed: 20578157]
9. Glynn SA, Boersma BJ, Howe TM, Edvardsen H, Geisler SB, Goodman JE, et al. A mitochondrial target sequence polymorphism in manganese superoxide dismutase predicts inferior survival in breast cancer patients treated with cyclophosphamide. Clinical cancer research : an official journal of the American Association for Cancer Research. Jun 15; 2009 15(12):4165–73. PubMed PMID: 19509150. Pubmed Central PMCID: 2697269. [PubMed: 19509150]
10. Lee WM. Drug-induced hepatotoxicity. The New England journal of medicine. Jul 31; 2003 349(5):474–85. PubMed PMID: 12890847. [PubMed: 12890847]
11. Wilson GJ, Bunpo P, Cundiff JK, Wek RC, Anthony TG. The eukaryotic initiation factor 2 kinase GCN2 protects against hepatotoxicity during asparaginase treatment. American journal of physiology Endocrinology and metabolism. Nov 1; 2013 305(9):E1124–33. PubMed PMID: 24002574. Pubmed Central PMCID: 3840205. [PubMed: 24002574]
12. Costantini P, Jacotot E, Decaudin D, Kroemer G. Mitochondrion as a novel target of anticancer chemotherapy. Journal of the National Cancer Institute. Jul 5; 2000 92(13):1042–53. PubMed PMID: 10880547. [PubMed: 10880547]
13. Choi JY, Nowell SA, Blanco JG, Ambrosone CB. The role of genetic variability in drug metabolism pathways in breast cancer prognosis. Pharmacogenomics. Jun; 2006 7(4):613–24. PubMed PMID: 16753008. [PubMed: 16753008]
14. Macmillan-Crow LA, Cruthirds DL. Invited review: manganese superoxide dismutase in disease. Free radical research. Apr; 2001 34(4):325–36. PubMed PMID: 11328670. [PubMed: 11328670]
15. Zelko IN, Mariani TJ, Folz RJ. Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. Free radical biology & medicine. Aug 1; 2002 33(3):337–49. PubMed PMID: 12126755. [PubMed: 12126755]
16. Fridovich I. Superoxide radical and superoxide dismutases. Annual review of biochemistry. 1995; 64:97–112. PubMed PMID: 7574505.
17. Lebovitz RM, Zhang H, Vogel H, Cartwright J Jr. Dionne L, Lu N, et al. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. Proceedings of the National Academy of Sciences of the United States of America. Sep 3; 1996 93(18):9782–7. PubMed PMID: 8790408. Pubmed Central PMCID: 38506. [PubMed: 8790408]
18. Williams MD, Van Remmen H, Conrad CC, Huang TT, Epstein CJ, Richardson A. Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese

superoxide dismutase knockout mice. The Journal of biological chemistry. Oct 23; 1998 273(43): 28510–5. PubMed PMID: 9774481. [PubMed: 9774481]

19. Ong MM, Wang AS, Leow KY, Khoo YM, Boelsterli UA. Nimesulide-induced hepatic mitochondrial injury in heterozygous Sod2(+/−) mice. Free radical biology & medicine. Feb 1; 2006 40(3):420–9. PubMed PMID: 16443156. [PubMed: 16443156]
20. Larosche I, Letteron P, Berson A, Fromenty B, Huang TT, Moreau R, et al. Hepatic mitochondrial DNA depletion after an alcohol binge in mice: probable role of peroxynitrite and modulation by manganese superoxide dismutase. The Journal of pharmacology and experimental therapeutics. Mar; 2010 332(3):886–97. PubMed PMID: 20016022. [PubMed: 20016022]
21. Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. Pharmacogenetics. Mar; 2003 13(3):145–57. PubMed PMID: 12618592. [PubMed: 12618592]
22. Ambrosone CB, Freudenheim JL, Thompson PA, Bowman E, Vena JE, Marshall JR, et al. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. Cancer research. Feb 1; 1999 59(3):602–6. PubMed PMID: 9973207. [PubMed: 9973207]
23. Woodson K, Tangrea JA, Lehman TA, Modali R, Taylor KM, Snyder K, et al. Manganese superoxide dismutase (MnSOD) polymorphism, alpha-tocopherol supplementation and prostate cancer risk in the alpha-tocopherol, beta-carotene cancer prevention study (Finland). Cancer causes & control : CCC. Aug; 2003 14(6):513–8. PubMed PMID: 12948282. [PubMed: 12948282]
24. Hung RJ, Boffetta P, Brennan P, Malaveille C, Gelatti U, Placidi D, et al. Genetic polymorphisms of MPO, COMT, MnSOD, NQO1, interactions with environmental exposures and bladder cancer risk. Carcinogenesis. Jun; 2004 25(6):973–8. PubMed PMID: 14729580. [PubMed: 14729580]
25. Pullarkat ST, Danley K, Bernstein L, Brynes RK, Cozen W. High lifetime incidence of adult acute lymphoblastic leukemia among Hispanics in California. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. Feb; 2009 18(2):611–5. PubMed PMID: 19208664. Pubmed Central PMCID: 3191882.
26. Bastaki M, Huen K, Manzanillo P, Chande N, Chen C, Balmes JR, et al. Genotype-activity relationship for Mn-superoxide dismutase, glutathione peroxidase 1 and catalase in humans. Pharmacogenetics and genomics. Apr; 2006 16(4):279–86. PubMed PMID: 16538174. [PubMed: 16538174]

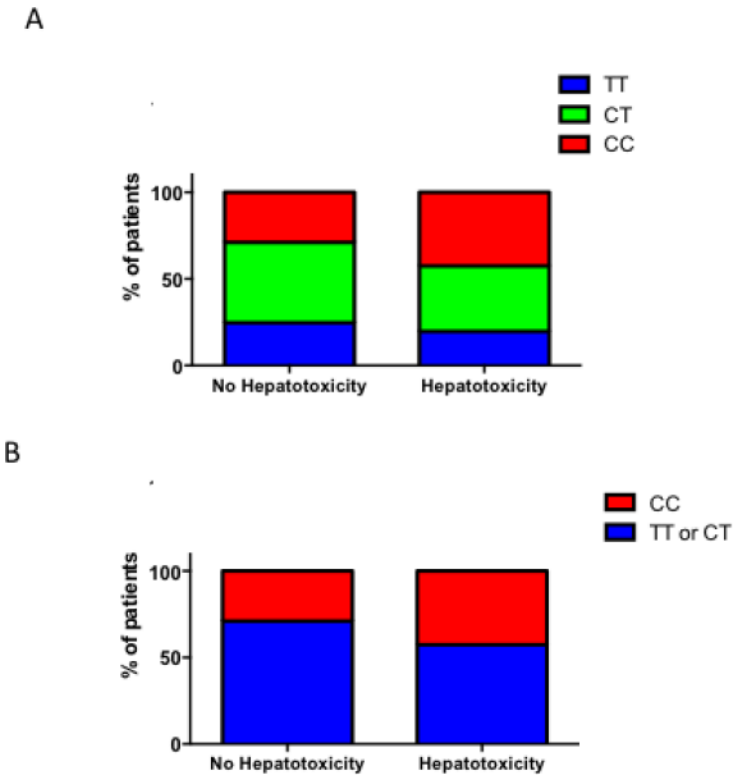

Figure 1. The rs4880 *SOD2* SNP genotype frequencies in adult patients with ALL and association with hepatotoxicity

(A) The frequency of the TT, CT, and CC genotypes in adult patients with or without hepatotoxicity. The CC genotype was significantly more frequent in patients with hepatotoxicity compared with patients without hepatotoxicity. (B) A recessive model was implemented, and showed that patients with the *SOD2* rs4880 CC genotype had significantly higher frequency of hepatotoxicity than those with the TT or CT genotypes (Fisher Exact test P=0.006; OR=2.5; 95% CI 1.3-4.8)

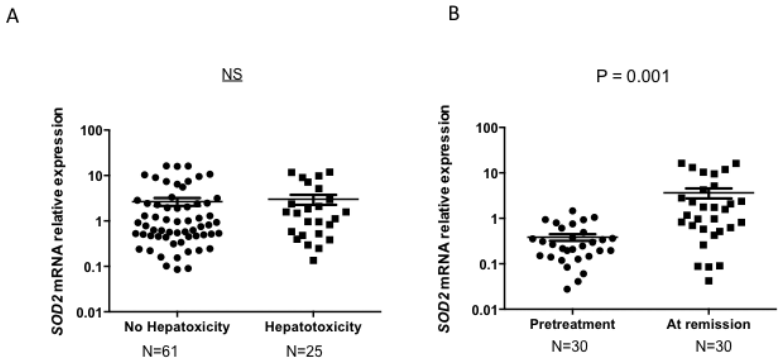

Figure 2. Analysis of SOD2 mRNA expression in adult patients with ALL and association with hepatotoxicity

(A) *SOD2* mRNA expression was measured by RT-PCR in samples obtained from patients with ALL (N=98) that completed induction therapy and two post remission cycles on CALGB10102 and compared according to their hepatotoxicity status (each circle represent different patient without hepatotoxicity and each square represent different patient with hepatotoxicity). (B) *SOD2* mRNA expression changes were analyzed in paired samples (pretreatment and post remission, N=30, circles represent pretreatment samples and squares represent post treatment samples).

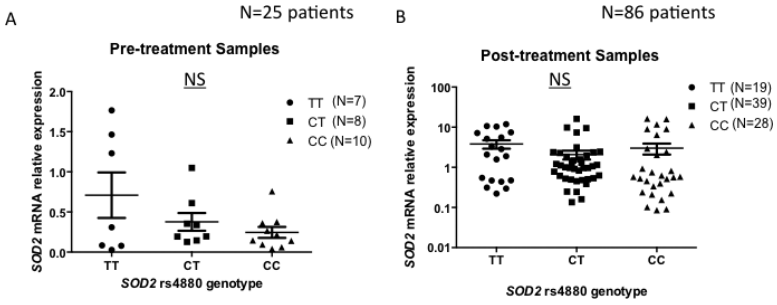

Figure 3. Analysis of SOD2 mRNA expression in adult patients with ALL and association with rs4880 SOD2 SNP genotypes

SOD2 mRNA expression levels in (A) pretreatment (N=25) and (B) remission (N=86) samples were examined according to rs4880 genotype. (Circles, squares and triangles represent TT, CT and CC genotypes, respectively).

Table 1

Hepatotoxicity rates in 221 adult patients with ALL received asparaginase-based treatment

Hepatotoxicity data	Number or patients (N)	Percent of patients (%)
Total	221	100
AST grade 3/4	51	23.08
ALT grade 3/4	82	37.10
Albumin grade 3	29	13.12
ALK-Pho grade 3/4	30	13.57
Bilirubin grade 3/4	53	23.98
Hepatic failure gratia 4/5	5	2.26
ALT and AST grade 3, either grade 4 or bilirubin grade 3/4	73	33.03

Table 2

The rs4880 *SOD2* SNP genotype frequencies in adult patients with ALL (bold) and comparison with its frequencies in European and Hispanics cohorts.

					CC vs CT vs TT analysis			CC vs CT+TT analysis	
Cohort	**total N**	**CC (%)**	**CT (%)**	**TT (%)**	**Chi square P value (including Hispanics)**	**Chi square P value (excluding Hispanics)**	**Chi square P Hispanics (only)**	**Fisher Exact P value (including Hispanics)**	**Fisher Exact P value (excluding Hispanics)**
ESP-Cohort	4368	0.207	0.504	0.288	0.029	0.16		0.01 *	0.069
Hap-Map-CEU	226	0.221	0.451	0.327	0.14	0.35		0.14	0.29
CEU-GENO	108	0.148	0.5	0.352	0.016	0.049		0.007	0.019
HSP-GENO	108	0.333	0.389	0.278			0.165		
Patients with ALL	192	**0.281**	**0.45**	**0.245**					
Patients with ALL (excluding Hispanics)	176	**0.267**	**0.466**	**0.267**					
Hispanics patients with ALL	16	**0.50**	**0.43**	**0.06**					

* The chi-square with Yates' correction was calculated instead of Fisher Exact text, due to the large number of samples

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Table 3

The rs4880 *SOD2* SNP genotype frequencies in adult patients with ALL and association with hepatotoxicity.

Genotype	Patients N (%)	CC N(%)	CT N(%)	TT N(%)	Chi-square test P value	Fisher Exact test P value (recessive model)
Total N (%)	190 (100)	55 (28.9)	88 (46.3)	47 (24.7)		
AST grade 3/4	42 (22.1)	16 (38.1)	21 (50)	5 (11.9)	0.069	0.18
ALT grade 3/4	68 (35.7)	19 (27.9)	30 (44.1)	19 (27.9)	0.76	0.86
Albumin grade 3/4	26 (13.7)	9 (34.6)	10 (38.4)	7 (26.9)	0.69	0.49
ALK-phos grade 3/4	27 (14.2)	10 (37)	9 (33.3)	8 (29.6)	0.35	0.36
Bilirubin* **grade 3/4**	43 (22.6)	18 (41.8)	14 (32.5)	11 (25.6)	0.067	0.05
Any Hepatotoxicity grade 4/5**	23 (12.1)	10 (43.4)	10 (43.4)	3 (13)	0.11	0.09
Any Hepatotoxicity grade 3/4/5	129 (67.9)	41 (31.7)	54 (41.8)	34 (26.35)	0.12	0.08
Both AST and ALT grade 3 Or eitherAST or ALT grade 4 Or Bilirubin 3/4	**61(32.1)**	**26 (42.6)**	**23 (37.7)**	**12 (19.6)**	**0.018**	**0.006**

*Based on total and direct bilirubin measurements

**Three patients had hepatic failure of Grade 5.